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Three binding sites for AraC protein are required for autoregulation of *araC* in *Escherichia coli*

(DNA loops/operators/repression/gene regulation/cooperative interactions)

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ABSTRACT **Three binding sites for AraC protein were shown to be required for the autoregulation of *araC*: *araI*$_1$, *araO*$_1$, and *araO*$_2$. Selective inactivation of AraC-binding sites on the DNA demonstrated that *araO*$_1$ and *araO*$_2$ are required *in vivo* to produce repression of *araC* in the presence of arabinose, whereas *araI*$_1$ and *araO*$_2$ are required in its absence. We found that the low-affinity site *araO*$_2$ is essential for *araC* autoregulation; *araO*$_1$ and *araI*$_1$ provide high-affinity AraC-binding sites, which allow cooperative binding at *araO*$_2$. Profound effects on the *araBAD* promoter and the *araC* promoter are produced by ligand-induced changes in AraC occupancy of functional sites on the DNA. We suggest that AraC exerts its multiplicity of controls through two alternative states of cooperative interactions with DNA and we illustrate this with a model. This model presents our interpretations of activation and repression of the *araBAD* operon and the autoregulation of the *araC* gene.**

Proteins that repress gene activity were believed for many years to act by binding to a single site (operator) within a promoter, blocking transcription initiation (1). More recently, additional operator sites at considerable distances from the promoter regions have been found to play a role in negative regulation, including the negative aspect of *araBAD* operon control [*gal* (2, 3), *lac* (4–6), *ara* (7–9), *deo* (10, 11), *nrd* (12)]. These multiple-repressor-binding sites are necessary for full repression of these operons. It has been proposed that the secondary operators serve to enhance repressor activity by stabilizing protein–DNA complexes through cooperative binding (13).

The *araC* gene, which encodes the transcriptional activator of the arabinose genes in *Escherichia coli*, is homeostatically autoregulated under inducing and noninducing conditions (14). *In vitro* studies initially suggested that *araC* is transcriptionally regulated by the competitive binding of AraC protein to a site (*araO*$_1$) congruent with the RNA polymerase-binding site of the *araC* promoter (15, 16) (Fig. 1). Direct selection of cis-acting, autoregulation-minus mutants in an *araC–lacZ* fusion strain gave primarily "promoter-up" mutations with increased affinities for polymerase rather than decreased binding of AraC (19). Without an *araO*$_1^-$ mutant that shows selective loss of AraC binding while retaining the ability to bind polymerase, the *in vivo* role of *araO*$_1$ in *araC* autoregulation cannot be established.

In addition to *araO*$_1$, there are three other AraC protein-binding sites (*araI*$_1$, *araI*$_2$, and *araO*$_2$) located near the *araC* gene promoter (Fig. 1). *araO*$_2$, which lies within the leader region of the *araC* gene, is essential for repression of the *araBAD* operon (7). Schleif and co-workers (7–9) have postulated that the cooperative binding of AraC molecules to

