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The DNA loop model for *ara* repression: AraC protein occupies the proposed loop sites *in vivo* and repression-negative mutations lie in these same sites

(DNA loops/distal regulatory elements/repression/induction/*in vivo* footprinting)

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ABSTRACT **Two sets of experiments have been performed to test the DNA loop model of repression of the *araBAD* operon of *Escherichia coli*. First, dimethyl sulfate methylation protection measurements on normally growing cells show that the AraC regulatory protein occupies the *araI* site in the presence and absence of the inducer arabinose. Similarly, the $araO_2$ site is shown to be occupied by AraC protein in the presence and absence of arabinose; however, its occupancy by AraC is greatly reduced when *araI* and adjacent sequences are deleted. Thus, AraC protein binds to $araO_2$ cooperatively with some other component of the *ara* system located at least 60 base pairs away. Second, the mutational analysis presented here shows that the DNA components required for repression of *araBAD* are *araI*, $araO_2$, and perhaps the *araBAD* operon RNA polymerase binding site.**

The L-arabinose operon of *Escherichia coli* has long been known to be positively regulated by AraC protein (1). Classical genetic (2–4) and biochemical (5–7) studies have also revealed that the *araBAD* genes are negatively regulated by AraC and that a site required for this repression is located upstream from all the sites required for induction (2, 8, 10).

Recently, a site required for repression, $araO_2$, was found and was determined to lie 210 base pairs upstream from the AraC binding site required for induction, *araI* (Fig. 1) (8). Surprisingly, the behaviors of strains with small deletions and insertions between the *araBAD* induction region and the $araO_2$ site suggest that repression of the *araBAD* operon involves the formation of a DNA loop that brings $araO_2$ near the induction region (8). Since repression of a constitutive *araBAD* promoter requires functional $araO_2$ and *araI* sites as well as the presence of AraC protein (7), the proposed loop likely involves AraC protein bound to $araO_2$ and *araI*.

Formation of a DNA loop by proteins bound at distal sites represents an undocumented, yet potentially versatile, mechanism of genetic regulation. Therefore, we have tested two critical predictions of the DNA loop model of *ara* repression. First, since the *araI* site appears to be involved in both repression and induction (7, 9), AraC protein should occupy the *araI* site in normally growing cells under repressing and inducing conditions. Second, mutations that are isolated solely on the basis that repression of *araBAD* is reduced ought to lie in $araO_2$ and *araI*.

We have used *in vivo* dimethyl sulfate footprinting techniques to address the first question. The results of these experiments show that the *araI* site is occupied in the presence and absence of arabinose. *araI* site occupancy in the presence of arabinose is as indicated by studies of induction (9), while its occupancy without arabinose is as required by the DNA loop model for repression.

In vivo footprinting experiments also show that AraC protein binds to the upstream operator, $araO_2$, both in the presence and absence of arabinose. However, no binding is seen at $araO_2$ if the *araI* site and adjacent sequences are deleted. This cooperativity strongly suggests direct interaction via a DNA loop between AraC protein at $araO_2$ and some other component(s) of the *araCBAD* regulatory region.

The results of genetic experiments were also as predicted by the DNA loop model. Mutations isolated solely on the basis that they reduced repression were found to lie in the $araO_2$ and *araI* sites. However, repression-defective mutations also were found in the *araBAD* operon RNA polymerase binding site, raising the possibility of the involvement of RNA polymerase in repression. Mutations constructed in other known protein binding sites in the regulatory region did not interfere with repression. The results of these standard genetic experiments suggest that only the *araI*, $araO_2$, and possibly RNA polymerase binding sites are involved in repression. These results strengthen previous results obtained by using "inverse genetics" in which regions of DNA are intentionally mutated and the results observed.

