Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 5444–5448, August 1988
Biochemistry

Alternative DNA loops regulate the arabinose operon in *Escherichia coli*

(repression/AraC protein/DNA–protein interaction/loop equilibria/regulation model)

LI HUO, KATHERINE J. MARTIN*, AND ROBERT SCHLEIF

Department of Biochemistry, Brandeis University, Waltham, MA 02254

Communicated by Peter H. von Hippel, April 4, 1988 (received for review December 17, 1987)

ABSTRACT **The *araCBAD* regulatory region of *Escherichia coli* contains two divergently oriented promoters and three sites to which AraC, the regulatory protein of the operon, can bind. This paper presents the results of *in vivo* dimethyl sulfate "footprinting" experiments to monitor occupancy of the three AraC sites and measurements of activity of the two promoters. These measurements were made both in the absence of the inducer arabinose and at various times after arabinose addition to growing cells containing the wild-type *ara* regulatory region or the regulatory region containing various deletions and point mutations. The data lead to the conclusion that two different DNA loops can form in the *ara* regulatory region. These loops are generated by AraC protein molecules binding to two different DNA sites and binding to each other. One of these loops predominates in the absence of arabinose and plays a major role in repressing activity of one of the promoters. Upon the addition of arabinose the amount of the first loop type, the repression loop, decreases and the amount of a second loop increases. Formation of this second loop precludes the counterproductive formation of the repression loop.**

AraC protein is a multifunctional regulatory protein of the genes required for uptake and catabolism of L-arabinose in *Escherichia coli* (1–4). This paper concerns the inducing and repressing activities of AraC on the two promoters located in the regulatory region between the *araC* and *araBAD* genes. One, P_{BAD}, serves the *araBAD* catabolic genes, and the other, P_C, serves the *araC* regulatory gene. In the presence of arabinose, AraC protein bound at the *araI* site, which is immediately adjacent to the RNA polymerase binding site of the P_{BAD} promoter, stimulates transcription of the *araBAD* genes (Fig. 1). The protein also represses mRNA synthesis from P_{BAD} in the absence of arabinose by a mechanism requiring the formation of a DNA loop (5–7). One end of this loop requires AraC protein binding at the $araO_2$ site, an operator that is located >200 base pairs (bp) away from the start site of *araBAD* transcription. Genetic experiments suggest that the other end of the loop is AraC protein bound to the *araI* site (6). AraC protein represses its own synthesis from the promoter P_C, most likely by binding to a third AraC protein binding site in the regulatory region, the operator $araO_1$, and directly blocking RNA polymerase entry to the promoter for synthesis of AraC protein, P_C (8, 9).

A variety of experiments have led to the conclusion that looping occurs in the *ara* system (5–7, 10). Additionally, DNA looping appears to be a common mechanism for gene regulation, as a number of other systems also show one or more of the properties displayed by the *ara* system and can be interpreted to loop so that two proteins bound to separated sites on DNA are in direct contact (11–22). Finally, the strength of the protein–DNA interactions and the protein–protein interactions in some systems (14, 16, 20, 22) permits loops generated on linear DNA to be observed in the electron microscope.

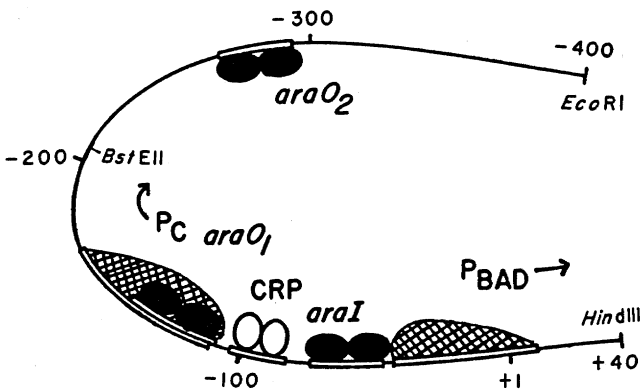

FIG. 1. Schematic drawing of the regulatory region of the *araCBAD* operon, showing the AraC binding sites, *araI*, $araO_1$, and $araO_2$, as well as portions of the flanking *araC* and *araB* genes. Numbering of base pairs is relative to the P_{BAD} transcription start site at +1. Solid, AraC protein; open, cAMP receptor protein (CRP); cross-hatch, RNA polymerase.

