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Novel Activation of *araC* Expression and a DNA Site Required for *araC* Autoregulation in *Escherichia coli* B/r

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Mutations in the *araC* gene have been isolated which alter both the activator and autoregulatory functions of AraC protein (L. G. Cass and G. Wilcox, J. Bacteriol. 166:892–900, 1986). In this study, the effect of each *araC* mutation on autoregulation was characterized in vivo and in vitro in the presence of L-arabinose. The effect of L-arabinose in some of these *araC* mutants revealed a novel activation of *araC* expression which was not observed in the *araC*$^+$ cell. Experiments were therefore focused on understanding the mechanism of this novel activation. We describe a systematic analysis of the effect of mutations within the known regulatory binding sites for *araBAD* and *araC* transcription on *araC* expression. Our results suggest that the novel activation of *araC* expression requires the AraC activator-binding site, *araI*, and the cyclic AMP receptor protein-cyclic AMP complex-binding site. We also found that in the absence of L-arabinose, the *araI* site was required for maximal autoregulation by the wild-type AraC protein.

The AraC protein functions as both a positive and negative regulator of gene expression in *Escherichia coli* (8, 17). Transcription of the *araBAD* operon is repressed by AraC protein in the absence of L-arabinose and is activated by AraC protein in the presence of L-arabinose (8, 17). In the presence or absence of L-arabinose, the *araC* gene is autogenously regulated (2, 12, 19). Maximal expression of both the *araBAD* operon and the *araC* gene requires the presence of the cyclic AMP (cAMP) receptor protein-cAMP complex (CRP-cAMP) (8, 17).

The *araBAD* operon and *araC* gene promoters are separated by 147 base pairs (bp) (33) and are transcribed in opposite directions (34). The DNA sequence of this *ara* regulatory region is known (10, 30). Genetic and biochemical analyses have defined the binding sites for the regulatory proteins AraC and CRP and for RNA polymerase (6, 19, 28) (Fig. 1). Based on these studies, models for the regulation of the *araCBAD* gene complex have been proposed, aspects of which are summarized as follows. (i) AraC protein in its activator conformation binds to a site, *araI*, adjacent to the RNA polymerase-binding site and stimulates initiation of transcription of the *araBAD* operon. (ii) Repression of *araBAD* transcription in the absence of L-arabinose requires AraC protein interaction at the AraC-binding sites $araO_2$, a site located within the transcribed but untranslated region of the *araC* gene, and *araI*. These two AraC-binding sites are separated by over 200 bp (6, 11). (iii) Autoregulation of *araC* gene expression requires AraC protein bound at $araO_1$, a site which overlaps the RNA polymerase-binding site of the *araC* promoter, preventing initiation of transcription (14). (iv) Positive regulation of the *araC* gene and the *araBAD* operon by CRP-cAMP requires the complex bound to a site located upstream from $araO_1$ and from the *araC* transcription initiation site (20, 26) (Fig. 1).

The *Escherichia coli araC* gene encodes a 292-amino-acid polypeptide, as predicted by DNA sequence analysis (23). Mutations in the *araC* gene have been isolated which affect both the activator and autoregulatory functions of AraC protein (5). The effect of each *araC* mutation on autoregulation was characterized in vivo and in vitro in the absence of L-arabinose. Previous studies have shown that in the wild-type cell, induction of *araC* expression by L-arabinose does not occur (2). In the present study, we have reexamined the effect of these *araC* mutations on autoregulation in the presence of L-arabinose. The effect of L-arabinose in some of these *araC* mutants revealed a novel activation of *araC* expression which is not observed in the *araC*$^+$ cell. Therefore, experiments were focused on understanding the mechanism of this novel activation. We describe a systematic analysis of the effect of mutations within the known regulatory binding sites for *araBAD* and *araC* transcription on *araC* expression. Our results suggest that the novel activation of *araC* expression requires the AraC activator-binding site, *araI*, and the CRP-cAMP complex-binding site. We also found that in the absence of L-arabinose, the *araI* site is required for maximal autoregulation by the wild-type AraC protein.

MATERIALS AND METHODS

Media, chemicals, and enzymes. The media and antibiotics used in this study have been described previously (5). [γ-^{32}P]ATP (crude; >5,000 Ci/mmol) was purchased from ICN. ^{125}I-labeled protein A (2 to 10 μCi/μg) was a product of New England Nuclear Corp. Restriction enzymes and polynucleotide kinase were from New England Biolabs and used as recommended by the manufacturer.

Bacterial strains and plasmids. The bacterial strains and plasmids used or constructed in this study are listed in Table 1. Strains constructed in this study are described in the text below.