MATERIALS AND METHODS

Media, Plasmids, Strains, and General Methods. Media and general methods were as described (11, 12). Plasmid pTD3 (9) contains 440 base pairs of the *araCBAD* regulatory region on a *Hin*dIII/*Eco*RI fragment with P_{BAD} driving *galK* of the pKO1 vector (13). The plasmid with the *araCBAD* induction region deleted and used for *in vivo* footprinting of $araO_2$, pLH1, was made by deleting from *Bst*EII (position −203) to *Bam*HI (position −46) of pTD3. $AraO_2$ deletions of plasmids with *araI* mutations were constructed by deleting from *Eco*RI to *Bst*EII. The isogenic $AraC^-$ strain SH321 and $AraC^+$ strain SH322 used for *in vivo* footprinting and galactokinase assays were provided by S. Hahn (7), as was the $RecA^-$ strain SH317 (F^- Thr^- Leu^- Dcm^- His^- $GalK^-$ Str^r Tn*10*:Srl $RecA^-$ Tet^r) used for isolation of repression defective mutations.

***In Vivo* Footprinting.** Cells were grown exponentially at 37°C in 600 ml of M10 minimal medium containing appropriate sugar and ampicillin to a cell density of 2×10^8 cells per ml. Addition of 1.2 ml of 100% dimethyl sulfate was followed by shaking the culture vigorously at 37°C for 30 sec. The reaction was stopped by adding 300 g of ice immediately followed by 100 ml of 0.27 M EDTA (pH 8.0). This addition prevents contamination of plasmid DNA by chromosomal DNA at later steps. In initial experiments, the plasmid was CsCl purified (11), but in later experiments the extraction was scaled down by a factor of 10 and the supernatant, after lysozyme digestion and centrifugation at 38,000 × *g* for 60

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

min, was extracted with water-saturated phenol to remove proteins, digested with RNase A, then passed through a 1 × 5 cm Bio-Rad A-50m column to separate RNA from DNA. All steps before phenol extraction were done at 4°C. The fractions containing DNA were pooled and DNA was precipitated with ethanol.

The plasmid DNA was then digested with an appropriate restriction enzyme, either *Hin*dIII or *Bst*EII, which cut at positions +40 and −203, respectively. The 5′ termini were labeled with [^{32}P]ATP, then a second restriction enzyme was used to produce a fragment 200 to 500 base pairs long. The labeled fragment was isolated and then cleaved at methylated bases using the piperidine reaction (14) and analyzed on a sequencing gel.

Isolation and Analysis of Repression-Defective Mutations. Plasmid pTD3 (9) was mutagenized with hydroxylamine (15). Half of the mutagenized control region, containing either the *$araO_2$* site or the *araI*, *$araO_1$*, cAMP receptor protein (CRP), and RNA polymerase binding sites, was isolated and inserted into unmutagenized pTD3 DNA containing the other half of the control region. Colonies containing plasmids with mutations that interfere with repression give increased levels of *galK* transcription from P_{BAD} under repressing conditions (absence of arabinose) and these turn red on MacConkey galactose indicator plates. Wild-type strains, which repress the synthesis of galactokinase, yield white colonies. Plasmids isolated from red colonies were sequenced by the method of Sanger *et al.* (16), using either of two synthetic oligonucleotides provided by Eli Lilly and Co., across the entire mutagenized control region.

Galactokinase assays were performed as described (7). AraC protein was prepared and gel electrophoresis DNA binding assays were done as described (17) using either a 140-base-pair *Aha* II to *Hin*dIII fragment containing the *araI* site or a 200-base-pair *Eco*RI to *Bst*EII fragment with the *$araO_2$* site.