Previous *in vivo* dimethyl sulfate methylation "footprinting" studies of the *araCBAD* operon revealed that in the absence of the two other AraC protein binding sites, $araO_2$ binds AraC protein so weakly that it is virtually unoccupied (9). That is, the presence of the other sites, even though they are located >100 bp away, greatly increases AraC protein occupancy of $araO_2$. Since AraC bound at $araO_2$ must be interacting with one or both of the other sites to generate this binding-site cooperativity, either they are communicating by sending a signal along the DNA between the sites or, more likely, the DNA is looped to permit the proteins bound to each site to interact directly.

Is *araI* the sole source of the binding-site cooperativity leading to AraC occupancy of $araO_2$? Here we report that AraC bound to $araO_2$ interacts not only with AraC bound to *araI* but also with AraC bound to $araO_1$ and, therefore, participates in the formation of an alternative loop in the *araCBAD* regulatory region. We find that both types of loops are involved in regulating both the P_C and P_{BAD} promoters. We also present data suggesting that only one or the other of the loops can exist at one time, that arabinose shifts the amounts of the two looped forms present, and that looping may restrict access of AraC protein and RNA polymerase to binding sites within the looped region.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CRP, cAMP receptor protein; P_{BAD}, promoter for *araBAD* operon; P_C, promoter for *araC* gene.

*Present address: Department of Biochemistry and Molecular Biology, 7 Divinity Avenue, Harvard University, Cambridge, MA 02138.

MATERIALS AND METHODS

Media, Strains, and General Methods. The isogenic AraC$^+$ and AraC$^-$ strains were SH322 and SH321 (6). Activity of P_{BAD} was monitored by measuring galactokinase (GalK) encoded by a plasmid in which wild-type or mutant *araCBAD* regulatory region was fused to *galK* (5). Cells containing the plasmid were grown in M10 minimal medium (23) at 35°C and assayed as described (6). Arabinose, when added, was at 0.2%. The activities of P_{BAD} and P_C were also measured by fusing the *araCBAD* regulatory region in either orientation to *lacZ* and quantitating β-galactosidase. The $araO_1$ deletion mutant KM270 was constructed by deleting 27 bp in the $araO_1$ region, positions −119 to −146 with respect to the transcription start of P_{BAD} at +1. Six base pairs of filled-out *Eco*RI linker have been inserted at the deletion site and 21 bp of compensating DNA have been inserted upstream at the *Bst*EII site. Thus, the wild-type spacing between *araI* and $araO_1$ has been maintained. The previous sequence at the *Bst*EII site was GGTAACC, and the new sequence with the 21-bp insertion is GGTAACTCTAGATCGATCTAGAG-TAACC. When β-galactosidase activity was to be measured, the cells were grown at 30°C in M10 minimal medium containing 0.2% casein amino acids and 0.2% glycerol. Cells were assayed (24) with the following modifications. Samples were treated with 20 μl of 0.1% $NaDodSO_4$ and 40 μl of chloroform instead of toluene and enzyme incubations were at 32°C instead of 28°C.

***In Vivo* Footprinting.** Methods were as described (7). All the footprint experiments utilized the plasmid pTD3 (5) or derivatives, in which the 440-bp complete *araCBAD* regulatory region is fused to the *galK* gene. One of the derivatives contained the wild-type-like *ara* regulatory DNA, ES51, in which restriction endonuclease cleavage sites have been created between regulatory sites to facilitate the manipulation of the DNA. ES51 differs from the wild-type *ara* regulatory region at the following positions: G→C change at −76, T→G at −77, T→C at −107, C→G at −162, A→C at −166, T→G at −219, C→A at −220, T→A at −248, C→T at −251, C→G at −290, and T→G at −291. These changes lie outside known protein binding sites and do not significantly alter regulation of P_C or P_{BAD}. The deletions used in the *in vivo* footprinting experiments were made by cutting ES51 with *Hin*dIII and another restriction enzyme. The phage M13 replication origin was inserted into pTD3 and a derivative containing the *araI*-site point mutation BRS33 (25). Gel binding assays (9) have shown that this mutation reduces the affinity of *araI* for AraC protein by a factor of 30 by increasing the dissociation rate. The M13 origin facilitated DNA sequencing and the oligonucleotide site-directed mutagenesis that was used to construct the $araO_1$ point mutation LH7 and the *araI*–$araO_1$ double point mutant plasmid pLH6. The occupancy of mutated $araO_1$, LH7, was reduced to about one-third of normal as measured by *in vivo* footprinting (data not shown).