Strain construction. (i) Construction of *araBAD* downpromoter derivatives of strain LA920. Plasmid pCGM1016 or pCGM1036 was introduced into the *araC-lacZ* fusion strain LA920 by transformation. Plasmids pCGM1016 and pCGM1036 contain the *araBAD* downpromoter alleles *araBp1016* and *araBp1036*, respectively (24). In addition, the plasmids contain part of *araA*, all of *araB*, and all of *araC*, therefore sharing homology with the LA920 chromosome. The transformants are Ara$^+$ since AraC protein produced by the *araC*$^+$ plasmid activates *araBAD* expression on the

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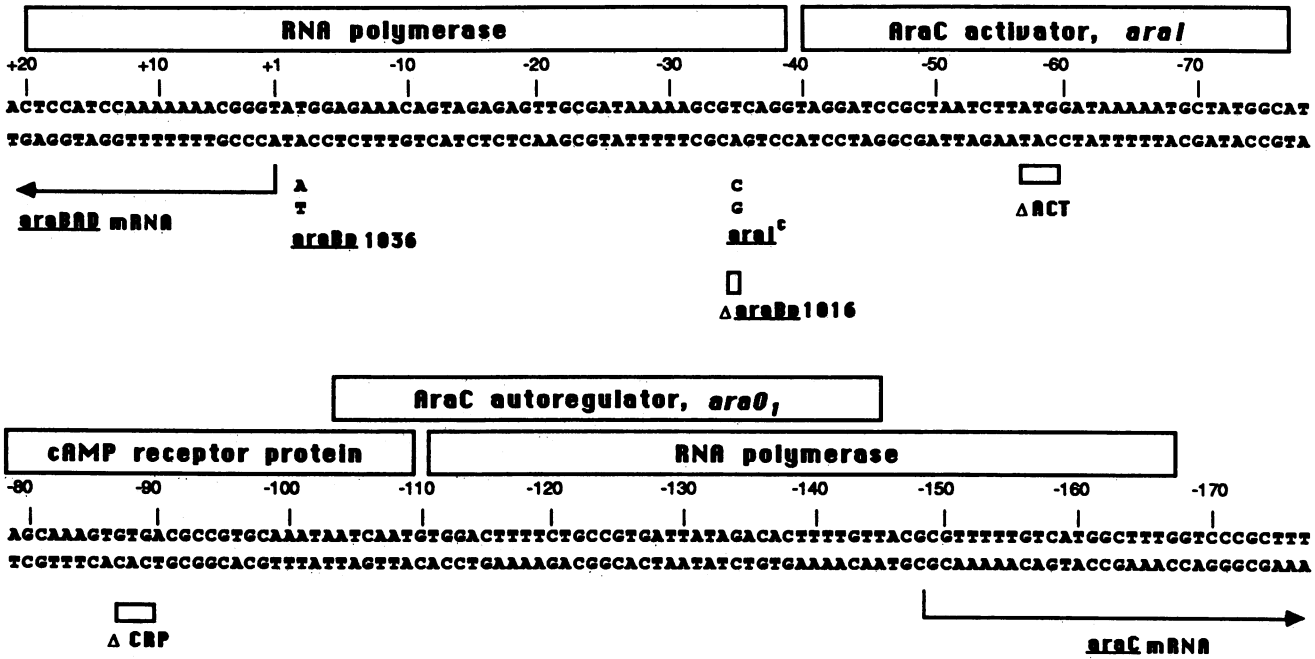

FIG. 1. Location of the *ara* regulatory region mutations within the *araB* and *araC* promoters. The DNA sequence (10, 28) and the DNA-protein contacts (19, 28) are shown with the start site of the *araBAD* mRNA at +1. The *araC* mRNA start site is indicated by the arrow at −148 (33). The *araI*(Con) mutation, previously designated *araI*^{c}, has been described (4). The sequence of the *araBAD* promoter mutants 1016 and 1036 was determined by Miyada et al. (24). The ΔACT deletion in the AraC activator-binding site and the ΔCRP deletion in the CRP-binding site have been described (25, 26). The solid-line bar adjacent to a number or letters indicates a deletion. The $araO_2$ site centered at −280 bp is not shown but is located within the leader region of the *araC* gene (6).

chromosome. An Ara$^+$ transformant was grown in TYE-tetracycline broth for 24 h and plated on MacConkey-L-arabinose-tetracycline (MAT) medium to obtain isolated colonies. A white Ara$^-$ homogenote in which the *araBp* mutation recombined onto the chromosome as shown in Fig. 2A was detected and purified. Repeated growth of the cells in the absence of tetracycline resulted in Tc^s colonies in which the plasmid was lost but the Ara$^-$ phenotype was maintained. The Ara$^-$ Tc^s strains containing the *ΔaraBp1016* and *araBp1036* alleles were designated LA923 and LA924, respectively. AraC protein produced by an *araC*$^+$ plasmid in *trans* to the downpromoter mutations was unable to activate *araBAD* expression.