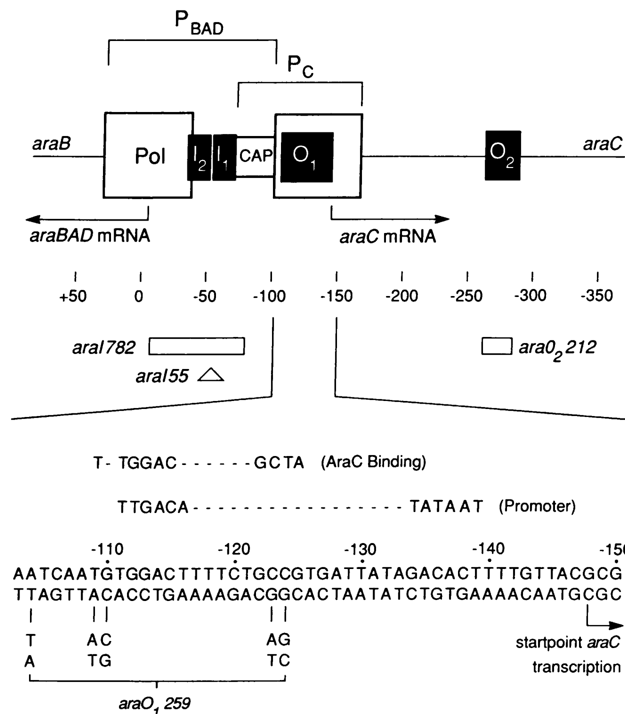

FIG. 1. Controlling region of the *araBAD* and *araC* operons, drawn to scale. The *araBAD* (P_{BAD}) and *araC* (P_c) promoters are shown along with the locations of the protein-binding sites. RNA polymerase (Pol)-binding sites, large open boxes; catabolite gene activation protein (CAP)-binding site, small open box; AraC protein-binding sites (I_2 for *araI*$_2$, I_1 for *araI*$_1$, O_1 for *araO*$_1$, and O_2 for *araO*$_2$), black boxes. Numbering of base pairs is relative to the *araBAD* transcription start site at +1. The positions of the *araO*$_2$ and *ara*(I_1I_2) deletions and the single base pair deleted in the *araI*$_2$ mutant are also indicated. The nucleotide sequence of the wild-type *araC* promoter from −103 to −150 is shown, with the four base substitutions in the *araO*$_1^-$ mutant indicated below. This mutant has a fifth substitution, a spontaneous $\frac{A}{T}$ to $\frac{T}{A}$ transversion at −104, which lies outside the consensus sequences. The locations of the AraC-binding consensus sequence (AraC Binding) (17) and the consensus promoter sequence (Promoter) (18) are shown in relationship to the mutations in the *araO*$_1^-$ strain.

araI and *araO*$_2$ results in *araBAD* repression. We have recently shown (17) that the *araI* site is separable into two adjacent regions, each containing a 17-base-pair (bp) AraC-binding consensus. These regions, designated *araI*$_1$ and *araI*$_2$, differ greatly in their affinities for AraC in the absence of inducer. We proposed that the induction of *araBAD* by arabinose is caused by a switching of the cooperative AraC binding from *araI*$_1$/*araO*$_2$ to *araI*$_1$/*araI*$_2$. This ligand-

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Abbreviations: X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside; P_C, *araC* promoter; P_{BAD}, *araBAD* promoter.
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induced transition allows the *araBAD* promoter to change from the repressed to the induced state, with a 1200-fold increase in its transcriptional activity.

The present study has been undertaken to determine (*i*) if $araO_1$ is involved in the *in vivo* autoregulation of *araC* and (*ii*) if the other AraC-binding sites ($araI_1$, $araI_2$, and $araO_2$) play a significant role in *araC* autoregulation. We selectively inactivated these sites in a strain carrying an *araC–lacZ* protein fusion and measured the activities of the fusion gene under inducing and noninducing conditions. A wild-type *araC* gene was introduced by lysogenization with a λ*para* transducing phage, so that only single copies of the *ara* genes were present. We were surprised to find that three AraC-binding sites were involved in *araC* autoregulation. In the presence of arabinose, $araO_2$ and $araO_1$ were required to repress *araC*, whereas, in the absence of inducer, $araI_1$ and $araO_2$ [the same sites that were shown to produce repression of *araBAD* (9)] were required. To our knowledge, no case has previously been reported where the cooperative interaction between protein molecules bound to widely separated DNA sites is *absolutely* required for repression. We present a model describing the ligand-dependent states of occupancy of all four AraC-binding sites and their respective roles in the regulation of *araC* and *araBAD* expression.

MATERIALS AND METHODS

Media and General Methods. Media used included Luria–Bertani medium (LB; ref. 10), mineral glycerol medium with or without 0.4% L-arabinose [per 100 ml: 0.7 g of K_2HPO_4, 0.3 g of KH_2PO_4, 0.1 g of $(NH_4)_2SO_4$, 10 mg of $MgSO_4 \cdot 7H_2O$, 0.2 ml of glycerol, 4 mg of L-leucine, 0.4 mg of thiamine, and $MnCl_2$ to a final concentration of 20 μM], MacConkey arabinose medium with or without 100 μg of ampicillin per ml (Difco MacConkey agar base with 1% L-arabinose), tryptone medium (per 100 ml: 1 g of Bactotryptone, 0.5 g of NaCl, and 0.4 mg of thiamine), and tryptone bottom agar (with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) (tryptone medium with 1.5% Bactoagar and 0.0028% X-Gal). DNA manipulations used in the constructions of bacterial strains and plasmids were as described (20).