P_C Expression Kinetics. All plasmids were derivatives of plasmid pTD4 (5), in which transcription of *galK* is driven by P_C. For the assay of the time course of P_C activity, the *ara* region of pTD4 was fused to the *lacZ* gene of pDL3 and cells were grown at 35°C for at least four generations. Aliquots of 10 μl of cells were taken every 2 min for β-galactosidase assays, and 1-ml aliquots were taken every 12 min for measurement of cell density.

RESULTS

Deletions Identify $araO_1$ as Aiding $araO_2$ Occupancy. To localize the source(s) of the cooperativity that assists binding of AraC protein to $araO_2$, we removed progressively larger amounts of the *ara* regulatory region from the *araI* end (Fig. 1). The results showed that either $araO_1$ or *araI* could provide the interactions necessary for AraC protein to occupy $araO_2$; that is, looping could occur from $araO_2$ to $araO_1$ or from $araO_2$ to *araI*.

First we shall discuss the results obtained in the absence of arabinose. Previous *in vitro* and *in vivo* experiments showed that the increase in the methylation rates of a guanine by dimethyl sulfate in AraC binding sites is a sensitive indication of the occupancy of the sites by AraC protein (5, 7). Although the precise relationship between the occupancy and increase in methylation rate is not known, it seems likely to be linear. Deletions of the *araI* or *araI*–CRP sites had only small effects on the apparent occupancy of $araO_2$ (Fig. 2*A*). This shows that neither the *araI* nor the CRP binding site is solely required for the cooperativity that gives AraC binding at $araO_2$ (looping). A still larger deletion, one removing $araO_1$ in addition to the CRP site and *araI*, almost completely eliminated binding to $araO_2$. This result shows that the looping present in the *araI* and *araI*–CRP site deletion strains involves $araO_1$.

In the presence of arabinose, the apparent occupancy of $araO_2$ was reduced by a factor of 2–3 on the wild-type plasmid. Upon deletion of *araI*, occupancy of $araO_2$ was further reduced. The occupancy of $araO_2$ was still further reduced upon deleting *araI* and the CRP binding site and was eliminated when *araI*, the CRP site, and $araO_1$ were all

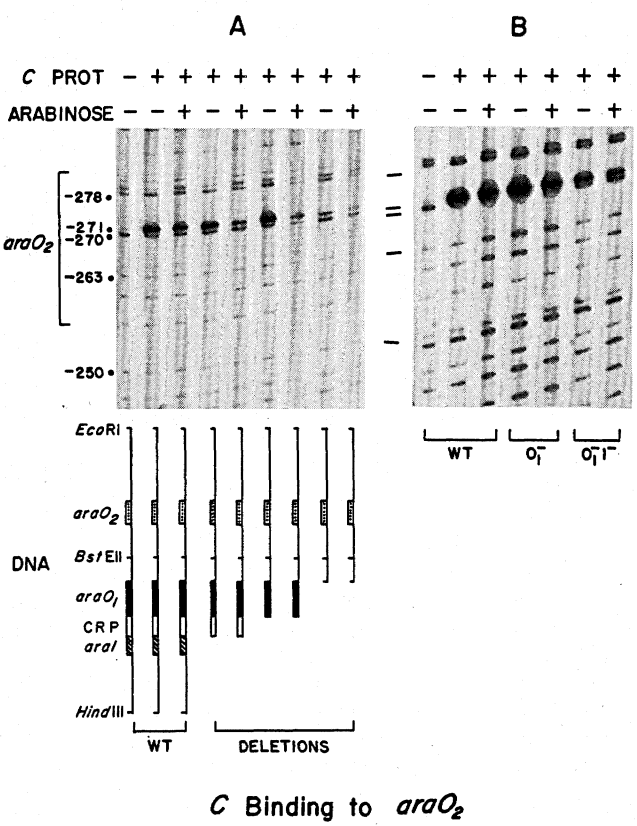

FIG. 2. *In vivo* dimethyl sulfate footprinting (7) of AraC (*C* PROT) binding to the $araO_2$ region in deletion and point mutation plasmids in the presence or absence of arabinose as indicated. (*A*) Below the autoradiogram, the *ara* regulatory region in the various deletion plasmids is shown to scale. Numbers at left indicate nucleotide positions relative to the *araBAD* transcription start site. WT, wild-type-like *ara* regulatory DNA ES51. (*B*) WT, wild-type *ara* regulatory DNA; O_1^-, DNA with the $araO_1^-$ point mutation (LH7), a G→T change at position −110; $O_1^-I^-$, LH6 DNA, which has the $araO_1^-$ mutation combined with the $araI^-$ mutation BRS33 [an A→T change at position −57 (25)].

deleted. These results provide evidence for the existence of both the $araO_2$–*araI* loop and the $araO_2$–$araO_1$ loop.