Construction of CRP Site and *$araO_1$*/*araC* Gene Promoter (P_C) RNA Polymerase Binding Site Mutations. The point mutation located within the CRP binding site, KM301, was isolated from pTD3 plasmid DNA containing a hydroxylamine-mutagenized induction region (positions −203 to +40). The mutation, a G·C to A·T change at position −98, was obtained by scoring for an uninducible, thus white, colony on a MacConkey indicator plate containing arabinose and galactose. A deletion of 27 base pairs of *ara* DNA within the overlapping *$araO_1$* and P_C RNA polymerase binding sites (positions −120 to −146) (Fig. 1) was constructed by fusing the filled-out *Eco*RI linker DNA of deletion plasmid pTD383 (9), in which DNA upstream of position −119 has been deleted, with the filled-out *Mlu* I site located at position −146 of pTD3. The filled-out *Eco*RI linker adds 6 base pairs at the site of the deletion. The remaining net deletion of 21 base pairs between *araI* and *$araO_2$* was partially compensated for by using pDL1, provided by Dong-Hee Lee, which contains 17 base pairs of DNA inserted at the *Bst*EII site (position −203). The spacing variants were constructed by various manipulations of the *Bgl* II site contained in the 17-base-pair insertion of pDL1.

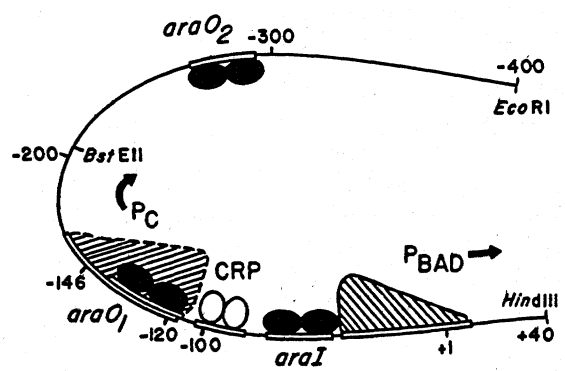

FIG. 1. Protein-binding sites of the L-arabinose *araCBAD* regulatory region, drawn to scale. Numbering of base pairs is relative to P_{BAD} transcription start site at +1. Hatched, RNA polymerase; solid, AraC protein; open, CRP–cAMP.

RESULTS

AraC Protein Occupies the *araI* and *$araO_2$* Sites *in Vivo*. Previous experiments with purified components have shown that the binding of AraC protein decreases the dimethyl sulfate methylation rate of one guanine and increases the methylation rate of an adjacent guanine residue within the *araI* and *$araO_2$* sites (8, 18, 19). These guanines with altered methylation rates are located in the center of the central major groove of the AraC binding site consensus (20) (positions −59 and −60 in *araI* and positions −270 and −271 in *$araO_2$*; see Fig. 3). We used this property to probe *in vivo* whether AraC protein is bound to these sites under normal growth conditions. After brief dimethyl sulfate treatment of growing cells carrying a plasmid containing the *araCBAD* regulatory region, the plasmid was partially purified and the methylation states of bases in the *ara* regulatory region were analyzed.

Fig. 2*A* shows the extent of methylation of bases within the *araI* site in an isogenic pair of strains—one lacking AraC protein, and one containing AraC protein. Since the pattern of protection and enhancement in the AraC$^+$ strain is the same as is seen *in vitro* with purified components, we conclude that it is AraC protein itself that is generating the altered methylation rates and that the binding of the protein to these sites can be monitored *in vivo* by this method.

Fig. 2*A* shows that the *araI* site is occupied *in vivo* in the presence and absence of arabinose. The *$araO_2$* site similarly is occupied in the presence and absence of arabinose (Fig. 2*B*). However, *$araO_2$* shows a decrease by a factor of 2 in