TABLE 1. Bacterial strains and plasmids

Strain[a] or plasmid	Genotype[b]	Reference
Strains		
LA920	*araC*::Mu d(Ap^r *lac*) Δ*leu* Δ(*lac-pro*)	26
LA921	LA920 Δ(*araBi*)*2000*	C. G. Miyada, this laboratory
LA922	LA920 Δ(*araCp*)*2001*	26
LA923	LA920 Δ(*araBp*)*1016*	This work
LA924	LA920 (*araBp*)*1036*	This work
LA925	LA920 [*araI*(Con)]*110*	This work
LA840	LA8390 (*araC-lacZ*) (Hyb)	5
Plasmids		
pBR322	*bla*$^+$ *tet*$^+$	31
pLGC1000	pBR322 Ω(*Pst*I-*Pvu*I *bla'*::LT2) *araB*' B/r *araC*$^+$	5
pLGC138	pLGC1000 (*araC183*)	5
pLGC210	pLGC1000 (*araC210*)	5
pLGC227	pLGC1000 (*araC227*)	5
pLGC231	pLGC1000 (*araC231*)	5
pLGC235	pLGC1000 (*araC235*)	5
pLGC264	pLGC1000 (*araC264*)	5
pLGC268	pLGC1000 (*araC268*)	5

[a] All strains are derivatives of *E. coli* B/r strain UP1000.
[b] Genetic nomenclature is as described by Bachmann (1) and Novick et al. (27).

(ii) Construction of an *araI*(Con) derivative of strain LA920. Strain LA921, a derivative of strain LA920, contains a 3-bp deletion in the AraC activator site (ΔACT), resulting in an *araBAD* promoter which is uninducible by L-arabinose (25) (Fig. 1). Strain LA921 was transformed with the plasmid pAH5 (15), and transformants were selected on MAT medium. Plasmid pAH5 contains part of *araA*, all of *araB*, the *araI*(Con) allele, and the (*araC0*)*766* deletion. The *araI*(Con) mutation allows a low level of expression of the *araBAD* promoter in the absence of AraC activation (7, 9). An Ara$^-$ transformant was grown in TYE-tetracycline broth for 24 h and plated on MAT medium to obtain isolated colonies. A slightly pink Ara$^+$ homogenote in which the *araI*(Con) mutation had recombined onto the chromosome (Fig. 2B) was isolated and subsequently cured of the plasmid as described for the construction of LA923 and LA924. The pink Ara$^+$ Tc^s strain was designated LA925. AraC protein produced by an *araC*$^+$ plasmid in *trans* to the *araI*(Con) mutation in LA925 activates *araBAD* expression to a wild-type induced level.

DNA hybridization. Chromosomal DNA from strains LA920, LA921, LA923, and LA925 was isolated by the procedure of Hull et al. (16). The chromosomal DNA was digested with *Hha*I, loaded onto a 1.8% agarose gel, and subjected to electrophoresis. Subsequently, DNA was trans-