Point Mutations Identify *araI* as Aiding $araO_2$ Occupancy. The experiments described above clearly identified $araO_1$ as a source of the interactions that enable $araO_2$ to be occupied by AraC protein. Those experiments less clearly identified the expected site, *araI*, also as a source of these interactions. To strengthen the evidence for looping between $araO_2$ and *araI*, we mutated $araO_1$ to reduce AraC binding there and then compared the occupancy of $araO_2$ in $araO_1^-araI^-$ and $araO_1^-araI^+$ strains. The difference in $araO_2$ occupancy between these two strains will then correspond to the binding-site cooperativity contributed by only *araI*.

The strain with the mutant $araO_1$ site, LH7, showed no reduction in looping as inferred from $araO_2$ occupancy (Fig. 2*B*). Presumably *araI* provided the cooperative interactions necessary for $araO_2$ occupancy. When a mutant *araI*, BRS33, was introduced into the strain, we observed significant reduction in $araO_2$ occupancy. Thus, this set of experiments showed that looping can also occur between $araO_2$ and *araI*.

The results shown in Fig. 2 indicate that, in appropriate deletion or point mutation strains, looping occurs from $araO_2$ to either $araO_1$ or *araI* in the presence or absence of arabinose. The decrease by a factor of 2–3 in the extent of the altered methylation pattern at $araO_2$ in the presence of arabinose likely results from reduction of looping and is discussed later. By themselves, these results do not indicate which loop might normally predominate in the presence or absence of arabinose, but they show that when formation of one loop is prevented, the remaining loop can form in the presence or absence of arabinose.

$araO_1$ Indirectly Affects Regulation of P_{BAD}. We examined P_{BAD} activity after damaging $araO_1$. This site is upstream from all the sequences required for activity of P_{BAD} in the absence of the $araO_2$ site. That is, deletions entering from upstream do not reduce transcription from P_{BAD} until they reach about 20 bp past $araO_1$ (26). However, reducing AraC protein binding to $araO_1$ while retaining the presence of $araO_2$ reduced the activity of P_{BAD}. We damaged $araO_1$ in two ways: (*i*) by introducing the double mutation previously mentioned that reduces the apparent *in vivo* occupancy of $araO_1$ by a factor of 3 and (*ii*) by deleting $araO_1$. The resulting activities of P_{BAD} were decreased (Table 1).

Under both noninducing and inducing conditions the effect of the $araO_1$ deletion was greatest when $araO_2$ and *araI* were correctly oriented with respect to each other around the DNA helix (data not shown).

***araI* Indirectly Affects Regulation of P_C.** It is reasonable that AraC protein should regulate its own synthesis by an autorepression scheme, and a plausible mechanism for achieving this is available since $araO_1$ overlaps P_C. Less obvious either in a mechanistic or in a physiological role is the fact that for about 10 min after the addition of arabinose to cells, P_C activity is about 5-fold higher than normal (27, 28). After this transient period of hyperactivity, the promoter activity falls to within a factor of 2 of the value it had before the addition of arabinose.

To test whether P_C, like P_{BAD}, might be affected by the *ara* loops, we altered looping in the *ara* regulatory system without directly affecting the P_C promoter or sequences transcribed from it. This was done by deleting *araI* so that only the $araO_2$–$araO_1$ loop should be able to form. Not only was the transient hyperactivity eliminated, it was reversed and the activity of P_C decreased after arabinose addition (Fig. 3).

To explore more carefully the transient hyperactivity of P_C, we measured apparent $araO_1$ and $araO_2$ occupancy during the period following arabinose addition. Fig. 4 shows that $araO_1$ was poorly occupied before addition of arabinose and that after arabinose addition its occupancy increased over a period of about 10 min. After arabinose addition, apparent $araO_2$ occupancy dropped quickly to about one-quarter of its pre-arabinose value, and then over about 10 min $araO_2$ apparent occupancy rose to about half its pre-arabinose value.

