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Arabinose-induced binding of AraC protein to *araI*$_2$ activates the *araBAD* operon promoter

(cleavage-inhibition patterns/DNA–protein interactions/gene regulation/positive control)

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ABSTRACT **The state of *Escherichia coli araI* DNA occupancy by AraC protein has been found to change from a two-turn to a four-turn occupancy upon the addition of the inducer arabinose. The *araI* site is separable into two contiguous regions, *araI*$_1$ and *araI*$_2$. *araI*$_1$ binds both ligand-bound and ligand-free AraC protein, whereas *araI*$_2$ binds AraC protein in the presence of arabinose only. A mutation in *araI* and a known mutation in *araC* led to the loss of *araI*$_2$ binding, while binding to *araI*$_1$ was unaffected. Both mutants failed to activate the promoter of the *araBAD* operon. We propose that *araI*$_2$ occupancy by AraC protein leads to RNA polymerase recognition of the *araBAD* promoter and that *araI*$_1$ acts as a switch mechanism allowing both the repressor and the activator forms of AraC protein to regulate the *araBAD* promoter.**

Transcription initiation at the promoter of the *Escherichia coli araBAD* operon is regulated both positively and negatively by the AraC protein (1). The site of AraC interaction required for activation (*araI*) is located very close to the RNA polymerase binding site (2–4), whereas the site for repression (*araO*$_2$) is located at a considerable distance from the start point of *araBAD* transcription (5–8) (Fig. 1). Recently, there has been considerable effort directed toward understanding how the *araBAD* promoter is down-regulated by a far-upstream site (7, 9, 10), whereas the mechanism of *araBAD* activation upon the addition of inducer remains largely unexplored. It is well known (5) that strains deleted of the *araO*$_2$ site still require L-arabinose to initiate transcription of *araBAD*, a fact that indicates that the relief of repression is not responsible for promoter activation. In cells containing *araO*$_2$, the relief of repression accompanies activation but is not the cause of it. Promoter activation must require some ligand-induced event at *araI*.

The state of *araI* occupancy by AraC protein is an important issue in understanding the mechanism of promoter activation, yet, with one exception (ref. 10; however, see *Discussion*), there has been no experiment reported that searched for a qualitative change in the interaction of AraC protein with *araI* upon induction. In this paper, we present data showing that, in the absence of the ligand L-arabinose, AraC protein binds to two turns on the DNA. The addition of arabinose leads to binding to an additional downstream sequence, representing two more turns on the DNA. We propose that the shift in the binding at *araI* from a two-turn occupancy to a four-turn occupancy corresponds to the transition from an inactive to an active promoter. Thus, the *araI* site is divisible into two regions, which we designate *araI*$_1$ and *araI*$_2$. In the absence of arabinose, AraC protein can bind only *araI*$_1$, but the addition of inducer allows AraC protein to bind both *araI*$_1$ and *araI*$_2$.

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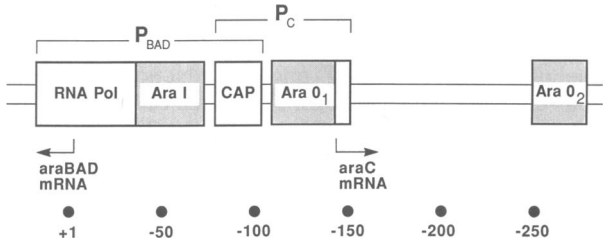

FIG. 1. The controlling region of the *araBAD* operon. The overlapping and divergent promoters of *araBAD* (P_{BAD}) and *araC* (P_C) are shown with the positions of the contact sites of RNA polymerase (RNA pol), AraC protein (*AraI*, *AraO*$_1$, and *AraO*$_2$), and the cAMP binding protein (CAP). The P_C RNA polymerase binding site (open box) overlaps *AraO*$_1$ (stippled box). The start points and directions of transcription are indicated by arrows. The numbers mark positions of nucleotides relative to the start point of *araBAD* transcription.