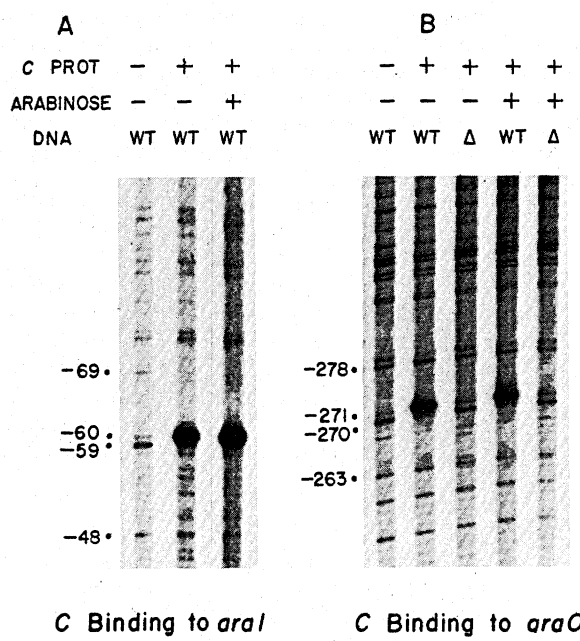

FIG. 2. Dimethyl sulfate methylation patterns of *ara* DNA in normally growing cells with (+) and without (−) arabinose and AraC protein (*C* PROT) as indicated. (*A*) DNA containing the *araI* site and labeled at position +40. (*B*) DNA containing the *$araO_2$* site and labeled at position −203. WT, wild-type *ara* DNA; Δ, pLH1 with DNA deleted from position −46 to −203 so that the only *ara* binding site remaining is *$araO_2$*.

methylation rate of the guanine at position −271 in the presence of arabinose, implying that either the site is less occupied by AraC protein in the presence of arabinose or that methylation rates of $araO_2$ are altered by a conformational change in the protein because of the presence of arabinose.

AraC Protein Binds to $araO_2$ Cooperatively with Some Other Component. If a DNA loop mediated by AraC protein does indeed form between the $araO_2$ and *araI* sites, then the binding to these sites could well be cooperative. A cooperativity consistent with this model is shown in Fig. 2*B*, although the required component(s) in the induction region of the proposed loop has not been determined. The results show that AraC protein is not appreciably bound to $araO_2$ *in vivo* under normal growth conditions if the DNA of the induction region of *araCBAD* including *araI*, the CRP binding site, $araO_1$, and the P_C RNA polymerase binding site, is deleted.

Repression Defective Mutations Lie in *araI*, $araO_2$, and the P_{BAD} RNA Polymerase Binding Site. Mutations that result in increased transcription from P_{BAD} in the absence of arabinose were obtained by screening after hydroxylamine mutagenesis of the P_{BAD}–GalK fusion plasmid pTD3. Of 110 candidates isolated and sequenced, a minimum of 33 independent mutations were located in 11 different positions. All mutations were G·C to A·T base-pair changes as is generated by cytosine deamination. The 11 different base-pair changes included both constitutive mutations, which express increased levels of galactokinase even in the absence of AraC protein, and nonconstitutive mutations, which, like wild-type P_{BAD}, require AraC protein for induction. The constitutive mutations were found to lie within the *araBAD* RNA polymerase binding site, or, in one case, to create a new promoter consensus sequence (21) elsewhere in the control region. The nonconstitutive mutations were found to lie in the *araBAD* RNA polymerase binding site and two AraC protein binding sites, *araI* and $araO_2$.

The two different mutations located in the $araO_2$ site, KM74 and KM166, are shown in Fig. 3 below the consensus sequence derived from six AraC protein binding sites (20). Interestingly, the two sites of mutation in $araO_2$ are the only consensus bases of $araO_2$ that are susceptible to hydroxylamine mutagenesis. These mutations result in ≈4-fold higher levels of uninduced transcription from P_{BAD} (Table 1). P_{BAD} expression of these mutants is normally inducible by arabinose. Equilibrium gel electrophoresis DNA binding assays performed at 100 mM KCl show that the affinity of AraC protein for $araO_2$ is reduced by a factor of 10–15 as a result of these mutations, from a K_d of $5.7 \pm 1.4 \times 10^{-10}$ M for wild-type to $8.0 \pm 2.8 \times 10^{-9}$ M for KM74 and $5 \pm 1 \times 10^{-9}$ M for KM166.