Table 1. Activity of P_{BAD}–*galK* or P_{BAD}–*lacZ* fusions: Damaging $araO_1$ impairs P_{BAD} inducibility

Strain	GalK units per cell		LacZ units $\times 10^{-4}$
	− Arabinose	+ Arabinose	(+ arabinose)
Wild type	1.5 ± 0.2	200 ± 15	2.5 ± 0.4
$araO_1$ deletion	0.8 ± 0.2	70 ± 10	1.3 ± 0.2
$araO_1$ mutation	—	—	1.7 ± 0.3

The cells used were the AraC$^+$ strain SH322, which is also GalK$^-$ and LacZ$^-$. The $araO_1$ deletion mutant was KM270 and the $araO_1$ point mutant was LH7.

DISCUSSION

Summary and Model. Four basic experimental results are presented in this paper. (*i*) Both the *araI* site and the $araO_1$ site potentiate binding by AraC protein at the much weaker AraC protein binding site, $araO_2$. (*ii*) Damaging $araO_1$, a site not in P_{BAD}, lowers the uninduced and induced levels of P_{BAD} activity. (*iii*) Damaging *araI*, which lies well upstream of P_C, eliminates the 10-min period of P_C hyperactivity that normally follows arabinose addition. (*iv*) After arabinose addition, apparent $araO_1$ occupancy by AraC increases over a 10-min interval during which apparent occupancy of $araO_2$ rapidly decreases and then increases again.

In light of the complexity of the *ara* system, we have found it most efficient first to present a model for *ara* regulation that is suggested by the data presented here and previously. Although this model undoubtedly is an oversimplification and is not fully proven, it serves as a convenient summary of much experimental data.

In the absence of arabinose, most copies of the *ara* regulatory region contain a loop between $araO_2$ and *araI* mediated by AraC protein bound to both of these sites (Fig. 5). This loop prevents AraC protein bound to *araI* from

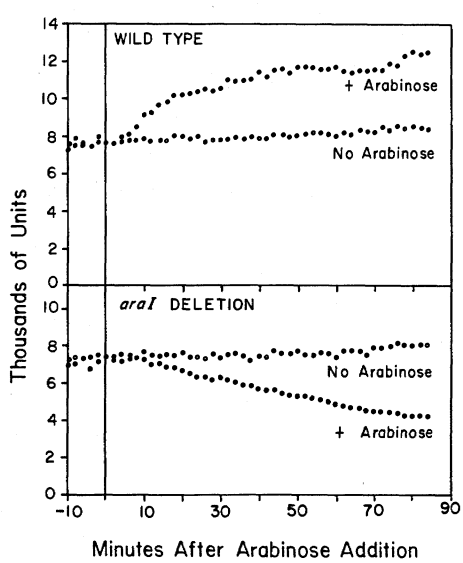

FIG. 3. Activity of P_C in P_C–*lacZ* fusion strains after addition of arabinose to *araI*$^+$ (wild-type) and *araI*-deleted cells. The plasmids were ES51 and ES51 deleted from the *Bam*HI site (located at −47) to the *Nhe* I site (located at −81).

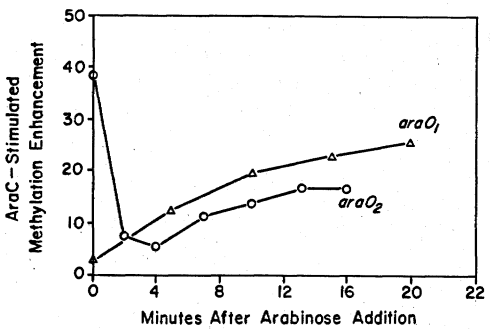

FIG. 4. AraC-stimulated methylation enhancement of $araO_1$ and $araO_2$ after addition of arabinose to the wild-type cells as measured by *in vivo* footprinting. The AraC$^+$ and AraC$^-$ strains were SH322 and SH321. The plasmid pLH2, which has the M13 origin inserted at the *Nde* I site of pTD3, was used for the footprinting. For quantitation of the enhancement in $araO_1$, the −113 band on the autoradiogram of the *in vivo* footprinting was scanned with an ISCO gel scanner (UV-5 series model 1312) and normalized by using the −74 band as a reference. For $araO_2$ methylation, the −271 band was scanned and normalized to the −250 band. One unit of the arbitrary scale of methylation enhancement was taken to be the methylation rate of the guanine in the AraC$^-$ strain. Therefore, the intensity of the band corresponding to the guanine in the AraC$^-$ strain was subtracted from the intensity of the band from the AraC$^+$ strain.