Further evidence for two discrete states of *araI* occupancy was obtained from two mutants, one a promoter mutant and the other an *araC* gene mutant. These mutants are defective in only *araI*$_2$ binding, and both fail to activate the *araBAD* promoter.

MATERIALS AND METHODS

Isolation of AraC Protein. AraC protein was isolated from a hyperproducing strain harboring a plasmid that carried a wild-type *araC* allele fused to the *tac* promoter (11). Details of the construction of the overproducing plasmid will be presented elsewhere (C.F., unpublished data).

For the isolation of AraC protein, cells were cultivated in 8 liters of minimal glycerol ampicillin medium containing per liter 7.0 g of K_2HPO_4, 3.0 g of KH_2PO_4, 1 g of $(NH_4)_2SO_4$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 4 mg of thiamine, 2.0 ml of glycerol, and 50 mg of sodium ampicillin and were induced with 100 μM isopropyl β-D-thiogalactoside. Purification steps were as described (8). The final product had an A_{280}-to-A_{260} ratio of 1.9 and appeared homogeneous by electrophoretic analysis in $NaDodSO_4$/polyacrylamide gels. Fig. 2 illustrates samples taken at successive steps during the isolation of AraC protein.

Spectrophotometric Measurement of AraC Concentration. The concentrations of purified AraC protein were determined spectrophotometrically by using the factor A_{260}, 1 mg/ml = 1.88. This value was obtained by amino acid analysis as follows. The absorbancy of a purified AraC protein solution in buffer was first determined against a buffer blank. Of this solution, 3.0 ml was then exhaustively dialyzed against distilled water, and a known quantity of norleucine was added to the dialyzed solution to monitor recovery. Aliquots of this solution were subjected to acid hydrolysis for 16, 24,

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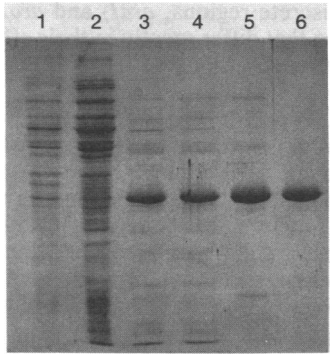

FIG. 2. NaDodSO$_4$/polyacrylamide gel electrophoresis showing the isolation of AraC from cells harboring the *tac* promoter-*araC* fusion plasmid. Samples were taken from successive steps during the isolation, precipitated with CCl_3COOH and analyzed on a NaDodSO$_4$/12.5% polyacrylamide gel. The isolation procedure was as described (8). Lanes: 1, whole cells (1/160,000 of the total preparation); 2, crude cell extract (1/80,000 of the total); 3, after phosphocellulose chromatography (1/7,000 of total); 4, after acid precipitation (1/7,000 of total); 5, after fractionation on a Sephadex G-100 column (1/2,250 of total); 6, after hydroxyapatite column chromatography (1/1,720 of total). The last three steps were necessary to remove numerous contaminants that made up about 20% of the total in lane 3.

48, and 72 hr. The hydrolysates were analyzed with a Beckman 120C amino acid analyzer. The yields of alanine and glutamic acid plus amides, in conjunction with the known amino acid composition of AraC protein (12), were used to determine the quantity of AraC protein in the solution before dialysis. The calculated specific absorbance value of 1.88 is based on the assumption that there was no significant loss of AraC protein during dialysis. Assays of AraC protein activity in a DNA-dependent protein-synthesizing system showed that there was no detectable loss of total AraC protein activity after dialysis when the quantity of AraC protein was large (about 6 mg).

