Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 3129–3133, May 1985
Biochemistry

A dimer of AraC protein contacts three adjacent major groove regions of the *araI* DNA site

(gene regulation/DNA–protein interaction/chemical modification of DNA)

WILLIAM HENDRICKSON* AND ROBERT SCHLEIF

Department of Biochemistry, Brandeis University, Waltham, MA 02254

Communicated by Mark Ptashne, January 11, 1985

ABSTRACT **Contact sites of AraC protein to the regulatory site *araI* of the *Escherichia coli araBAD* operon have been determined by the chemical-interference technique. DNA fragments were chemically modified an average of once per molecule, and fragments that no longer bound AraC were separated by gel electrophoresis from the DNA fragments still able to bind the protein. The contact sites were then determined by comparing the positions of modifications in the two DNA samples. Strong contacts were found with guanines in three consecutive major groove regions and the adjacent phosphates along one side of the DNA. The conserved bases of the AraC-binding DNA consensus sequence are also found in the same positions. The gel electrophoresis assay was used to determine the stoichiometry of binding, and AraC protein was found to bind the *araI* and *araO*$_1$ regulatory sites as a dimer. Therefore, AraC appears to bind DNA differently from the other well-characterized regulatory proteins such as phage λ repressor.**

A variety of physical and genetic techniques have been used to develop a picture for one mode of DNA sequence recognition by proteins. Each of the monomers of phage λ repressor, λ cro protein, *lac* repressor, and cAMP receptor protein contains a highly conserved sequence that appears to form a DNA-contacting domain (1–8). The two DNA-contacting domains on these dimeric proteins bind two adjacent major groove regions of the DNA centered in a region of dyad symmetry.

AraC protein, which plays an inducing and repressing role in regulating the *araBAD* operon (9–16), does not possess any region of amino acid sequence with close homology to the consensus of the DNA-contacting domains of the above-mentioned proteins (8, 17). Also, AraC protein appears to associate with a more extensive DNA region, protecting about 40 base pairs from nuclease digestion (9, 10). Therefore, we have used the binding-interference technique developed by Siebenlist and Gilbert (18) to examine the DNA contact sites that this protein makes with DNA to look for different possible modes of protein–DNA interaction. These studies reveal that AraC protein binds one face of the DNA in three adjacent major groove regions of *araI* site. AraC protein binds this site to induce or repress the *araBAD* promoter (15, 16). Consistent with the apparent contacts, the consensus of AraC binding sites contains three regions of conserved bases, and these lie in the three regions of contact.

In view of the unusual binding pattern shown by AraC protein, we wished to determine if the protein binding unit is comprised of two, three, or some other number of subunits of AraC. We used the gel electrophoresis assay of DNA binding to accurately quantitate specifically bound protein and DNA. The results of these experiments show that AraC protein binds to *araI* as a dimer and to *araO*$_1$, the site to which AraC binds to repress the *araC* promoter, also as a dimer.

MATERIALS AND METHODS

Strains, Plasmids, and General Methods. Strains, plasmids, and general methods were as described (15, 19–21). Plasmid pTD383 contains the *araI* site on a 170-base-pair fragment that is released by *Eco*RI and *Hin*dIII digestion. The *araO*$_1$ site is on a 300-base-pair fragment that is released by *Eco*RI and *Hin*dIII digestion of plasmid pTD242. AraC protein was purified as described (21).

Binding Interference Assay. The assay is based on the method of Siebenlist and Gilbert (18). Phosphates of a ^{32}P-end-labeled DNA fragment were ethylated by adding 100 μl of saturated ethylnitrosourea (Sigma) in 95% ethanol to a 100-μl DNA sample in 50 mM sodium cacodylate, pH 8.0/0.1 mM EDTA and incubating at 50°C for 30–60 min. Purines were methylated by adding 1 μl of dimethyl sulfate to the DNA in 200 μl of 50 mM sodium cacodylate, pH 8.0/10 mM $MgCl_2$/0.1 mM EDTA and incubating for 1 min at 20°C. This reaction was stopped by adding 50 μl of 1 M Tris·HCl, pH 7.5/1 M 2-mercaptoethanol/1.5 M sodium acetate/0.05 M magnesium acetate/1 mM EDTA/0.1 mg of tRNA per ml. All reactions were timed to yield approximately one modification per DNA molecule. Modified DNA samples were precipitated with ethanol, washed, dried under vacuum, and resuspended in 10 mM Tris·HCl, pH 8.0/1 mM EDTA (TE buffer).