occasionally entering its inducing state, and hence it holds the uninduced level of P_{BAD} low. The loop may be small enough to reduce access both of AraC to $araO_1$ and of RNA polymerase to P_C, both of which are located within the loop. Under these conditions, $araO_1$ is poorly occupied and the promoter overlapping $araO_1$ is relatively inactive. Upon the addition of arabinose and its binding to AraC protein, the $araO_2$–*araI* loop opens. Once the loop is opened, most of the AraC protein molecules bound at the low-affinity $araO_2$ site are released, while most of the AraC molecules bound at *araI* remain in place. The combination of the absence of looping and the presence of bound arabinose drives AraC protein molecules bound at *araI* into their inducing conformation, and P_{BAD} is induced. Without the steric constraint generated by the $araO_2$–*araI* loop, RNA polymerase has increased access to the *araC* promoter P_C, and the activity of this promoter sharply increases. About 10 min later, most $araO_1$ sites have become occupied by AraC. The occupancy of $araO_1$ also leads to the formation of a DNA loop involving the $araO_1$ and $araO_2$ sites on about half of the copies of *ara* DNA.

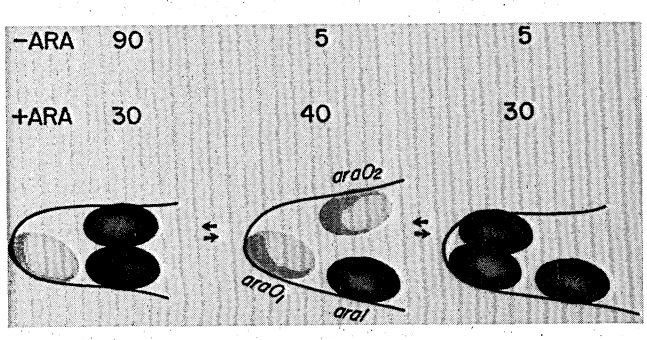

FIG. 5. The three major looping states of the *ara* system. Ellipses represent AraC protein molecules. Numbers are our estimates of the fraction (%) of the DNA molecules present in each of the three states before (−ARA) or long after (+ARA) the addition of arabinose. Left-hand state represents the molecules possessing a loop between $araO_2$ and *araI*, in which case *araBAD* is assumed to be uninducible. AraC protein bound to *araI* in the middle, unlooped, state and the alternative looped state on the right is free to flicker into its inducing conformation or to be driven by arabinose into its inducing conformation. Proteins depicted in grey were shown by footprinting to be present at appreciably lower amounts than those depicted in black.

This loop or AraC occupancy of either $araO_1$ or $araO_2$ represses P_C.

Discussion of the Data. We have used *in vivo* footprinting techniques to show that the binding of the AraC regulatory protein to its upstream operator site, $araO_2$, requires cooperative interactions that can be provided by either of two other AraC binding sites, $araO_1$ or *araI*, implying the existence of two *ara* loops.

One of the *ara* loops, that involving AraC bound at $araO_2$ and *araI*, was previously shown to generate repression of *araBAD* (5–7). This repression occurs in the absence of the inducer arabinose and reduces the level of *araBAD* transcription by a factor of about 8. Apparently, this loop prevents or substantially inhibits AraC bound at the induction site *araI* from entering its inducing state.

The second *ara* loop, that involving AraC bound at $araO_2$ and $araO_1$, was previously unknown. The existence of this second loop suggests a regulatory scheme in which one loop generates *araBAD* repression and the other loop prevents formation of the first loop, thereby assisting induction. Therefore, we tested whether removing the $araO_1$ site affects *araBAD* expression. In support of the idea, our results show that damaging $araO_1$ reduces both the induced and the uninduced expression of P_{BAD}, suggesting that the change increases the amount of the repression loop under noninducing and inducing conditions.