DNase I Cleavage-Protection Experiments. The procedure used was essentially as described (2) except that reactions were scaled down so that each binding mixture contained 500 fmol of DNA at a concentration of 10 nM. DNA fragments were 5′-end labeled as described (13) at a *Bst*EII restriction site located at −203 (12) and then cleaved with *Hae* III at position 44. [The numbering of nucleotides is based on the assignment of +1 to the start point of *araBAD* transcription (14).] The 247-base-pair (bp) fragment (from −203 to 44) was purified by polyacrylamide gel electrophoresis. The amount of fragment was determined by its UV absorbance with the factor A_{260}, 1 μg/ml = 0.020.

Isolation of the Promoter-Null Mutant 30-033. Strain 30-033 was obtained by the selection for arabinose-resistant, arabinose-negative revertants of the *araD139* mutation (15). The *araD139*-carrying mutant, deficient in L-ribulose 5-phosphate 4-epimerase, is both Ara$^-$ and arabinose-sensitive on eosin/methylene blue arabinose or McConkey arabinose agar. Spontaneous revertants that are resistant to arabinose inhibition arise with high frequency and contain secondary mutations that affect the production of AraA protein and/or AraB protein. These proteins catalyze the conversion of L-arabinose to L-ribulose 5-phosphate, the metabolite whose accumulation inhibits the growth of the *araD139*-carrying mutant. From about 1200 arabinose-resistant clones isolated, a small number of *araBAD* promoter-null mutants were found. The locations of the second site mutations were first mapped by crossing against F′ *ara* homogenotes (16), then the mutations were cloned in *ara*-transducing phages (17, 18) by marker rescue, subcloned in either pBR322 or phage M13, and sequenced by the Maxam–Gilbert chemical modification or the Sanger dideoxy method (13, 19). The mutation in 30-033 is the deletion of a T·A base pair at position −55. This deletion removes one of two central base pairs (both T·A) in *araI*, which spans the region from −73 to −38.

Measurements of Activation *in Vivo*. Activation of the *araBAD* operon was measured by L-arabinose isomerase assays (20); the synthesis of this enzyme is regulated by the *araBAD* operon promoter. Strains used for assays carried single copies of the promoter and *araC* gene, and the wild-type strain 20-000 served as a control. Cells were grown at 37°C in minimal glycerol medium with and without L-arabinose (final concentration, 0.4%), and cell-free extracts