Strain Constructions. The scheme for the construction of strains used in our studies is outlined in Fig. 2. AraC-binding sites were inactivated by three different mutagenic procedures. Oligonucleotide-directed *in vitro* mutagenesis (21) was used to delete 20 bp (from −264 to −283, inclusive) of the $araO_2$ site, including the entire AraC-binding consensus sequence (17). This method was also used to create a mutant with four base substitutions in the AraC-binding site $araO_1$ (Fig. 1). Digestion of *ara* DNA cut by *Bam*HI (at −44) by the exonuclease BAL-31 resulted in the deletion of 76 bp (from −7 to −82, inclusive) containing $araI_1$, $araI_2$, and half of the *araBAD* polymerase-binding site. We also used a previously identified, spontaneous chromosomal mutation in $araI_2$, which deletes the base pair at position −55 and eliminates AraC binding to $araI_2$ *in vitro* (17).

The plasmid pNL20 was used as an integration and rescue plasmid. It contains *ara* DNA from 1816 to −44, including the entire *araB*-coding region and part of the *araBAD* promoter. Also contained on the plasmid are most of the *lacZ* gene (374–3455), the distal end of the *araC* gene plus about 800 bp of downstream sequence (from −877 to −2006), and pBR322 DNA from 2066 clockwise to 25. Fig. 2 shows this plasmid after a *Sau*3A restriction fragment derived from *ara* (from −44 to −330) had been cloned into the *Bam*HI site at the *ara/lac* junction [formed by joining the *ara Bam*HI site at −44 to the *Bam*HI site at 374 in the *lacZ* gene on the plasmid pMC1871 (Pharmacia P-L Biochemicals)]. This method of cloning was used to construct plasmids carrying each AraC-binding site mutation and resulted in the fusion of the sixth codon of *araC* to the eighth codon of *lacZ*. Those plasmids that had a functional *araBAD* promoter (the ones carrying the $araO_1^-$ and $araO_2^-$ mutations) were put into NL20-185a, a haploid strain with a 3-bp deletion (from −53 to −55) that rendered it $araBAD^-$. Integration events were detected as red papillae on the white colonies on MacConkey arabinose/ampicillin agar. The plasmids that carried a mutant *araBAD* [due to the $araI_2^-$ and $ara(I_1I_2)^-$ mutations] were put into the ara^+ strain NL20-000. Integration events resulted in white papillae on the red colonies on MacConkey arabinose/ampicillin plates. All strains containing integrated plasmids were purified, inoculated into LB, incubated overnight at 44°C, diluted, and plated on MacConkey arabinose plates. Cells cured of the plasmid, but retaining the *araC–lacZ* fusions, were white on MacConkey arabinose, sensitive to ampicillin, and blue on tryptone plates containing X-Gal.