The addition of arabinose to cells initiates a 10-min period of hyperactivity of the *araC* promoter (27). This previously unexplained phenomenon can be understood in light of the alternative-looping scheme and the footprinting data presented here. The rapid loss of $araO_2$ occupancy upon addition of arabinose indicates that the repression loop opens. This would generate a temporary overpopulation of the unlooped state in which $araO_1$ would be largely unoccupied and in which RNA polymerase should have free access to P_C. During this period, P_C would be hyperactive and $araO_2$ would be unoccupied, presumably due to the absence of looping. Then, after about 10 min, when we observe $araO_1$ and $araO_2$ to become occupied, indicating formation of the $araO_2$–*araI* loop, the hyperactivity of P_C should, and does, cease. Deleting *araI* has the predicted effect on the transient hyperactivity of P_C. In the absence of *araI*, there should be no transient overpopulation of the unlooped state and hence no transient hyperactivity, and indeed, we saw none.

It is of interest to estimate quantitatively the amounts of the repression loop and of the second, "antirepression" loop present during noninducing and inducing conditions. The approximate levels of the two types of loops can be calculated from the available data on the activity of P_{BAD}. The results of such calculations (Table 2) show that, subject to the simplifying assumptions mentioned in the legend, arabinose addition decreases the fraction of molecules containing the $araO_2$–*araI* repression loop from 90% to 33% and increases the fraction of molecules containing the $araO_2$–*araI* antirepression loop from 5% to about 40% (Fig. 5).

Additional Considerations. Two types of looping are formally possible amongst *araI*, $araO_1$, and $araO_2$. One is a double looped structure simultaneously involving several dimers of AraC protein and all three DNA sites, and the other is the mutually exclusive situation in which only one loop may exist at any one time. If a complex involving all three DNA sites formed, then eliminating one of the sites would seem likely to weaken the structure and lessen the probability that the remainder of the looped structure would form. On the other hand, with alternative looping, eliminating one loop form would increase the amount of the other loop form. Since we observed the latter for either loop, we favor models based on alternative looping as we have described above.

We might worry that forming the 140-bp $araO_2$–$araO_1$ loop would be energetically difficult. Although unstrained circles

Table 2. Inducibility and looping frequencies

Strain	Relative P_{BAD} activity	Frequency		
		$araO_2$–*araI* loop	$araO_2$–$araO_1$ loop	Unlooped state
Wild type				
Uninduced	1	0.90	0.05	0.05
Induced	100	0.33	0.37	0.3
$araO_2$ deletion				
Uninduced	10	–	–	1.00
Induced	150	–	–	1.00
$araO_1$ deletion				
Uninduced	0.5	0.95	–	0.05
Induced	70	0.53	–	0.47

The input data are the set of numbers of P_{BAD} activity. These are the typical activities of P_{BAD} reported previously (6) except for the induced activity when $araO_2$ is deleted, for which its value was found to be 150 (ref. 29 and Dong-Hee Lee and Rob Lobell, personal communication). Entries for the looped or unlooped states were calculated by building ("bootstrapping") from the activities of the promoter with the assumptions that P_{BAD} is inactive on molecules containing an $araO_2$–*araI* loop and that the partitioning of the molecules among the various states is governed by equilibrium constants that are affected by the presence of arabinose. For example, the amount of the $araO_2$–*araI* loop in uninduced wild-type cells was calculated to be 0.9 from the fact that deletion of $araO_2$ increases the uninduced level by a factor of 10. The estimate that the remaining 0.1 of the molecules are evenly split between the $araO_2$–$araO_1$ loop and the unlooped state derives from the factor of 2 reduction in P_{BAD} activity upon deletion of $araO_1$. Two decimal places of precision are presented to assist the reader in reproducing the calculations, not because we believe such precision is experimentally significant.

as small as 120 bp can be formed with DNA containing bent sequences (30), the *ara* appears not to contain such sequences. Of course, if the protein were flexible, then a significant portion of the 360° bending around a loop could be provided by the protein, and the DNA would need to bend less and less as the loop size decreased. Similar flexibility in the protein may help explain the small (52-bp) loop size that has been observed with λ phage repressor (13).

The activity of P_C is repressed both before the addition of arabinose and more than 10 min after the addition of arabinose. This repression could come from three sources: (*i*) direct competition between binding of AraC to $araO_1$ and binding RNA polymerase to P_C, since $araO_1$ and P_C overlap; (*ii*) RNA polymerase binding at P_C being hindered by the $araO_2$–*araI* loop; and (*iii*) AraC bound at $araO_2$ blocking elongation by RNA polymerase. The $araO_1$ site overlaps the RNA polymerase binding site for P_C, and one set of experiments showed that binding to these two sites was mutually exclusive (9). RNA polymerase binding at P_C, which lies in the middle of the $araO_2$–*araI* 210-bp loop, should be sterically hindered by the existence of the loop. In an analogous situation DNase could not easily cleave phosphodiester bonds on the inside face of DNA circles of dimensions similar to those of the loop (31).