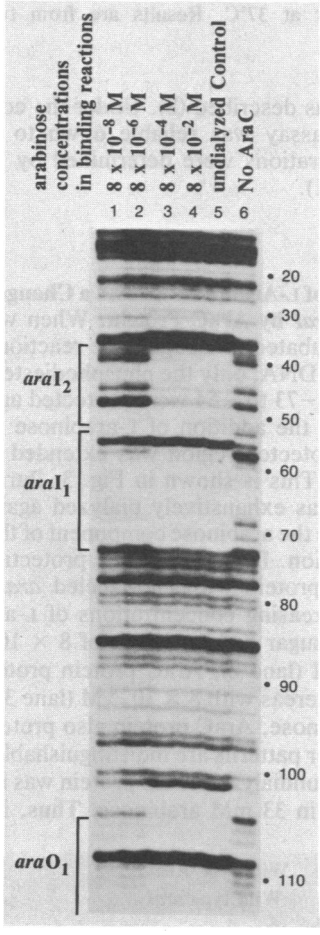

FIG. 3. DNase protection experiment showing the arabinose-dependent protection of *araI*$_2$ by AraC. The binding mixtures (50 μl) were as described (2) and contained 10 mM Tris·HCl (pH 7.9), 10 mM $MgCl_2$, 5 mM $CaCl_2$, 100 mM dithiothreitol, and 90 mM KOAc. The end-labeled DNA fragment was present at 10 nM; AraC protein and L-arabinose were at the concentrations indicated. The mixtures were incubated for 16 min at 25°C and then were treated with DNase I at 0.2–0.3 g/ml for 30 sec. The digestions were stopped by the addition of 12.5 μl of "DNase stop" (3 M NH_4OAc/0.25 M EDTA/0.15 mg of sonicated salmon sperm DNA per ml). The fragments were precipitated twice with ethanol, dissolved in 80% (vol/vol) deionized formamide/10 mM NaOH/1 mM EDTA/0.1% xylene cyanol/0.1% bromophenol blue, heated 1 min at 90°C, and subjected to electrophoresis in 8% polyacrylamide/7 M urea gels. A 250-bp DNA fragment containing *araI* was 5′-end-labeled at position −208, incubated with AraC protein concentrations of L-arabinose from 80 nM to 80 mM as indicated (lanes 1–4), and challenged with DNase I. Lanes: 1–4, 400 mM dialyzed AraC; 5, 200 nM undialyzed AraC with 33 mM added arabinose and AraC that had not been made sugar-free by extensive dialysis; 6, no AraC. The nucleotide positions indicated by the numbers on the right are negative relative to the transcription initiation site at +1. The bracket marked *araI*$_2$ indicates the region showing arabinose-dependent protection.

Table 1. Loss of promoter activation in mutant 30-033

Cells	Strain 20-000 (wild type)	Strain 30-033 (*araI* mutant)
Induced	182	0.04
	173	0.03
Uninduced	0.16	<0.02
	0.14	—

Induced and uninduced cells were grown eight generations in minimal glycerol medium with and without 0.4% L-arabinose, respectively. The cells were harvested at $\approx A_{600} = 1$ and cell-free extracts were prepared and assayed for L-arabinose isomerase activity (8) and their protein concentrations (21). One unit of L-arabinose isomerase activity allows the formation of 1 μmol of L-ribulose per hr at 37°C. Results are from two independent determinations.

were prepared as described (8). Under the conditions used, the isomerase assay was reliable down to 0.02 unit/mg. Protein concentrations were determined by the method of Lowry *et al.* (21).

RESULTS

The Addition of L-Arabinose Causes a Change in the State of Occupancy at *araI* by AraC Protein. When wild-type AraC protein was incubated in a sugar-free reaction mixture with ^{32}P-labeled *araI* DNA, only the phosphodiester bonds within the region from −73 to −54 were protected against DNase I cleavage. Upon the addition of L-arabinose to the binding mixture, the protected region was extended further downstream to −38. This is shown in Fig. 3. Purified wild-type AraC protein was exhaustively dialyzed against sugar-free buffer to remove the arabinose component of the buffers used during its isolation. DNase cleavage protection patterns of dialyzed AraC protein with end-labeled *araI* DNA in the presence of increasing concentrations of L-arabinose were obtained. At a sugar concentration of 8×10^{-8} M (lane 1) and 8×10^{-6} M (lane 2), AraC protein protected only the region $araI_1$, whereas with 8×10^{-4} M (lane 3) and 8×10^{-2} M (lane 4) arabinose, AraC protein also protected the $araI_2$ region. The latter patterns are indistinguishable from the one in lane 5, where undialyzed AraC protein was incubated with the same DNA in 33 mM arabinose. Thus, it appears that there are two discrete regions, $araI_1$ and $araI_2$, of approximately equal size that differ greatly in their affinity for AraC protein in the absence of inducer.

A Promoter-Null Mutation in *araI* Prevents the Binding of Wild-Type AraC Protein to $araI_2$. The promoter-null mutant 30-033 is deleted of one of the two central A·T base pairs in *araI*. Its phenotype is arabinose negative, and assays of *araBAD* promoter activity indicated that this mutation abolished promoter function almost entirely (Table 1).

A DNA fragment containing the 30-033 mutant promoter was titrated against various concentrations of purified wild-type AraC protein in the presence of arabinose (Fig. 4). Binding to $araI_1$ was indistinguishable from that with the wild-type promoter (see Fig. 3), but $araI_2$ was only partially protected. The region containing nucleotides −38, −39, and −40, normally protected by AraC protein in the presence of arabinose, was completely exposed to DNase I digestion. The loss of promoter function is associated with the loss of AraC protein binding to the $araI_2$ region.