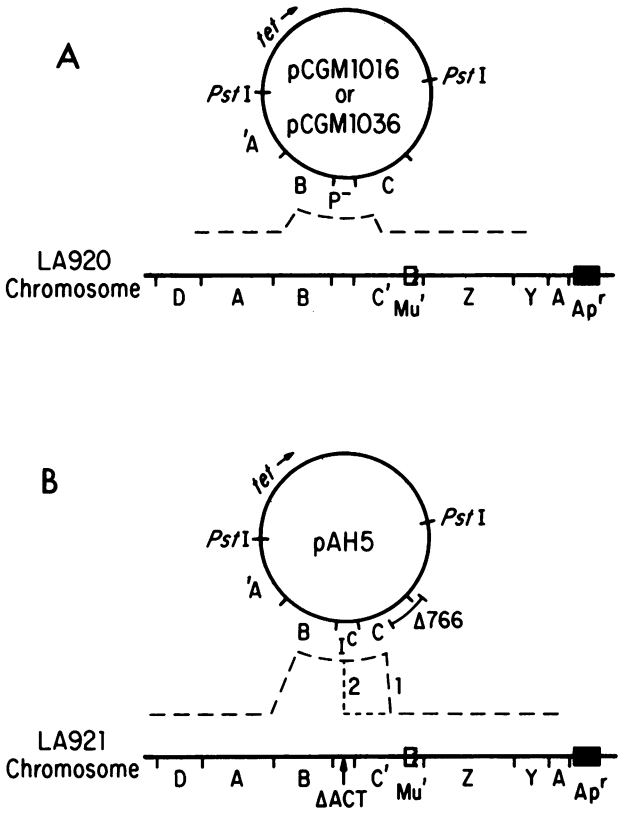

FIG. 2. Recombination events that place *araBp* or *araI*(Con) mutations onto the *araC-lacZ* fusion chromosome. (A) Recombination event between plasmid pCGM1016 or plasmid pCGM1036 and the LA920 chromosome. (B) Possible recombination events between plasmid pAH5 and the LA921 chromosome. For the purpose of this diagram, the symbol P^- refers to the *ΔaraBp1016* or the *araBp1036* mutation in the *araBAD* promoter. The symbol I^c refers to the *araI*(Con) mutation in the *araBAD* promoter. The symbol ΔACT refers to the 3-bp deletion in the AraC activator-binding site.

ferred to nitrocellulose as described by Maniatis et al. (21). In one hybridization, a 2.2-kilobase (kb) *Bst*EII fragment isolated from pHM7 (15) containing the entire *ara* control region was used as a probe. The *Bst*EII fragment was nick translated (29), and hybridization was performed as described previously (21). Another nitrocellulose filter of the same chromosomal digests was hybridized with an 18-base oligodeoxyribonucleotide containing the ΔACT mutation (25). The 18-mer was 5'-end labeled with [γ-^{32}P]ATP as described by Miyada et al. (25). The hybridization conditions which were used allowed the labeled probe to bind to both its exact complement and the wild-type sequence. A subsequent wash for 1.5 min at 49°C removed the probe from any inexact complement, allowing identification of chromosomal DNA containing the ΔACT mutation.

Enzyme assays. (i) β-Galactosidase assay. Cultures (5 ml) of strain LA920 containing the desired *araC* plasmid were grown in supplemented M9 salts medium containing tetracycline (15 μg/ml) at 37°C for 16 h. These cells were used to inoculate 50 ml of the same medium, and growth was monitored with a Klett-Summerson colorimeter (filter 42). Cultures were inoculated to 10 U (3.3×10^7 cells per ml), and at 70 U (2.3×10^8 cells per ml) L-arabinose was added to a final concentration of 0.4% (wt/vol). Growth was continued for one doubling, at which time 50 μg of chloramphenicol per ml was added and the samples were placed on ice. Cell extracts were prepared, and β-galactosidase activity was measured as described by Miller (22).

TABLE 2. Effect of L-arabinose on autoregulation in the *araC* mutants[a]

Plasmid	Uninduced[b]		Induced[c]	
	β-Galactosidase sp act	% of sp act with pBR322	β-Galactosidase sp act	% of sp act with pBR322
pLGC1000	152 ± 5	6.6	149 ± 3.1	6.5
pLGC264	791 ± 36	34	786 ± 43	34
pLGC268	863 ± 85	37	865 ± 40	38
pLGC235	1,663 ± 103	72	2,326 ± 134	101
pLGC227	2,018 ± 164	87	3,270 ± 221	143
pLGC210	2,079 ± 127	90	2,273 ± 40	99
pLGC183	2,091 ± 160	90	2,273 ± 113	99
pLGC231	2,260 ± 68	98	2,529 ± 69	110
PBR322	2,314 ± 130	100	2,292 ± 26	100

[a] Strain LA920 was transformed with the *araC*-containing plasmids, and enzyme levels were measured in crude extracts. Results of four experiments expressed as mean ± average error. β-Galactosidase specific activity is expressed as nanomoles of *O*-nitrophenol formed per minute per milligram of protein at 28°C.

[b] Uninduced values are taken from reference 5, Table 3.

[c] β-Galactosidase activity was measured in cells induced with 0.4% L-arabinose.

(ii) L-Arabinose isomerase assay. Cultures (100 ml) of strain LA925 containing the desired *araC* plasmid were grown in supplemented M9 salts medium containing tetracycline (15 μg/ml) at 37°C for 14 h. The stationary-phase cells were harvested, cell extracts were prepared, and L-arabinose isomerase was assayed as described by Englesberg et al. (7).