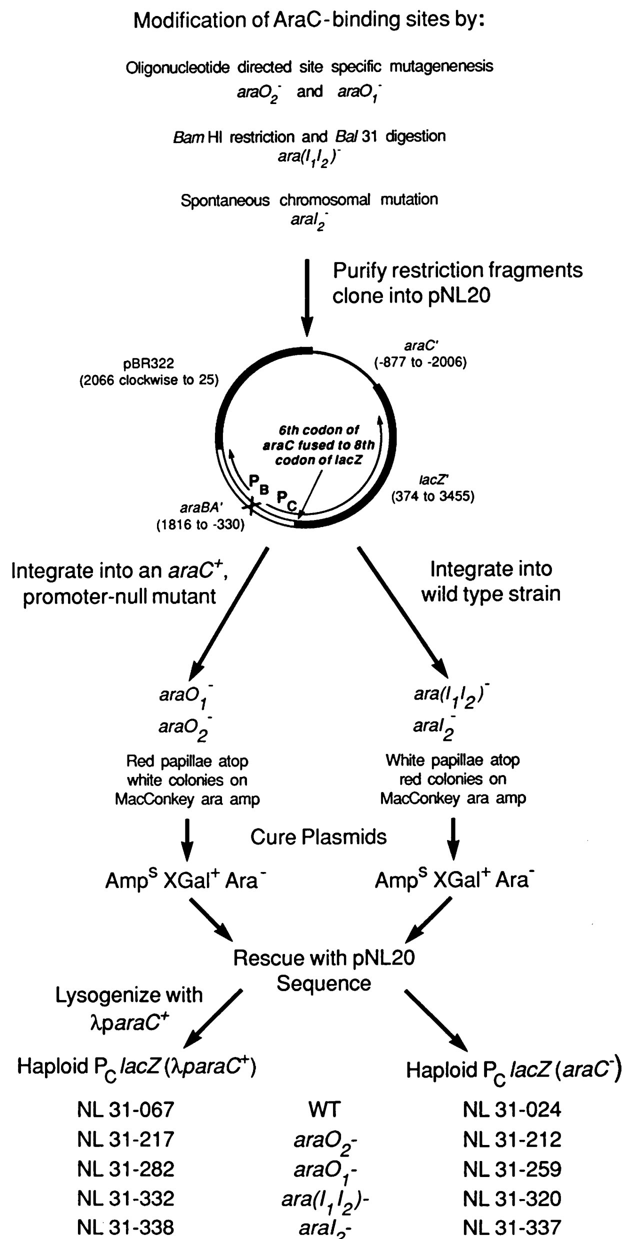

FIG. 2. Scheme used to construct strains for testing autoregulation.

To confirm the genotypes of these strains, the *araC–lacZ*

haploids were transformed with pNL20 and grown in LB with ampicillin with several transfers. Rescued promoters from the $araO_1^-$ (NL31-259) and $araO_2^-$ (NL31-212) strains (which are P_{BAD}^+) gave rare recombinant $AraB^+$ plasmids, which were easily detected after transformation of NL20-314, a $recA^-$ $araB^-$ (*araB716*, a deletion from 436 to 634) strain. Plasmid DNA from Ara^+ colonies was isolated; DNA containing the *ara* regulatory region was cloned into an M13 phage and sequenced by the Sanger dideoxy method (22). Rare recombinant plasmids from the P_{BAD}^- strains [NL31-337 $araI_2^-$ and NL31-320 $ara(I_1I_2)^-$] were detected as blue colonies on X-Gal plates after transformation of NL20-272, which is *recA* and contains the *araC766* deletion (from −626 to −1698). The regulatory region DNA from these plasmids was also cloned and sequenced.

We lysogenized our haploid *araC–lacZ* fusion strains with a $\lambda paraC^+$ to make $AraC^+$ derivatives. Stable lysogens were isolated, and the presence of $\lambda paraC^+$ prophages was verified by their ability to produce high-titer lysates that complemented *araC766*. These strains, NL31-217 ($araO_2^-$), NL31-282 ($araO_1^-$), NL31-332 [$ara(I_1I_2)^-$], and NL31-338 ($araI_2^-$), were shown to be single lysogens by their sensitivity to *λcI90 c17* (23).

RESULTS

A Mutation of $araO_1$ with Unimpaired Polymerase Binding. The AraC-binding site $araO_1$ was thought to be the operator responsible for *araC* autoregulation (15, 16). This assumption was based primarily on two facts: (*i*) $araO_1$ overlaps the RNA polymerase-binding site of the *araC* promoter and (*ii*) AraC protein and RNA polymerase compete for binding to this site in the presence of arabinose *in vitro*. *In vivo* experiments showing the effect of $araO_1$ on autoregulation have not been possible due to the lack of mutants. We constructed an $araO_1^-$ mutant ($araO_1259$) by site-directed mutagenesis. The mutant $araO_1259$ contained five base substitutions, four of which lie within a 17-bp AraC-binding consensus (17). This mutation left intact the −35 and −10 hexanucleotides of the overlapping polymerase binding site, as shown in Fig. 1. DNase I footprinting (DNase protection) showed that there was a reduction by a factor of 8 in the affinity of the mutant $araO_1$ for AraC protein as compared with the wild type (Fig. 3). As indicated below, these changes appeared to have no effect on polymerase binding.