DNA recovered from the gel electrophoresis binding assay (see below) was cleaved at the positions of the modifications. To display methylated guanines, DNA was heated at 90°C in 10% piperidine for 30 min. To display both methylated guanines and adenines, the DNA was resuspended in 43 μl of 20 mM sodium acetate/1 mM EDTA, then 7.5 μl of 1 M NaOH was added, and the sample was incubated at 90°C for 30 min. Cleavage at ethylated phosphates was carried out as for the guanine-plus-adenine reaction. After incubation, samples with NaOH were neutalized with 7.5 μl of 1 M HCl and 50 μl of 20 mM Tris·HCl (pH 7.5). Samples were precipitated by addition of 4 μg of calf-thymus DNA, 150 μl of 70% ethanol, and 1 ml of 1-butanol; the butanol precipitation was repeated after resuspending the samples in 50 μl of 1% $NaDodSO_4$; and the DNA was dried under vacuum and resuspended in sequencing sample buffer (22). Positions of the cleavages were determined by electrophoresing samples on 8% denaturing acrylamide gels in parallel with samples prepared by the guanine-greater-than-adenine sequencing reactions (23).

Binding Reactions and Gel Electrophoresis. DNA binding reactions and electrophoresis to separate protein–DNA complexes were as described (21). The modified DNA (0.05–0.1 pmol; 2×10^5 dpm) was incubated with excess AraC protein

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.

*Present address: Department of Microbiology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA 17033.

(0.1–0.3 pmol) in 25 μl of binding buffer (10 mM Tris·HCl, pH 7.5/50 mM KCl/1 mM EDTA/5% glycerol/1 mM dithiothreitol/50 μg of bovine serum albumin per ml/50 mM arabinose or fucose/0.05% Nonidet P-40) for 10 min. A 20-fold molar excess of *ara* plasmid DNA was then added for 10 min (arabinose-containing buffer) or for 30 sec (fucose-containing buffer) to allow dissociation of complexes with modifications in critical positions of the binding site. The times allowed for dissociation were 1/10th the half-time found for AraC dissociation from unmodified DNA in these buffers (21). The samples were electrophoresed for 1 hr in 6% acrylamide/0.1% methylene bisacrylamide gels. The gels were soaked in 90 mM Tris borate/1 mM EDTA, pH 8.3, to improve subsequent recovery of the DNA and exposed to x-ray film for 20 min, and the DNA was recovered by using NA45 DEAE membranes (Schleicher & Schuell) as described (21).

Stoichiometry. To obtain AraC protein of known specific activity, strain RFSOP8, which harbors an AraC protein-overproducing plasmid, was grown for at least six doublings in 1–2 ml of M10 medium containing 1–2 mCi (1 Ci = 37 GBq) of $^{35}SO_4$ (carrier-free H_2SO_4; New England Nuclear), 0.1 mM $MgSO_4$, 1 mM $MgCl_2$, 0.5% glucose, and 5 μg of thiamine per ml to a density of 1×10^8 cells per ml. Cells were harvested, washed in lysate buffer (50 mM potassium phosphate, pH 7.0/50 mM KCl/1 mM EDTA/5% glycerol/1 mM dithioerythritol/16 μg of phenylmethylsulfonyl fluoride per ml) and resuspended at 10^9 cells per ml of lysate buffer. Cells were disrupted by sonication, and the debris was removed by centrifugation in a microcentrifuge. Polyethyleneimine (Polymin-P; pH 7.8) was added slowly with stirring to a final concentration of 0.5%. After 10 min on ice, samples were centrifuged in a microcentrifuge for 10 min, and the supernatant was applied to a 60-μl Bio-Rex 70 (Bio-Rad) column equilibrated in C buffer (50 mM potassium phosphate, pH 7.0/20 mM L-arabinose/0.5 mM dithioerythritol/0.1 mM EDTA). The column was washed with 200 μl of C buffer, and bound protein was eluted by adding 60-μl aliquots of C buffer plus KCl increasing from 0.1 to 0.4 M. AraC protein, generally was eluted in the 0.4 M KCl fraction. The fractions were collected directly into tubes containing 1 μl of 3% Nonidet P-40 and 3 μg of bovine serum albumin, 12 μl of glycerol were added, and the protein was used immediately in gel binding assays.