DISCUSSION

The *araI* site, located immediately upstream of the RNA polymerase binding site in the *araBAD* operon, is complexed with AraC protein both in the presence and absence of the inducer arabinose (10). Our DNase I cleavage-protection results revealed that the region protected by AraC protein in the presence of inducer spans four turns of DNA, whereas only two turns of *araI* DNA are protected in the absence of inducer (Fig. 5).

The protection patterns of $araI_1$ and $araI_2$ show considerable similarity. $araI_1$ is 20 bp in length, or about two full turns of the DNA helix. AraC protein is known to contact two guanine residues at positions −70 and −69 within the first major groove (2, 23) and six flanking phosphate groups (23). Interaction with the second major groove is less pronounced, as evidenced by the interaction with the G residue at −59 (2, 23) and three phosphate groups bordering it (23). $araI_2$ also spans two turns on the DNA helix. AraC protein contacts two guanine residues at positions −48 and −49 within the first major groove in $araI_2$ (2, 23) and six phosphate groups flanking it (23). The similarity in the protection patterns of the first major grooves in $araI_1$ and $araI_2$ is striking. The weakest contacts are made with the fourth turn of the DNA. No

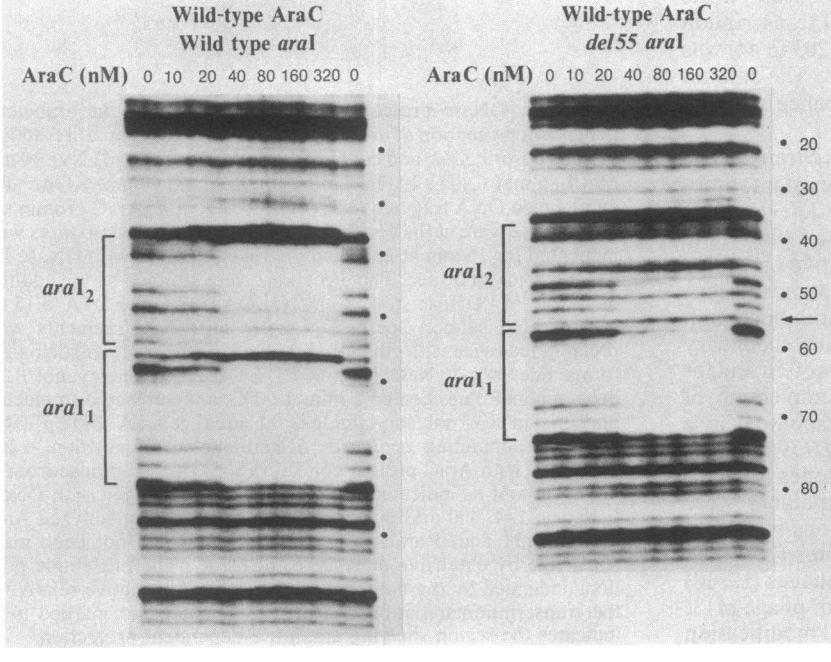

FIG. 4. DNase protection experiment showing the incomplete $araI_2$ protection in the deletion 55 *araI* (mutant 30-033) promoter. The binding mixtures were all 10 nM in DNA and 33 nM in arabinose, with wild-type AraC at the concentrations indicated. The nucleotide positions indicated by the numbers on the right are negative relative to the transcription initiation site at +1. The arrow indicates the position of the deleted base. Note that AraC did not protect the residues at −38, −39, and −40, nor did it cause enhanced cleavages at −35 and −36 of the mutant promoter. The band that appears at −52 in all lanes of wild-type *araI* represents a nick in the DNA fragment, which is observed even in the absence of DNase I treatment. For unknown reasons, the nick was observed only when the fragment was derived from certain plasmids and not from others, such as the one used to produce the fragment in Fig. 2.