Effect of $araO_1$ on Autoregulation *in Vivo*. To detect the effect of $araO_1259$ on *araC* autoregulation, we put the mutation in cis to a chromosomal *araC–lacZ* fusion. This mutation, in the absence of an intact *araC* gene, had little effect on the *araC* promoter (Table 1, line 4), indicating that the base substitutions in $araO_1259$ did not significantly alter polymerase binding. We introduced into this strain a single copy of *araC* by way of a *λpara*. When the lysogen was tested for its degree of autoregulation, we found a large disparity between the induced and noninduced cells (Table 1, lines 5 and 6). Unlike the wild-type control, which showed repression by AraC in the presence and absence of inducer (Table 1, lines 1–3), the $araO_1259$ lysogen showed a complete loss of autoregulation in the presence of arabinose (Table 1, line 6), whereas the normal repression (by a factor of 10) was observed in the absence of the sugar (Table 1, line 5). This unexpected finding suggested that the mechanism of *araC* autoregulation might be more complex than previously assumed. Ligand-induced alterations in the occupancy of various AraC-binding sites have been demonstrated (17). The regulation of *araC* may also involve such changes, since the role of $araO_1$ in autoregulation changes with the presence of inducer. To test this possibility and to locate the site(s) responsible for the repression of *araC* in the absence

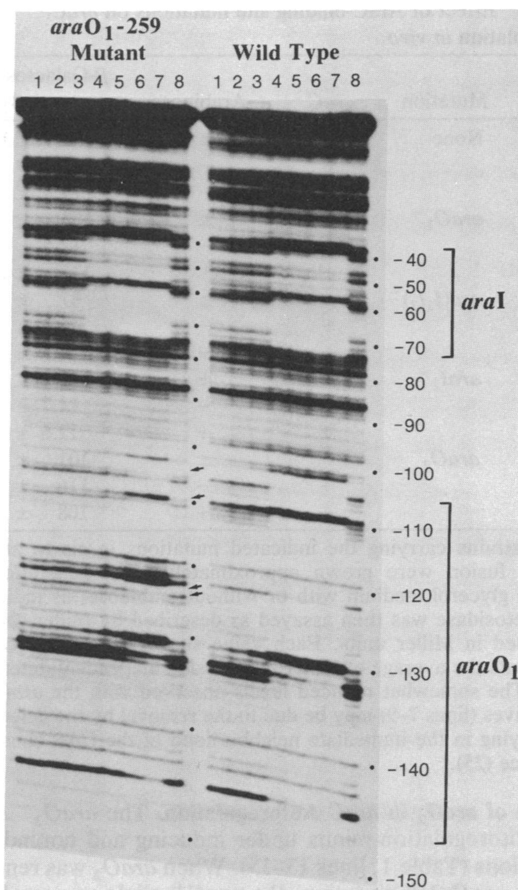

FIG. 3. DNase protection experiment showing decreased $araO_1$ protection in the $araO_1259$ mutant. The binding mixtures (50 μl) were as described (17). End-labeled DNA fragments were at 7.5 nM, L-arabinose was 33 nM, and AraC protein was at the concentrations indicated: lanes 1 and 8, no AraC; lanes 2, 10 nM; lanes 3, 20 nM; lanes 4, 40 nM; lanes 5, 80 nM; lanes 6, 160 nM; and lanes 7, 320 nM. Nucleotide positions are indicated on the right. Arrows indicate the positions of mutated bases in the $araO_1259$ DNA. The promoter fragment containing the $araO_1259$ mutation exhibited the same degree of protection at 320 nM AraC (lane 7) as did the wild-type fragment at 40 nM AraC protein (lane 4). No difference in the protection of *araI* was observed.

of arabinose, we tested the remaining nearby AraC-binding sites for their involvement in *araC* autoregulation.