The two repression-defective mutations in *araI*, KM76 and KM82, are shown above the AraC binding site consensus in Fig. 3. The phenotypes of these mutations are also shown in Table 1. The mutations have no effect on levels of transcription observed in the absence of AraC protein or on fully induced transcription. However, in repressing conditions (the presence of AraC and the absence of arabinose) the

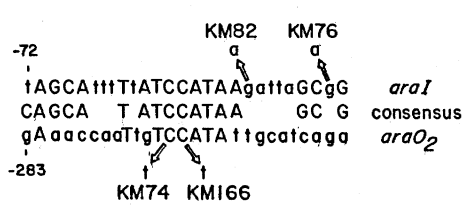

FIG. 3. Locations of repression-defective mutations in the *araI* and $araO_2$ sites relative to the conserved bases of six AraC protein binding sites (20). Uppercase letters, conserved bases. DNA sequences are written 5′ to 3′ and numbering is relative to P_{BAD} transcription start site +1.

Table 1. P_{BAD} expression of cells with repression defective mutations in *araI* or $araO_2$

Plasmid	AraC⁺ strain: Without arabinose	AraC⁺ strain: With arabinose	AraC⁻ strain
WT	1.5 ± 0.2	215 ± 30	2.0 ± 0.4
KM74	7.6 ± 0.3	173 ± 13	2.3 ± 0.5
KM166	6.1 ± 0.4	189 ± 11	2.1 ± 0.5
Δ$araO_2$	9.1 ± 1.5	200 ± 34	2.4 ± 0.6
KM76	4.6 ± 0.8	193 ± 22	2.6 ± 0.6
KM82	5.5 ± 1.0	220 ± 33	2.9 ± 0.3

Expression is reported as galactokinase units (nmol of galactose phosphorylated per min per ml of cells at OD_{550} of 1.0) measured in cells containing P_{BAD}–*galK* fusion plasmids with the indicated *ara* mutation. WT, wild-type *ara* DNA; KM74, C → T at position −271 in $araO_2$; KM166, C → T at position −270 in $araO_2$; Δ$araO_2$, pTD383 (9) wild-type *ara* DNA with sequences deleted upstream of position −119; KM76, G → A at position −47 in *araI*; KM82, G → A at position −54 in *araI*.

mutants' levels of transcription are ≈4-fold higher than wild-type levels.

The *araI* site mutants have repression-defective phenotypes. It is conceivable, however, that their elevated uninduced expression levels result from increased induction-like activity at *araI* rather than decreased repression activity from *araI*. An increased induction-like activity might have been revealed by expression levels greater than those of wild type after repression was eliminated by deletion of $araO_2$. This was not seen (data not shown), suggesting that the *araI* mutations predominantly interfere with repression.

Equilibrium binding assays show that the mutations alter the affinity of AraC protein for the *araI* site in the presence of arabinose or the anti-inducer fucose by <50%, not a surprising result since the mutations do not lie in conserved bases of *araI* (20).

The phenotypes of mutations within the RNA polymerase binding site that result in increased levels of transcription in the absence of arabinose are listed in Table 2. Three general phenotypes are apparent: (*i*) a repressible but uninducible constitutive mutation at position −38 (previously isolated and described, Cip-5) (7); (*ii*) inducible but nonrepressible constitutive mutations at positions −36, −12, and −10 (−10 mutation was previously isolated as a −35/−10 double mutation I^c/X^c) (22); and (*iii*) two nonconstitutive inducible, but repression defective, mutations at positions −19 and −15. Thus, although mutations in the RNA polymerase binding site include several complex phenotypes, one of these is identical to that observed for the repression-defective mutations in *araI* and $araO_2$.