Finally, we note that the addition of arabinose shifts the loops from *araI* toward $araO_1$. That is, AraC protein bound to these two sites behaves differently. Although having the DNA sequence to which a protein is bound act as an allosteric effector of the protein is not a familiar concept in biochemistry, AraC protein does in fact change its ability to repress P_{BAD} when bound to a mutant *araI* site (7).

We thank Sharon Jenkins for constructing ES51; Dong Hee Lee for his gift of pDL3; and Pieter Wensink, Alan Brunelle, Jim Tobin, John Carra, Robert Lobell, Dong Hee Lee, and Ranjan Sen for discussions and comments on the manuscript. This work was supported by grants from the National Science Foundation and the National Institutes of Health.

1. Sheppard, D. & Englesberg, E. (1967) *J. Mol. Biol.* **25,** 443–454.
2. Stoner, C. & Schleif, R. (1983) *J. Mol. Biol.* **171,** 369–381.
3. Kolodrubetz, D. & Schleif, R. (1981) *J. Mol. Biol.* **151,** 215–227.
4. Schleif, R. (1987) in *Escherichia coli and Salmonella Typhimurium, Cellular and Molecular Biology*, ed. Neidhardt, F. (Am. Soc. Microbiol., Washington, DC).
5. Dunn, T., Hahn, S., Ogden, S. & Schleif, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5017–5020.
6. Hahn, S., Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180,** 61–72.
7. Martin, K., Huo, L. & Schleif, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3654–3658.
8. Casadaban, M. (1976) *J. Mol. Biol.* **104,** 557–566.
9. Hendrickson, W. & Schleif, R. (1984) *J. Mol. Biol.* **178,** 611–628.
10. Hahn, S., Hendrickson, W. & Schleif, R. F. (1986) *J. Mol. Biol.* **188,** 355–367.
11. Buck, M., Miller, S., Drummond, M. & Dixon, R. (1986) *Nature (London)* **320,** 374–378.
12. Mossing, M. & Record, T., Jr. (1986) *Science* **233,** 889–892.
13. Hochschild, A. & Ptashne, M. (1986) *Cell* **44,** 681–687.
14. Krämer, H., Niemöller, M., Amouyal, M., Revet, B., von Wilcken-Bergmann, B. & Müller-Hill, B. (1987) *EMBO J.* **6,** 1481–1491.
15. Borowiec, J. A., Zhang, L., Sasse-Dwight, S. & Gralla, J. D. (1987) *J. Mol. Biol.* **196,** 101–111.
16. Griffith, J., Hochschild, A. & Ptashne, M. (1986) *Nature (London)* **322,** 750–752.
17. Majumdar, A. & Adhya, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6100–6104.
18. Dandanell, G. & Hammer, K. (1985) *EMBO J.* **4,** 3333–3338.
19. Dandanell, G., Valentin-Hansen, P., Løve Larsen, J. E. & Hammer, K. (1987) *Nature (London)* **325,** 823–826.
20. Théveny, B., Bailly, A., Rauch, C., Rauch, M., Delain, E. & Milgrom, E. (1987) *Nature (London)* **329,** 79–81.
21. Whitson, P., Hsieh, W., Wells, R. & Matthens, K. (1987) *J. Biol. Chem.* **262,** 4943–4946.
22. Mukherjee, S., Erickson, H. & Bastia, D. (1988) *Cell* **52,** 375–383.
23. Schleif, R. F. & Wensink, P. (1981) *Practical Methods in Molecular Biology* (Springer, New York).
24. Miller, J. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 353–355.
25. Smith, B. R. (1978) Dissertation (Brandeis Univ., Waltham, MA).
26. Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180,** 201–204.
27. Hahn, S. & Schleif, R. (1983) *J. Bacteriol.* **155,** 593–600.
28. Ogden, S., Haggerty, D., Stoner, C., Kolodrubetz, D. & Schleif, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3346–3350.
29. Lichenstein, H., Hamilton, E. & Lee, N. (1987) *J. Bacteriol.* **169,** 811–822.
30. Ulanovsky, L., Bodner, M., Trifonov, E. & Chodner, M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 862–866.
31. Drew, H. & Travers, A. (1985) *J. Mol. Biol.* **186,** 773–790.