Mutations in the Other AraC-Binding Sites. There are three other AraC-binding sites near the transcriptional startpoint of the *araC* gene: $araO_2$, $araI_1$, and $araI_2$ (Fig. 1). We selectively inactivated these sites and obtained $araO_2^-$ ($araO_2212$), $araI_2^-$ (*araI55*), and $ara(I_1I_2)^-$ (*araI782*) derivatives of the *araC–lacZ* fusion strain. The *araI55* mutation prevents AraC binding at $araI_2$ while retaining wild-type affinity for AraC at $araI_1$ (17). $AraO_2212$ was a deletion of 20 bp of $araO_2$ DNA that included the entire AraC-binding consensus sequence (17). The *araI782* deletion left the CAP consensus sequence intact but removed $araI_1$, $araI_2$, and part of the *araBAD* polymerase-binding site (Fig. 1).

Role of $araI_1$ in *araC* Autoregulation. The $araI_1$ site was found to be an indispensable element in *araC* autoregulation only in the absence of inducer. Inactivation of $araI_1$ by the $ara(I_1I_2)^-$ deletion affected autoregulation in the absence of inducer (Table 1, line 8) but not in its presence (Table 1, line 9), a situation that is the exact converse of that seen with the $araO_1259$ mutant. That it was $araI_1$ not $araI_2$ that was involved with *araC* regulation was shown by the observation that the $araI_2^-$ mutation (*araI55*) did not affect autoregulation (Table 1, lines 10–12).

Table 1. Effect of AraC-binding site mutations on *araC* autoregulation *in vivo*

Strain	Mutation	*araC*	L-Arabinose	β-Galactosidase activity
31-024	None	−	±	132 ± 13
31-067		+	−	12.7 ± 0.5
31-067		+	+	14.8 ± 1.6
31-259	$araO_1^-$	−	±	105 ± 2
31-282		+	−	12.5 ± 0.6
31-282		+	+	132 ± 21
31-320	$ara(I_1I_2)^-$	−	±	87 ± 2
31-332		+	−	46.4 ± 2.9
31-332		+	+	4.3 ± 0.1
32-337	$araI_2^-$	−	±	110 ± 5
31-338		+	−	14.7 ± 0.3
31-338		+	+	12.6 ± 0.4
31-212	$araO_2^-$	−	±	101 ± 6
31-217		+	−	116 ± 11
31-217		+	+	108 ± 6

The strains carrying the indicated mutations in cis to an *araC–lacZ* fusion were grown approximately eight generations in mineral glycerol medium with or without arabinose, as indicated. β-Galactosidase was then assayed as described by Miller (24) and expressed in Miller units. Each value shown in the last column represents the average of either four or six independent determinations. The somewhat reduced levels observed with the $ara(I_1I_2)^-$ derivatives (lines 7–9) may be due to the removal by the deletion of bases lying in the immediate neighborhood of the CAP consensus sequence (25).

Role of $araO_2$ in *araC* Autoregulation. The $araO_2^-$ strain was autoregulation-minus under inducing and noninducing conditions (Table 1, lines 13–15). When $araO_2$ was removed by the $araO_2212$ mutation, the $araC^+$ allele was unable to repress β-galactosidase synthesis when the cells were grown in either arabinose or glycerol medium. Thus, three discrete AraC-binding sites are involved in maintaining the repressed state of the *araC* gene. $araO_2$ and $araO_1$ are both required for autoregulation in the presence of inducer, whereas $araO_2$ and $araI_1$ are required when inducer is absent. At any given time, two of the three sites are operative; the $araO_2$ site must remain intact for repression to occur, whereas $araO_1$ and $araI_1$ alternate to maintain repression in the presence and in the absence of inducer, respectively.

DISCUSSION

The promoters of *araC* and *araBAD* are subject to transcriptional control by the AraC protein. When the inducer is absent, *araBAD* and *araC* are repressed by AraC. Upon addition of the inducer, AraC becomes an activator of the *araBAD* operon while continuing to repress the *araC* gene. These highly selective and diversified actions seem to demand an unusual degree of functional complexity in the AraC protein.