Plasmid DNA was purified by CsCl/ethidium bromide gradient centrifugation followed by gel filtration on agarose A-15m (Bio-Rad) to remove RNA (19), and the DNA concentration was determined spectrophotometrically by assuming $\epsilon_{260} = 1.3 \times 10^4\ M^{-1}\cdot cm^{-1}$ (per mol of base pairs). DNA fragments containing the *araI* or *araO₁* sites were excised by complete restriction digestion as monitored by gel electrophoresis. Then, both the *ara* DNA and remaining 3-kilobase vector DNA fragments were precipitated with ethanol and resuspended in TE buffer. The recovery of DNA in the precipitation (>95%) was monitored by adding ^{32}P-labeled tracer *ara* fragment after the restriction enzyme digestions.

After gel electrophoresis to separate AraC–DNA complexes from free DNA and protein, gel slices were prepared for scintillation counting by grinding in a glass vial and incubating overnight in 10% tissue solubilizer (Amersham) in 3 ml of ACS fluid (Amersham) at 55°C. Two vol of ACS fluid were then added before counting. The counting efficiency was determined by adding known amounts of $^{35}SO_4$ or $^{32}PO_4$ to similarly prepared blank samples.

RESULTS

Determination of Purine and Phosphate Contacts by AraC Protein. Dimethyl sulfate was used to methylate the N-7 of guanines in the major groove and the N-3 of adenines in the minor groove of DNA, while ethylnitrosourea was used to ethylate phosphates of the DNA backbone (18). A small DNA fragment containing the *araI* site was ^{32}P-labeled at one end and then treated so that, on average, each molecule was modified once. The modified DNA was incubated in the appropriate buffer with an excess of purified AraC protein. After the binding reaction, a large excess of unlabeled *ara* plasmid DNA was added for a brief period to sequester unbound protein. Under these conditions, AraC protein dissociated appreciably only from binding sites containing modifications that reduce the binding affinity. Acrylamide gel electrophoresis (21) then was used to separate the free DNA molecules (modified at positions that interfere with protein binding) from the AraC–DNA complexes (presumably modified only at noncrucial sites). Protein–DNA complexes and the faster-migrating free DNA were each recovered from the gels, and the DNA samples were cleaved at the modified bases so that their positions could be determined from the lengths of the resulting DNA fragments run on sequencing acrylamide gels.

Autoradiograms of the sequencing gels are shown in Fig. 1. In the lanes prepared from free DNA, bands appeared at the protein contact positions, while in the lanes prepared from DNA isolated from protein–DNA complexes, bands appeared at all positions except those required for binding. Protein binding was impaired by premethylation of guanines in any of five positions: −49 and −70 of the top strand (Fig. 1*A*), and −48, −59, and −69 of the bottom strand (Fig. 1*B*). Thus, three regions of contacts in the major groove of the DNA were observed, each spaced about 10 base pairs apart. Experiments that detected methylation of adenines in the minor groove of the DNA revealed no strong contacts.

Three similarly spaced regions of strong phosphate contacts were indicated by the preethylation experiments. Phosphate contacts were apparent at positions on the 5′ side of bases at −49 to −52, −62, and −70 to −73 of the upper strand (Fig. 1*A*) and of bases at −46, −47, −56, −57, −66, and −67 of the lower strand (Fig. 1*B*). That is, phosphate contacts were found on each side of the three regions of guanine contacts. An additional, weak contact was seen at position −41 on the upper DNA strand. No other purine or phosphate contacts were seen in this region in three experiments. The purine and strong phosphate contacts are centered in the DNA region protected by AraC from DNase digestion (9, 10). However, the site of the weak interaction at −41 is just outside the protected region. To detect possible conformational differences between AraC protein in the inducing [(+)-L-arabinose] and repressing [(+)-D-fucose] states, the contact sites of the protein in the presence of each of these two sugars was determined (Fig. 1). Although the DNA binding affinity of the protein for the *araI* site in the presence of fucose is 45-fold weaker than in the presence of arabinose (21), we saw no difference in the pattern of contacts.

The position of AraC protein bound to the *araI* site can be visualized from its contacts on a representation of the DNA helix (Fig. 2). The protein apparently binds along one face on the DNA. Guanines are contacted in each of three adjacent major groove regions, and the pattern of phosphate contacts suggests that the protein contacts the DNA backbone on each side of the three major groove binding regions. The AraC binding consensus sequence has three conserved regions and they correspond well to the contact-site data (Fig. 2*A*). Each set of guanine contacts is centered in a conserved region of the sequence, and the entire sequence forms an imperfect inverted repeat centered in the middle major groove of the DNA.