Immunoblot analysis. Growth conditions and preparation of cell extracts were those used for β-galactosidase assays with the following modification: cell extract buffer (buffer B) consisted of 0.15 M potassium acetate, 0.01 M Tris-acetate, pH 7.4, 1 mM EDTA, 0.4% (wt/vol) L-arabinose, and 50 μg of phenylmethylsulfonyl fluoride per ml. Antibody preparation and immunoblot analysis were carried out as described previously (5, 32).

RESULTS

Effect of L-arabinose on autoregulation in *araC* mutants. The effect of each *araC* mutation on the autoregulatory function of AraC protein was determined from cultures grown in the presence of L-arabinose. The ability of the plasmid-encoded AraC protein to repress the expression of the *araC-lacZ* fusion strain LA920 was measured. The level of β-galactosidase was assayed, and the results of these assays are shown in Table 2. The β-galactosidase levels determined from uninduced cultures were reproduced from reference 5 and are shown for comparison with the induced levels. In the absence of AraC protein, the level of expression of the *araC-lacZ* fusion was not affected by L-arabinose. In the presence of the wild-type AraC protein, the level of *araC-lacZ* expression was not affected by L-arabinose, as previously reported (2). Five of the seven *araC* mutations resulted in complete loss of autoregulation in the presence of L-arabinose. The level of β-galactosidase in each of the five mutants was higher than in uninduced cultures. In the mutant 227H, the increased β-galactosidase level could not have resulted solely from a loss in autoregulation. The β-galactosidase level of the *araC-lacZ* fusion strain containing plasmid pLGC227 was increased to 143% of the fully derepressed level (pBR322). This demonstrates an activation

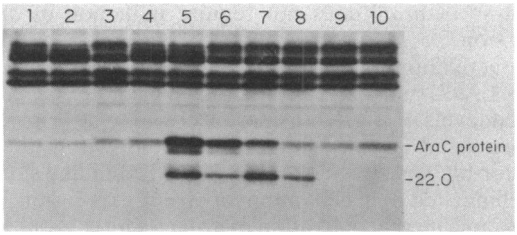

FIG. 3. Autoradiograph of immunoblot analysis of wild-type and mutant 227H AraC protein. Cell extracts were prepared from induced or uninduced cultures of strain LA920 or strain LA840 containing the desired *araC* plasmid, and 90 μg of total protein was loaded in each lane. Lanes 1 to 6, LA920 cell extracts. Lane 1, pBR322, induced; lane 2, pBR322, uninduced; lane 3, pLGC1000, induced; lane 4, pLGC1000, uninduced; lane 5, pLGC227, induced; lane 6, pLGC227, uninduced. Lanes 7 to 10, LA840 cell extracts. Lane 7, pLGC227, induced; lane 8, pLGC227, uninduced; lane 9, pLGC1000, induced; lane 10, pLGC1000, uninduced. The positions of the AraC and 22-kDa proteins are shown.

of *araC* expression in the presence of L-arabinose that is not observed in the wild-type cell.

The *araC-lacZ* fusion in strain LA920 is an operon fusion constructed by using the Mu d(Ap^r *lac*) phage (3). Though unlikely, the increase in β-galactosidase expression in the presence of L-arabinose might reflect transcription initiation from within the extra Mu d DNA between the *araC* promoter and the *lacZ* gene and not a stimulation of *araC* transcription. To examine this possibility, an *araC-lacZ* protein fusion strain was used. β-Galactosidase was assayed in induced and uninduced cultures of strain LA840 (5) containing plasmid pLGC227 (data not shown). In the absence of L-arabinose, the level of autoregulation retained in the mutant 227H was 19% of that in the *araC*$^+$ cell (pLGC1000). In the presence of L-arabinose, the β-galactosidase level increased to 131% of the level in LA840 containing plasmid pBR322. As in the operon fusion strain, the level of repression by the wild-type AraC protein remained constant in the presence or absence of L-arabinose. Thus, a novel activation of the *araC-lacZ* expression was seen in both the operon and the protein fusion strains containing plasmid pLGC227.

Immunoblot analysis of intracellular AraC protein. In the presence of L-arabinose, the *araC* mutation on plasmid pLGC227 resulted in activation of transcription of the chromosomal *araC-lacZ* fusion. The altered AraC protein, if able to activate expression of its own synthesis on the plasmid, would result in an increase in the intracellular AraC protein concentration over the uninduced level in the same mutant. The intracellular level of AraC protein was measured by immunoblot analysis as described in Materials and Methods. The autoradiogram of the immunoblot is shown in Fig. 3. The levels of intact AraC protein and the 22-kilodalton (kDa) breakdown product from induced cultures of both LA920 and LA840 containing plasmid pLGC227 were significantly greater than the amount in uninduced cultures (lanes 5 to 8). The wild-type AraC protein concentration was not altered by L-arabinose. The total amount of both the wild-type and the mutant 227H protein in the LA840 cultures was less than the amount in the LA920 cultures. The reason for this difference in AraC protein levels is unclear. The presence of L-arabinose resulted in an increased concentration of AraC protein encoded by plasmid pLGC227 and an activation of the *araC-lacZ* fusion by this altered AraC protein.