Our findings that there is a ligand-induced change in the state of occupancy of AraC-binding sites near these promoters and that such changes have profound effects on P_{BAD} (17) and P_C activities have led us to believe that AraC exerts this multiplicity of control through alternate states of cooperative binding to DNA. This simple model is shown in Fig. 4. We postulate that, in the absence of inducer, a single interaction between ligand-free AraC molecules facilitates their cooperative binding to $araI_1$ and $araO_2$; this configuration produces repression of *araBAD* (7, 9) and autoregulation of *araC*. Upon the introduction of arabinose, AraC undergoes a ligand-induced conformational change that precludes its cooperative binding to $araI_1$ and $araO_2$. Instead, two different interactions become established between ligand-bound AraC molecules: the binding to $araI_1/araI_2$ leads to P_{BAD} activation (17) and the binding to $araO_1/araO_2$ restores $araO_2$ occupancy and maintains *araC* repression.

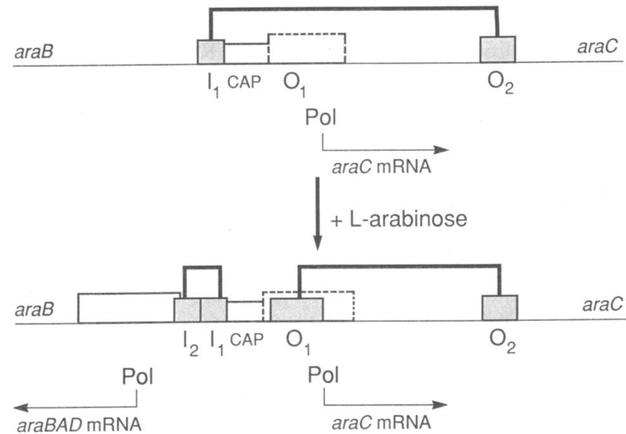

FIG. 4. Integrative model for *araC* autoregulation, *araBAD* activation, and repression. The cooperative interactions of AraC protein bound to different sites are responsible for autoregulation in the absence (*Upper*) and presence (*Lower*) of L-arabinose. Proteins: AraC protein, shaded boxes; catabolite gene activator protein, small open boxes; RNA polymerase, large open boxes. Protein-binding sites are designated as in Fig. 1. Bold lines connecting AraC protein boxes show cooperative interactions. Dashed polymerase boxes signify partial occupancy by RNA polymerase due to the autoregulation of *araC*.

The above model accounts for the various phenotypes observed after selective inactivations of the four AraC-binding sites. The loss of $araI_2$ function prevented *araBAD* activation (17) without affecting autoregulation. Inactivation of $araI_1$ abolished *araBAD* activation (ref. 8; N.L. and C. Francklyn, unpublished data), *araBAD* repression (9), as well as *araC* autoregulation in the absence of arabinose but not in its presence. Inactivation of $araO_1$ eliminated *araC* autoregulation in the presence of inducer only and had no effect on either the activation or the repression of *araBAD* (ref. 9; unpublished data). Removal of $araO_2$ was accompanied by the loss of *araBAD* repression but not its activation (7), and the autoregulation of *araC* was completely abolished by the $araO_2$ deletion.

The model is based on two key facts: (*i*) the DNA sites that bind AraC have very different affinities for the protein (15, 16) and (*ii*) the binding of ligand induces a conformational change in AraC that alters the sites of cooperative binding on the DNA (ref. 17; this paper).

The $araO_2$ site possesses a very low affinity for the AraC protein (16) and does not bind AraC *in vivo* in the absence of cooperativity (9). We propose that the role of $araI_1$ and $araO_1$ in maintaining *araC* autoregulation is to provide the sites required for cooperative binding of AraC to the low-affinity site $araO_2$. The alternate participation of these two high-affinity sites is compatible with *in vivo* binding data (9). It is not known whether AraC is bound to $araO_1$ *in vivo* in the absence of inducer. This high-affinity site (16), even if bound, has now been shown to have no effect on *araC* transcription in glycerol medium. Congruency of protein-binding sites does not necessarily preclude simultaneous occupancy (17, 26, 27).

We believe that the conformation of AraC protein plays a direct role in determining which cooperative interaction will occur. The conformational change in AraC induced by the binding of L-arabinose (28) is necessary for its cooperative binding to $araI_1$ and $araI_2$, which produces *araBAD* activation (18). Our experiments suggest that the $araO_1/araO_2$ interaction leading to *araC* repression also requires the ligand-bound conformation of AraC protein.