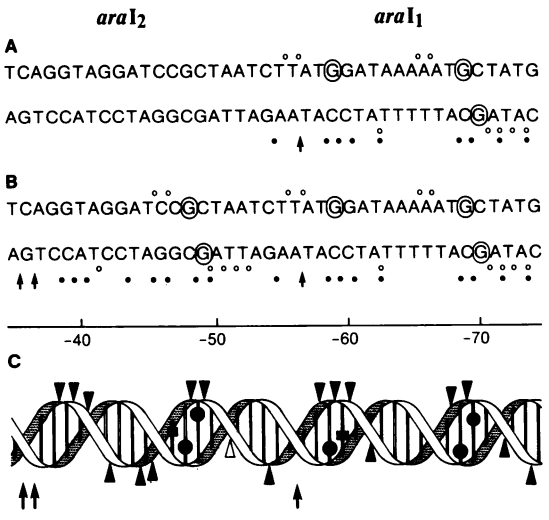

FIG. 5. DNA sequence and topology of the $araI_1$ and $araI_2$ regions. (*A*) The $araI_1$ region. The positions of phosphate bonds that were protected against DNase I cleavage by ligand-free AraC are indicated by closed circles. The position of enhanced DNase I cleavage is shown by the arrow. (Data from Fig. 3.) An "activation-minus" *araC* mutant described by Brunelle *et al.* showed ethylation interference (open circles) and methylation interference (circled guanines) in the same region (22). (*B*) The $araI_2$ region is downstream of $araI_1$ and is protected by wild-type AraC only in the presence of arabinose. Phosphates that were protected against DNase I cleavage by AraC in the presence of arabinose are indicated by closed circles. Positions of enhanced cleavage are shown by arrows. (Data from Fig. 2). Circled guanines were protected against methylation by AraC arabinose complex (2) and showed methylation interference (23). Phosphates showing ethylation interference are indicated by open circles (23). Note the resemblance of the right halves of $araI_1$ and $araI_2$. (*C*) $araI_1$ and $araI_2$ each correspond to two turns of a DNA duplex. ▲, Positions of protected phosphate bonds; △, position of a DNase I cleavage site not affected by the AraC–arabinose complex; ↑, enhanced cleavage sites; ●, protected guanines; and ■, guanines showing enhanced methylation. The sequences of 11 AraC-binding sites in *E. coli* (2, 7, 24) and *Salmonella typhimurium* (25) are shown below along with a consensus sequence. Sequences: 1, *E. coli* $araI_1$; 2, *E. coli* $araI_2$; 3, *E. coli* $araI_1$ (*araE* promoter); 4, *E. coli* $araI_2$ (*araE* promoter); 5, *S. typhimurium* $araI_1$; 6, *S. typhimurium* $araI_2$; 7, *E. coli* $araO_1$ (−109 to −125); 8, *E. coli* $araO_1$ (−130 to −146); 9, *S. typhimurium* $araO_1$ (−109 to −125); 10, *S. typhimurium* $araO_1$ (−130 to −146); 11, *E. coli* $araO_2$ (−267 to −283). Underlined bases agree with the consensus sequence.

1. (−56) TATGGATAAAAATGCTA (−72)
2. (−35) TCAGGTAGGATCCGCTA (−51)
3. (−57) TATGGATTAAATTGCTG (−73)
4. (−36) TCAGGTCGGAAACAGCA (−52)
5. (−56) TATGGACAAAAATGCTA (−72)
6. (−35) TCAGGCAGGATCCGCTA (−51)
7. (−109) TGTGGACTTTTCTGCCG (−125)
8. (−130) TATAGACACTTTTGTTA (−146)
9. (−109) TGTGGACATTCCAGCCA (−125)
10. (−130) TATAGACACTTCTGTTA (−146)
11. (−267) TATGGACAATTGGTTTC (−283)

Consensus T-TGGAC------GCTA

specific guanine residue is contacted within the fourth major groove, and only a single phosphate bordering the fourth major groove showed ethylation interference with AraC protein binding (23). However, our DNase I protection experiments indicated that a fourth turn of the DNA is shielded by AraC protein and that $araI_2$ is the same size as $araI_1$.