Mutations in the CRP Site and $araO_1$/P_C RNA Polymerase Binding Site Do Not Interfere with Repression. We tested the possible effects on repression of mutations in the other sites

Table 2. P_{BAD} expression of cells with RNA polymerase binding site mutations

Location	AraC⁺ strain: Without arabinose	AraC⁺ strain: With arabinose	AraC⁻ strain
−38	14 ± 2	16 ± 5	56 ± 5
−36	40 ± 2	240 ± 50	17 ± 1
−19	6.0 ± 0.1	170 ± 10	2.5 ± 0.2
−15	4.3 ± 0.4	150 ± 20	1.4 ± 0.1
−12	23 ± 4	193 ± 50	24 ± 4
−10	286 ± 20	195 ± 10	130 ± 10
WT	1.5 ± 0.2	215 ± 30	2.0 ± 0.4

Expression is reported as galactokinase units. Locations of mutations are relative to *araBAD* transcription start site +1.

of the *araCBAD* regulatory region. A mutation of the $araO_1$ site that overlaps the P_C RNA polymerase binding site deletes 27 base pairs of *ara* DNA (positions −119 to −146) (Fig. 1). Six base pairs have been inserted at the deletion site and various amounts of DNA have been inserted upstream at the *Bst*EII site to regenerate the wild-type separation between $araO_2$ and the *araBAD* induction region as well as spacing variants of +2, −2, −3, −4, −7, and −14. The phenotypes of the resulting mutants show that in the absence of $araO_1$ and P_C, repression still occurs. Also, like wild-type *ara* DNA (8), the behavior of strains deleted of $araO_1$ and P_C with small net insertions and deletions between $araO_2$ and *araI* show that the magnitude of repression varies with the relative angular orientation around the DNA helix axis of $araO_2$ with respect to *araI*. The degree of repression, however, appears about 2-fold stronger in the absence of $araO_1$ and P_C: wild-type strains show 1.5 ± 0.2 galactokinase units, while strains containing the $araO_1$ and P_C RNA polymerase binding site deletion show 0.8 ± 0.2 galactokinase units.

A point mutation of the CRP binding site was obtained by screening for an uninducible colony after hydroxylamine mutagenesis of the induction region of *araBAD*. To measure repressibility of this mutant, it was fused to the repressible constitutive promoter mutation located at position −38 (Cip-5) (7). Galactokinase assays of transcription levels of the resulting double mutant show that the presence of AraC protein without arabinose causes a repression of the constitutive promoter by a factor of 4 (from 19.5 ± 3.5 to 4.5 ± 0.7 galactokinase units). This is the same factor by which AraC protein will repress this constitutive promoter when the wild-type CRP site is present (53 ± 3 to 14 ± 2 galactokinase units), although the CRP site is also seen to stimulate transcription 3-fold. Thus, an intact CRP binding site is not required for repression.

DISCUSSION

In this paper, we present the results of two types of experiments designed to test the DNA loop model (7, 8) for repression of the *araBAD* operon. According to this model, repression, which occurs in the absence of arabinose, is generated by the association of AraC protein bound at the *araI* site with AraC protein bound at a site 200 base pairs away, the $araO_2$ site. As a result, a loop is formed in the intervening DNA, and AraC protein at the *araI* site is held in its repressing conformation. As reported here, both *in vivo* dimethyl sulfate footprinting and genetic experiments support this model.

Induction of P_{BAD} has been shown to require AraC protein bound at *araI* (9), and the loop model implies that AraC protein must also bind at *araI* in the absence of induction. Thus, if the loop model is correct, AraC must be bound at *araI in vivo* under both inducing and repressing conditions. This is what we have found.

Additional evidence supporting the loop model for repression is that AraC protein does not produce an *in vivo* dimethyl sulfate footprint at $araO_2$ if the other binding sites in the *araCBAD* regulatory region—including *araI*, $araO_1$, the P_C RNA polymerase sites, and the CRP site—have been deleted. It is most likely that this absence of a footprint at $araO_2$ indicates that AraC protein is not bound there. This observation indicates an interaction that results in cooperative binding between AraC bound at $araO_2$ and some other component of the *araCBAD* regulatory region. We need to investigate the effects of smaller deletions and point mutations to define further this cooperativity.