The transfer of AraC binding from *araI*$_1$ to *araO*$_1$ upon the addition of inducer must transiently disengage AraC bound at *araO*$_2$, since the latter site is incapable of noncooperative binding (9). This may account for the arabinose-induced transient derepression of the *araC* gene. It has been reported that *araC* expression increases in the first 15 min after arabinose addition before autoregulation is reestablished and *araC* expression returns to preinduction levels (29, 30). We suggest that the decrease in autoregulation following the addition of arabinose represents a transient escape synthesis when *araI*$_1$/*araO*$_2$-mediated autoregulation is replaced by that resulting from the *araO*$_1$/*araO*$_2$ interaction.

The alternate states of AraC occupancy depicted in Fig. 4 represent a dynamic equilibrium, governed by the ligand-dependent changes in AraC conformation. There is considerable evidence, however, suggesting that the requirement for arabinose in the allosteric transition of AraC is not absolute and that a very small amount of AraC activator is present even in the uninduced cell (7, 31, 32). This activator is responsible for the 12-fold stimulation of the *araBAD* promoter seen when repression is prevented by the elimination of the *araO*$_2$ site. We propose that the repression of *araBAD* exists in the uninduced cell because the *araI*$_1$/*araO*$_2$ interaction precludes the *araI*$_1$/*araI*$_2$ interaction. An AraC molecule bound at *araI*$_1$ is capable of entering into associative interactions with either *araO*$_2$ or *araI*$_2$, depending on its conformational state. In the absence of inducer, AraC at *araI*$_1$ is locked into a cooperative interaction with *araO*$_2$, preventing its participation in an *araI*$_1$/*araI*$_2$ association. We envision that these mutually exclusive cooperative interactions constitute the basis for *araBAD* repression, since this promoter has no affinity for RNA polymerase whatsoever in the absence of AraC protein (33). This model for the mechanism of *araBAD* repression predicts that any modification that favors the binding of AraC to *araI*$_1$/*araO*$_2$ would enhance repression, and any modification that favors the binding of AraC to *araI*$_1$/*araI*$_2$ would decrease it. In view of the proximity of *araI*$_2$ and the polymerase-binding site at P_{BAD} (17), it would not be surprising if a promoter mutant that strengthened the polymerase interaction with DNA also favored the occupancy of *araI*$_2$ by AraC, at the expense of the *araI*$_1$/*araO*$_2$ interaction. This may explain the finding that some mutations that reduced *araBAD* repression map within the RNA polymerase-binding domain (9).

It has been suggested that when a protein binds cooperatively to widely separated sites, the intervening DNA forms a loop (see ref. 13 for a review). Looping has been incorporated into the repression models of many operons (2–12). The repression of *araBAD* in the absence of inducer has been postulated to involve the formation of a DNA loop (7, 9, 17), since the phasing of the two sites involved in repression, *araI*$_1$ and *araO*$_2$, is critical (7), an observation that supports the idea of loop formation (34, 35). There is yet no experimental evidence suggesting that a DNA loop forms between *araO*$_1$ and *araO*$_2$. Examination of the *araC* leader sequence (36) shows that the center-to-center distance between the AraC-binding consensus sequences (17) within *araO*$_1$ and *araO*$_2$ is 158 bp, which represents an integral number of helical turns (15.0) in B-form DNA. The phasing of *araO*$_1$ and *araO*$_2$ therefore suggests the existence of a similar DNA loop structure.

How AraC occupancy generates repression of *araC* remains to be elucidated. The binding of AraC could either directly block polymerase progress (37) or produce a DNA conformation unfavorable for polymerase entry at the *araC* promoter. Further work is needed to determine the mechanism.

We have proposed that AraC protein exerts positive and negative transcriptional regulation and mediates cellular response to inducer by two alternate states of DNA occupancy. Like the phage λ cI protein, the mode of action of AraC—i.e., whether positive or negative—is governed by the DNA sites that are occupied (38). The widespread occurrence of multiple, and often widely separated, binding sites on the DNA in different biological systems (13, 39, 40) suggests their importance in regulation. The *ara* system provides a model where a single protein interacts with its four cognate sites to produce a multiplicity of controls.

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