Characterization of *araC-lacZ* expression *cis* to *ara* regulatory site mutations in the *araC*$^+$ and *araC227* cell. In the presence of L-arabinose, the altered AraC protein encoded on pLGC227 is able to activate *araBAD* expression to 50% of the wild-type level (5) and results in a novel activation of its own gene expression. At what site does this novel activation occur? Mutations within the binding sites for the *ara* regulatory proteins AraC and CRP and for RNA polymerase that affect promoter activity have been isolated and characterized on a molecular level (24–26). The effect of these *ara* regulatory site mutations on this novel activation of *araC* gene expression was measured. To use this approach, strains derived from LA920 were constructed in which the regulatory site mutations were placed *cis* to the *araC-lacZ* fusion. Two previously described strains, LA921 and LA922, contain a 3-bp deletion in the AraC activator binding site (ΔACT) and in the CRP-cAMP-binding site (ΔCRP), respectively (25, 26) (Fig. 2). The ΔCRP deletion at −88 affected *araC* expression, decreasing expression to 33% of wild-type levels and creating an *araC* promoter insensitive to catabolite repression (26). The ΔACT mutation at −58 removed AraC activator binding at *araI*, eliminating activation of transcription of the *araBAD* promoter (25). The binding of AraC at *araI*, expression of *araBAD*, or both events might be involved in this novel activation of *araC*.

In this study, mutations in the RNA polymerase-binding site which affect the *araBAD* promoter but not AraC activator binding at *araI* were placed *cis* to the *araC-lacZ* operon fusion. Mutations *araBp1016* and *araBp1036*, which decrease *araBAD* expression (24), and the *araI*(Con) mutation, which allows low level expression of *araBAD* in the absence of AraC activator (7, 9), were transferred from plasmids to the fusion chromosome by in vivo recombination. The construction of these strains is described in Materials and Methods, and the recombination events placing the mutations onto the chromosome are shown in Fig. 2. The 1-bp deletion in *araBp1016* and the transition in the *araI*(Con) mutation located at −35 created a new *Hha*I restriction site (4) (Fig. 1). Southern blot analysis of *Hha*I-digested chromosomal DNA of strain LA923(*araBp1016*) and LA925[*araI* (Con)] confirmed the transfer of these mutations to the chromosome (Fig. 4a). A 2.2-kb fragment containing the entire *ara* regulatory region was used as a probe. A *Hha*I fragment spanning the −35 region from LA920 and LA921 DNA was absent in LA923 and LA925 DNA and a new *Hha*I site was generated.

In the construction of LA925, it was necessary to confirm that the recombination event placing the *araI*(Con) mutation onto the LA921 chromosome replaced the ΔACT mutation at −58 with the wild-type DNA from the plasmid (Fig. 2B, recombination event 2). The Southern blot analysis shown in Fig. 4b demonstrates that the ΔACT probe hybridized only to LA921 chromosomal DNA and not to the LA925 chromosomal DNA.

L-Arabinose isomerase activity of strain LA925 containing *araC* plasmids. The LA925 strain was initially characterized with respect to the effect of the *araI*(Con) allele on *araBAD* expression. The level of L-arabinose isomerase was measured in uninduced cultures of strain LA925 carrying plasmid pLGC1000, pLGC227, or pBR322; the specific activity (± standard error) was 0.7 ± 0.2, 4.1 ± 0.5, and 6.9 ± 0.7 μmol of L-ribulose formed per min per mg of protein at 30°C, respectively. Strain LA925 shows a low level of L-arabinose isomerase activity in the absence of AraC activation (pBR322), which confirmed, in vivo, the presence of the *araI*(Con) allele. The wild-type AraC protein produced in *trans* reduced the level of L-arabinose isomerase 10-fold. This repression of *araBAD* indicates that the $araO_2$ site,