The cooperative binding of AraC at $araO_2$ is seen both in the presence and absence of arabinose. This result was not predicted and suggests that the DNA loop may be present during induction as well as repression. We note a decrease by a factor of 2 in the degree of enhancement of the methylation rate of the guanine at position −271 of $araO_2$ when arabinose is present, although this is difficult to detect in the exposure presented. It is possible that this observed decrease in methylation rate reflects a decrease in the occupancy of $araO_2$ and extent of loop formation in the presence of arabinose, which is sufficient to allow induction of the operon. It is also possible that loop opening is not required for induction. The different methylation rates may reflect altered conformations of AraC within a loop that always exists.

The *in vivo* footprints of *araI* are also important independent of the loop model. *In vitro* experiments have found that the affinity of AraC protein for *araI* is significantly greater in the presence of arabinose than in the absence of arabinose (17–19). This fact led to a preliminary mechanism for induction of the operon—that is, the binding of arabinose to AraC protein permits the protein to bind *araI*, thereby activating transcription of the operon (18, 19). Despite its attractiveness, this model appears incorrect. The reason is that the estimated *in vivo* concentration of AraC protein (23) and the affinity of AraC protein for *araI* (17) suggest that AraC protein occupies *araI in vivo* in the presence and absence of arabinose. However, a direct demonstration of this is essential. Our *in vivo* footprints show that AraC is always bound at *araI*. Thus, induction of *araBAD* by arabinose results from a changed property of AraC protein bound at *araI* and not from modulation of *araI* site occupancy.

If the loop model for repression of *araBAD* is correct, mutations that reduce repression should be located in two sites: *araI* and $araO_2$. These mutations were found, as were repression negative mutations in the *araBAD* RNA polymerase binding site.

The repression defective mutations in $araO_2$ have properties consistent with previously characterized deletion mutations (8). The $araO_2$ mutations change consensus bases in the binding site, resulting in significantly reduced affinity of the mutated $araO_2$ site for AraC protein. These properties show that the presence of AraC protein at $araO_2$ is required for repression, but that it plays no necessary role in induction.

The two repression-defective mutations in the *araI* site do not affect induction or the affinity measured *in vitro* of AraC protein for the *araI* site. In the absence of data suggesting the involvement of any other proteins at the *araI* site, these results suggest that the conformation of the protein bound to *araI* under repressing conditions is altered by the mutations. That binding of a protein to a mutant DNA sequence could lead the protein to adopt a variant conformation is to be expected on basic principles but appears not to have been observed before.

In addition to the *araI* and $araO_2$ sites, mutations with repression-defective phenotypes were also found in the RNA polymerase binding site of P_{BAD}. Like the mutations in *araI* and $araO_2$, two of these promoter mutations increase the basal level of *araBAD* transcription only in the presence of AraC protein. These mutations raise the possibility that RNA polymerase is normally present in the proposed repression loop. However, in light of the simplicity of a model where repression is generated by a direct interaction between AraC protein molecules bound at $araO_2$ and *araI*, we prefer a mechanism in which the repression-negative mutations in the polymerase site would then alter the conformation or reaction rates of a RNA polymerase molecule that is only transiently present in the looped AraC·DNA repression complex.