Stoichiometry of AraC Protein Binding. Although AraC protein appears to be a dimer in solution (24), its unusual pattern of DNA contact sites raises the possibility that the protein might bind with some other structure, such as a trimer. The gel electrophoresis assay is a convenient method

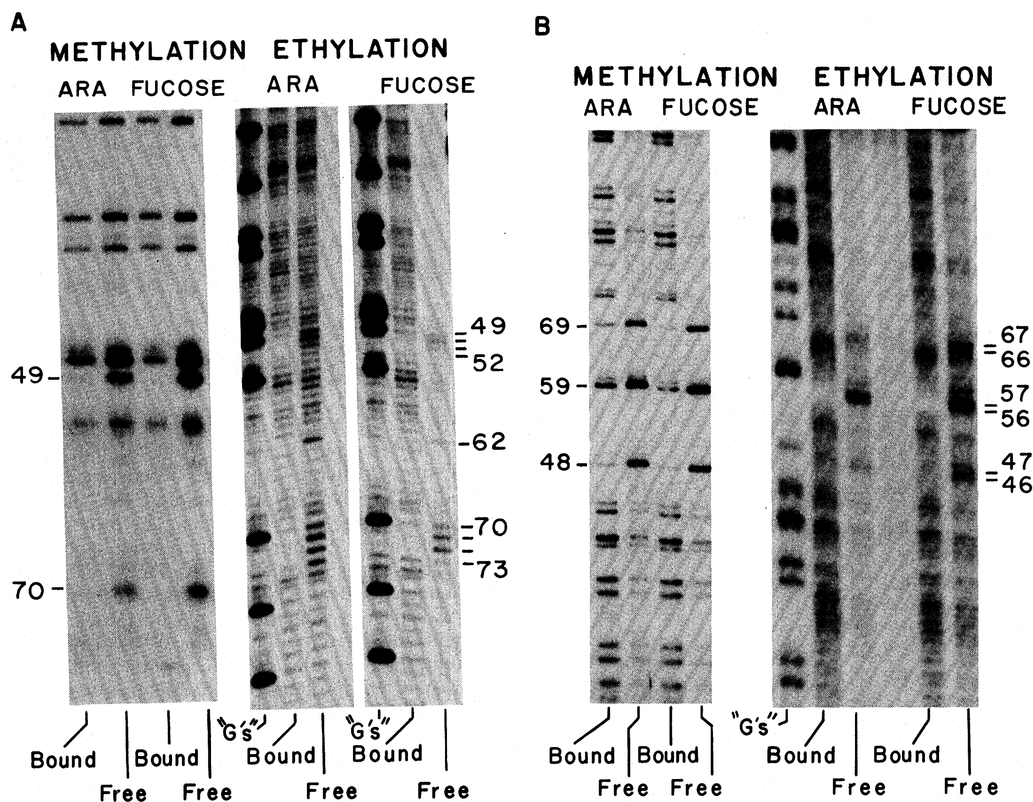

FIG. 1. Purine and phosphate contacts of AraC protein at *araI*. (*A*) DNA fragments containing the *araI* site were ^{32}P-end-labeled in the upper strand at the *EcoRI* site. DNA was premethylated or preethylated and then incubated with AraC protein in buffer containing arabinose (lanes ARA) or D-fucose (lanes FUCOSE). Designations at the bottom of lanes: bound, gel lanes with modified DNA isolated from AraC–DNA complexes; free, gel lanes with DNA isolated from the free DNA bands; "G's," size standards prepared by the guanosine-more-than-adenosine procedure. The positions of apparent protein contacts relative to the transcriptional start are indicated. Modified phosphates are on the 5′ side of the indicated bases. (*B*) DNA fragments were labeled on the lower strand at the *Hin*dIII site, and protein contacts were analyzed as in *A*.

for separation of specifically bound AraC protein–DNA complexes from free DNA and inactive protein (21). This method has been used to determine the stoichiometry of cAMP receptor protein binding by using *in vitro* labeling or labeled cAMP to quantitate the recovered proteins (25, 26).