The lack of close contacts between AraC protein and the bases lying within the second major groove of $araI_2$ explains why *araI* ($araI_1$ + $araI_2$) was previously thought to span only three turns on the DNA helix (23). Although DNase I is a much larger probe, it does not seem likely that its size could account for the protection of an entire additional turn of the DNA, especially since this additional turn lies completely to one side of the three major grooves of *araI* that make specific contacts.

Examination of the sequences of $araI_1$ and $araI_2$ revealed a number of conserved bases. Other AraC-binding sites, in *E. coli* (2, 7, 24) and *S. typhimurium* (25), also show conservation of these bases. A consensus AraC-binding sequence is presented in the legend to Fig. 5. In every case except $araO_2$, the sequence protected by AraC can be divided into two consensus sites. These sites are located on the DNA so that each base within a site is separated from the corresponding base in a neighboring site by 21 bp. This arrangement allows AraC protein to bind to the same face of the DNA at both sites. A second feature of the consensus sequence is its lack of apparent dyad symmetry. The binding of AraC protein to this sequence, unlike the binding of CAP to its consensus sequence, is asymmetric. Lastly, there is a clustering of A or T residues near the center of the consensus sequence, suggesting that the regions of DNA comprising the minor grooves that face AraC may be especially susceptible to conformational distortion. These features shared by AraC binding sites argue that both activator and repressor forms of AraC protein establish similar contacts with DNA, perhaps engaging the same amino acid residues in the protein. The individual sites differ greatly, however, in their affinity; this is also seen with other protein contact sites (26, 27). $araI_2$ possesses no affinity for ligand-free AraC protein. The addition of arabinose, which changes AraC protein conformation (28), allows the binding of AraC protein to $araI_1$ and $araI_2$ in cooperative manner (N.L., unpublished data). We propose that binding to $araI_2$ results from an association between neighboring AraC protein molecules made possible by the ligand-induced conformational change. This association strengthens the specific contacts with $araI_2$ DNA.

The molecular events accompanying the change from a two-turn to a four-turn occupancy at *araI* remain to be elucidated. The AraC consensus lacks any apparent dyad symmetry, a fact that could account for the asymmetric binding by AraC to the two major groves of each *araI* region (Fig. 5). The protection of each *araI* region could result from the binding of either a monomer or a dimer that presents a slightly asymmetric binding surface. Hendrickson and Schleif (23) have reported that it is a dimer of AraC protein that binds to *araI* in the presence of either the inducer arabinose or the anti-inducer D-fucose. (No data was presented regarding sugar-free AraC–*araI* complexes.) Determination of the stoichiometry of binding in the absence of ligand and confirmation of the stoichiometry in the presence of ligand are needed. Irrespective of the number of monomers associated with each *araI* site, the addition of inducer produces a change so that twice the number of protein is bound.

Two mutants illustrate the fact that binding to $araI_2$ is essential for promoter activation. One of these is the promoter mutant 30-033. This promoter could not be activated by wild-type AraC protein in the presence of arabinose (Table 1), and its $araI_2$ region was not protected by AraC *in vitro* (Fig. 4). A second mutant, with a defect in its *araC* gene, has been reported by Brunelle *et al.* (22), although the cause of the mutant phenotype was not apparent at the time. This *araC* gene mutant was isolated as an "activation-minus" mutant, which failed to activate the *araBAD* promoter even though the mutant protein bound inducer and showed only a slight loss in its affinity for *araI* DNA. Brunelle has shown that the mutant AraC protein, in the presence of arabinose, contacts both major grooves in $araI_1$ (G residues at −70, −69, and −59) and the nine flanking phosphate groups but does not contact any of the guanine residues (−48 and −49) or any of the seven phosphate groups in $araI_2$ that are contacted by

wild-type AraC protein. Clearly, this mutant protein is capable of binding $araI_1$, and it is the loss of $araI_2$ binding that must be responsible for its activation-minus phenotype.