In conclusion, the results we have presented in this paper strengthen the evidence for DNA looping in the *ara* system. We point out, however, that direct irrefutable evidence for the existence of the DNA loop does not yet exist. The formation of DNA loops *in vivo* would, however, be a versatile mechanism for both prokaryotic and eukaryotic gene regulation. Looping could well occur in the *gal* operon

of *E. coli* (24), and many of the properties of yeast upstream activation sites (25) and enhancers (26) and silencers (27) of metazoan cells could be explained by DNA loops that bring these elements near promoter regions. DNA loops not only facilitate bringing together multiple proteins at a site but also allow a large stretch of intervening DNA to participate in a regulatory mechanism. This intervening DNA could contain additional sites where different regulatory proteins could function in altering the potential for loop formation. Such a mechanism could explain some aspects of yeast *MATα2* gene repression (28) and the artificially created LexA protein repression of *Gal4* (29), as suggested by Brent (30). This may also be the mechanism by which CRP–cAMP breaks repression of *araBAD* in the presence of arabinose from a site removed from the promoter yet within the proposed DNA loop (7, 31).

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1. Sheppard, D. & Englesberg, E. (1967) *J. Mol. Biol.* **25,** 443–454.
2. Englesberg, E., Squires C. & Meronk, F. (1969) *Proc. Natl. Acad. Sci. USA* **62,** 1100–1107.
3. Gielow, L., Largen, M. & Englesberg, E. (1971) *Genetics* **69,** 289–302.
4. Schleif, R. & Lis, J. (1975) *J. Mol. Biol.* **95,** 417–431.
5. Greenblatt, J. & Schleif, R. (1971) *Nature (London) New Biol.* **233,** 166–170.
6. Wilcox, G., Meuris, P., Bass, R. & Englesberg, E. (1974) *J. Biol. Chem.* **249,** 2946–2952.
7. Hahn, S., Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180,** 61–72.
8. Dunn, T., Hahn, S., Ogden, S. & Schleif, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5017–5020.
9. Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180,** 201–204.
10. Wilcox, G., Al-Zarban, S., Cass, L. G., Clarke, P., Heffernan, L., Horwitz, A. H. & Miyada, C. G. (1982) in *Promoters: Structure and Function*, eds. Rodriquez, R. & Chamberlain, M. (Prager, New York), pp. 183–194.
11. Schleif, R. & Wensink, P. (1981) *Practical Methods in Molecular Biology* (Springer, New York).
12. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
13. McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C. & Rosenberg, M. (1981) in *Gene Amplification and Analysis*, eds. Chirikjian, J. & Papas, T. S. (Elsevier/North-Holland, Amsterdam), Vol. 2, pp. 383–415.
14. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
15. Busby, S., Irani, M. & deCrombrugghe, B. (1982) *J. Mol. Biol.* **154,** 197–209.
16. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
17. Hendrickson, W. & Schleif, R. (1984) *J. Mol. Biol.* **178,** 611–628.
18. Ogden, S., Haggerty, D., Stoner, C., Kolodrudetz, D. & Schleif, R. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3346–3350.
19. Lee, N., Gielow, W. & Wallace, R. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 752–756.
20. Hendrickson, W. & Schleif, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 3129–3133.
21. Hawley, D. & McClure, W. (1983) *Nucleic Acids Res.* **11,** 2237–2255.
22. Colome, J., Wilcox, G. & Englesberg, E. (1977) *J. Bacteriol.* **129,** 948–958.
23. Kolodrubetz, D. & Schleif, R. (1981) *J. Mol. Biol.* **149,** 133–139.
24. Irani, M. H., Orosz, L. & Adhya, S. (1983) *Cell* **32,** 783–788.
25. Guarente, L. (1984) *Cell* **36,** 799–800.
26. Gluzman, Y. & Shenk, T., eds. (1983) *Enhancers and Eukaryotic Gene Expression* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
27. Brand, A. H., Breeden, L., Abraham, J., Strenglanz, R. & Nasmyth, K. (1985) *Cell* **41,** 41–48.
28. Johnson, A. D. & Herskowitz, I. (1985) *Cell* **42,** 237–247.
29. Brent, R. & Ptashne, M. (1984) *Nature (London)* **312,** 612–615.
30. Brent, R. (1985) *Cell* **42,** 3–4.
31. Hahn, S., Hendrickson, W. & Schleif, R., *J. Mol. Biol.*, in press.