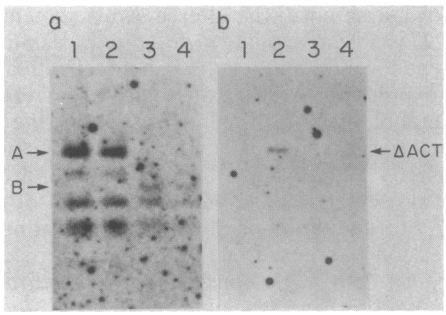

FIG. 4. Autoradiogram of Southern blot analysis of *Hha*I-digested chromosomal DNA. A 500-ng amount of *Hha*I-digested chromosomal DNA was loaded in each lane. Lane 1, LA920; lane 2, LA921; lane 3, LA923; lane 4, LA925. (a) Confirmation of a new *Hha*I restriction site in the −35 region mutants. A 2.2-kb *Bst*EII fragment spanning the *ara* regulatory region was used as a probe. Arrow A indicates the *Hha*I fragment containing the wild-type −35 region. Arrow B indicates the smaller *Hha*I fragment created by the new *Hha*I restriction site at −35. (b) Confirmation of recombination event 2 (Fig. 2B) in construction of LA925. An 18-mer synthetic oligonucleotide containing the ΔACT mutation was used as a probe (22). The arrow indicates the *Hha*I fragment from LA921 containing the ΔACT deletion.

located at −280, was present in the *araC-lacZ* fusion. The ability of the altered AraC protein from pLGC227 to repress *araBAD* expression was measured. The *araC* mutation impaired the repressor function of AraC protein, retaining 45% of the wild-type activity. This value was determined by linear regression analysis; the derepressed L-arabinose isomerase level (pBR322) was set to 0% wild-type activity and the level in the *araC*$^+$ cell (pLGC1000) to 100% wild-type activity. Thus, *araC* mutation 227 alters both repression of *araBAD* and repression of its own gene transcription.

Regulation of *araC-lacZ* expression *cis* to the *ara* regulatory mutations. The level of *araC* expression *cis* to *ara* regulatory site mutations was measured in the presence of wild-type or mutant 227H AraC protein. The *araC-lacZ* fusion strain LA920 and its derivatives were transformed with the *araC* plasmids, and β-galactosidase was assayed. The results of these assays are shown in Table 3. The mutations in the RNA polymerase-binding site of the *araBAD* promoter did not affect the activation of *araC* expression by AraC227 proteins (LA923, LA924, and LA925). As in the parent strain, LA920, the level of β-galactosidase in induced cultures was higher than in uninduced cultures, ranging from 135 to 155% of the fully derepressed level. The downpromoter mutations *araBp1016* and *araBp1036* eliminated *ara BAD* induction without removing AraC activator binding at *araI*. Therefore, our results suggest that the mechanism of activation of *araC* does not require induction of *araBAD* expression.

The activation of *araC-lacZ* expression was not seen in strains LA921 and LA922 containing plasmid pLGC227. The induced levels of β-galactosidase were 40 to 50% lower than the uninduced levels. The 3-bp deletions within the AraC activator-binding site and the CRP-cAMP-binding site therefore eliminated the activation of *araC* expression. These results indicate that activation of *araC* requires AraC protein bound at *araI* and CRP-cAMP bound at the CRP site.

The results presented in Table 3 also show the effect of the *ara* regulatory site mutations on *araC-lacZ* expression in the *araC*$^+$ cell. In uninduced cultures, all of the strains containing the wild-type AraC protein except LA921 had approximately the same low levels of β-galactosidase. In strain LA921, the β-galactosidase level was at least fivefold higher than in the other strains. The ΔACT mutation *cis* to the *araC-lacZ* fusion resulted in a decrease in the level of autoregulation of the wild-type cell. This result suggests that the *araI* site is in part necessary for autoregulation of *araC* expression. As seen in strain LA921 containing plasmid pLGC227, the level of *araC* expression decreased in the presence of L-arabinose to 40% of the uninduced level.

DISCUSSION

An analysis of the effect of L-arabinose on *araC* mutants altered in the ability to autoregulate and to activate *araBAD* expression identified a novel activation of *araC* gene expression. This activation of *araC* expression was not evident in the wild-type cell. The ability of plasmid-encoded AraC protein to repress the expression of an *araC-lacZ* fusion was determined by assaying β-galactosidase in cultures grown in the presence or absence of L-arabinose. The level of β-galactosidase in five of the seven mutants was higher in induced cultures than in uninduced cultures. The level of β-galactosidase in one of the *araC* mutants, 227H, was above the fully derepressed level (Table 2). This demonstrates activation of the *araC* promoter. The altered AraC protein encoded on plasmid pLGC227 stimulated transcription to 143% of the fully derepressed level. The presence of L-arabinose resulted in a higher level of AraC protein in the cell than in uninduced cultures (Fig. 3). Thus, there was activation of both expression of the *araC-lacZ* fusion and the level of AraC protein encoded by plasmid pLGC227.