Thus, the *araI* region consists of two physically separable and functionally distinct sites. The $araI_1$ site possesses a relatively high affinity for AraC protein in both its ligand-bound (activator P2) and ligand-free (repressor P1) forms. Two additional binding sites, $araO_2$ and $araI_2$, are incapable of binding AraC protein when present alone, but each binds AraC cooperatively with $araI_1$. The cooperative binding of ligand-free AraC protein to $araI_1$ and $araO_2$ produces repression of the *araBAD* operon (7). The cooperative binding of ligand-bound AraC protein to $araI_1$ and $araI_2$ produces activation, as is proposed in this paper. We propose that, upon the addition of the inducer arabinose, the interaction between ligand-free AraC protein bound at $araI_1$ and the far upstream site $araO_2$ is replaced by the interaction between ligand-bound AraC protein at $araI_1$ and $araI_2$. Therefore, $araI_1$ is essential for both repression and activation and acts as a switch to allow both the repressor and the activator forms of the AraC protein to control the *araBAD* promoter. Arabinose flips the switch and increases the activity of the promoter 1200-fold.

The above model brings together the repression data of the DNA loop model (7, 10) and our data on the ligand-induced occupancy of $araI_2$ by AraC protein. It shows that induction is not simply due to a change in the property of AraC protein bound at *araI* (10) but results from a change in the state of *araI* occupancy. The proposal that there is no change in the state of occupancy at *araI* upon inducer addition (10) was based on using methylation enhancement as a probe for AraC binding *in vivo*. This does not permit distinction between the binding of AraC protein to $araI_1$ alone or to $araI_1$ and $araI_2$ simultaneously, since there is no methylation enhancement site within $araI_2$ on the DNA strand examined (2, 23). Our model extends the original proposal by Englesberg (5) that the AraC protein is biologically active in both its ligand-bound and ligand-free states by binding specifically to different regions of the DNA.

How does $araI_2$ occupancy lead to promoter activation? Examination of the location of $araI_2$ relative to the transcriptional start point of *araBAD* operon shows that this site may well overlap the region of RNA polymerase binding. Although the *araBAD* promoter does not bind polymerase in the absence of AraC protein (2), a fact which has precluded binding experiments with polymerase alone, at least part of $araI_2$ falls within the region protected by RNA polymerase in other *E. coli* promoters (29). Furthermore, there is one nucleotide within $araI_2$ that appears to affect both AraC and polymerase functions. A base substitution mutation at position −38 had a dual effect on the *araBAD* promoter (10). This mutation renders the promoter unresponsive *in vivo* to stimulation by *araC*, and, in an AraC$^-$ cell, this mutant promoter is constitutive, possessing 26% the level of wild-type, fully induced activity (10). Activator–polymerase contacts have been shown to be important in promoter activation in other systems such as phage λ (30). Interestingly, phage λ *c*I protein binds adjacent upstream sites O_R1 and O_R2 much like what we observed with AraC protein at $araI_1$ and $araI_2$ in the presence of arabinose. *In vivo* the phage λ repressor stimulated the P_{RM} promoter 8-fold (30) via binding O_R sites by increasing the rate of polymerase isomerization to the open complex (31). The extent of AraC stimulation of *araBAD* is at least 25-fold larger. The *ara* promoter DNA does undergo a conformational change in the presence of the activator CAP (8). Perhaps AraC is required at $araI_2$ to produce a new DNA conformation that favors polymerase action and, simultaneously, to provide a surface where protein–protein interactions with the polymerase serve to initiate the selection of an otherwise unrecognized promoter.

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