The mechanism of *araC* activation was investigated by measuring the effect of mutations within the regulatory binding sites for *araBAD* and *araC* transcription on *araC* expression. Activation of *araC-lacZ* fusion expression by the mutant 227 AraC activator required the AraC activator-binding site *araI* and the CRP-cAMP complex-binding site (Table 3, Fig. 1). Mutations within the RNA polymerase-

TABLE 3. Effect of *ara* regulatory site mutations on *araC-lacZ* fusion expression[a]

Plasmid	β-Galactosidase sp act (nmol of *O*-nitrophenol/min per mg of protein at 28°C)											
	LA920 (wt)		LA921 (ΔACT)		LA922 (ΔCRP)		LA923 (*araBP1016*)		LA924 (*araBp1036*)		LA925 [*araI*(Con)]	
	U	I	U	I	U	I	U	I	U	I	U	I
pBR322	2,314	2,292	2,837	2,529	549	579	2,359	2,215	2,315	2,240	2,498	2,467
pLGC227	2,018	3,270	2,653	1,427	496	329	1,810	3,308	1,896	3,033	1,973	3,822
pLGC1000	152	149	809	476	107	92	129	176	132	158	123	140

[a] The *araC-lacZ* fusion strains listed were transformed with the *araC*-containing plasmids, and enzyme levels were measured in crude extracts. Results are expressed as the mean of at least two experiments. The average error was never greater than 9%. β-Galactosidase activity was measured in cultures grown in the absence of L-arabinose (U) or in the presence of 0.4% L-arabinose (I).

binding site of the *araBAD* promoter which abolished activation of *araBAD* transcription had no effect on activation of *araC* expression. Therefore, activation of transcription at the *araBAD* promoter is not necessary for activation of transcription at the *araC* promoter.

The activator-binding site lies adjacent to the CRP-cAMP-binding site (Fig. 1). A model consistent with the requirement for a functional *araI* site and a CRP-cAMP site is one in which AraC protein bound at *araI* mediates activation through CRP-cAMP bound at the *araC* promoter. Because of the proximity of the two binding sites, the AraC activator might make direct contact with the CRP-cAMP complex. This model requires that AraC activator be able to bind at the *araI* site. As previously reported (5), the AraC protein encoded on plasmid pLGC227 retained 50% of the $araC^+$ ability to activate *araBAD*, suggesting that the AraC protein retained partial ability to bind at *araI*.

The activation of *araC* expression in the presence of L-arabinose was not observed in the $araC^+$ cell (Table 2). Thus, the activator function observed in the *araC* mutants may not exist in the wild-type AraC protein. The activation mediated by the mutant 227 AraC protein may reflect an altered protein structure, unable to bind at $araO_1$ but able to interact at *araI* in a novel manner. Alternatively, the activator function may be present in the wild-type protein but masked by the level of autoregulation.

The experiments presented here suggest that maximal autoregulation of *araC* expression involves the *araI* site. The ΔACT mutation (in *araI*) *cis* to the *araC-lacZ* fusion resulted in a decrease in the level of autoregulation in the $araC^+$ cell (Table 3). In the absence of L-arabinose, the level of β-galactosidase was fivefold higher than in the $araI^+$ *araC-lacZ* fusion strain. While it is possible that the *araI*-dependent autoregulation of *araC* expression operates only at high concentrations of AraC protein, it is most likely that the high-copy-number plasmid amplifies rather than creates the autoregulation.

A mutation that affects autoregulation of the *araC* gene in *Salmonella typhimurium* has been isolated which is also located outside of the $araO_1$ site. The non-self-regulatory phenotype resulted from a transversion at −271 (J. H. Lee, Ph.D. thesis, University of California, Los Angeles, 1982). This mutation is located in a region which corresponds to the AraC protein-binding site $araO_2$ of *E. coli*, which is centered at −280 (6). What role might a functional *araI* site or $araO_2$ have in autoregulation of *araC*? One possible model is that presented for repression of *araBAD* expression (6, 11). A functional *araI* site and an $araO_2$ site are required to mediate *araBAD* repression. It has been proposed that in the repressed state, AraC protein bound at these distant sites might interact directly via a loop in the intervening DNA, a state that prevents transcription of the *araBAD* promoter. This interaction might simultaneously inhibit *araC* transcription proceeding through $araO_2$.

Since this work was completed, a model has been proposed consistent with the data reported in this paper (13, 18).

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