Obtaining biologically active, pure protein with high specific activity can be difficult, and inaccuracy can occur in measurements of the amount of protein that retains specific binding activity. To avoid these problems, AraC protein was labeled to a precisely known specific activity by extended growth of cells synthesizing the protein in a medium of known $^{35}SO_4$ specific activity. Cells were grown for at least six doublings so that the specific activity of the sulfur-containing amino acids in the cellular protein equals that of the medium. From the known number of sulfur atoms in the protein, its precise specific activity was calculated. Although AraC protein can be detected easily in gel binding assays with crude lysates of cells, an excessive amount of other cellular protein migrates into the gel, creating an unacceptably high background. Therefore, a rapid microprocedure was used to partially purify the AraC protein obtained from the labeled cells (Fig. 3).

Precisely known concentrations of DNA fragments containing the *araI* or *araO$_1$* sites were prepared. The concentration of *ara* plasmid DNA was measured spectrophotometrically, and then the fragment was released by quantitative restriction enzyme digestion. No further purification of the DNA fragments was necessary because the vector DNA in the sample does not interfere with the assay. When needed, a trace of $^{32}PO_4$-end-labeled DNA fragment was added for quantitation of recoveries.

The protein–DNA complexes formed sharp bands that were located by ethidium bromide staining (Fig. 3*B*). They were cut out of the gels, and the protein-to-DNA ratios for AraC binding to the *araI* and *araO$_1$* sites were determined by comparing the ^{35}S-labeled protein with ^{32}P-labeled DNA recovered (Table 1). For both DNA binding sites a ratio of 2 (1.9 ± 0.1 for *araI* and 2.1 ± 0.1 for *araO$_1$*) was obtained as the average of four different labeling/purification/assay determinations, indicating that the dimer found in solution also is the DNA-binding form. The amount of nonspecific background in the gel with a particular AraC preparation varied by ±10% in duplicate gel lanes. However, the magnitude of this background varied up to a factor of 2 between different AraC preparations.

DISCUSSION

These chemical modification studies show that the purines and phosphates that are contacted or closely approached by AraC protein when it binds the *araI* site are distributed along only one face of the DNA. These contacts are in three major groove regions along a line that is parallel to the DNA helical axis. This picture for the binding of the protein is supported by the AraC protein binding DNA consensus sequence. The guanine contacts are centered in each of three conserved regions, whereas the positions in the minor groove of the DNA on this same face that could contact the protein are not conserved. Furthermore, although the chemical modification experiments could have detected adenine contacts in the minor groove, none were prominent.

For other well-studied regulatory proteins in *E. coli*, two protein monomers bind adjacent major grooves of the DNA

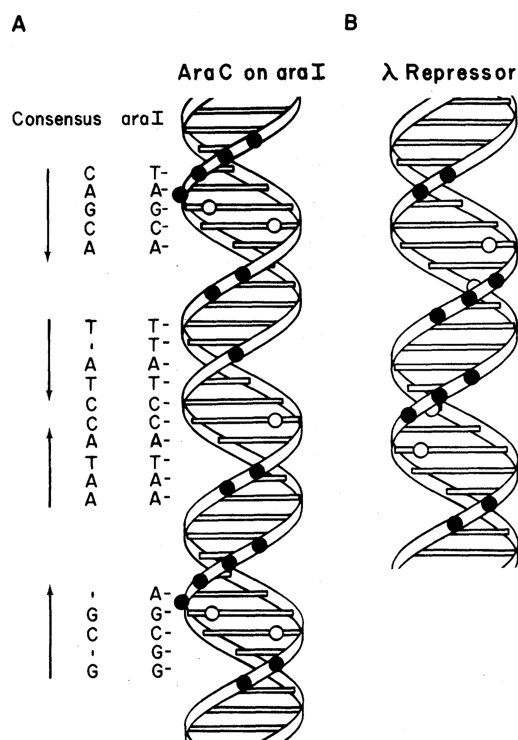

FIG. 2. AraC protein binds three adjacent major grooves of the DNA. (A) The data of Fig. 1 are shown on a model of the DNA helix drawn with 10.5 base pairs per helical turn. ○, Guanine contacts; ●, phosphate contacts. The consensus sequence of the six AraC binding sites at the *araC, araBAD, araE, araFG,* and *araH* promoters (refs. 9 and 30; unpublished data) is shown along with the *araI* sequence. Sequences protected from DNase digestion are listed below with capitals indicating homology in at least four sites:
araI: atgccatAGCAtttTtATCCATAAgattaGCgGatc
araE: atgtggCAGCAattTaATCCATAttta-tGCtGtttccga
araO1: cgtAaCAaaagtgTCtATAAtcacgGCaGaaaagtccacatt
araH: actattCAGCAggaTaATgaATAcagaggGgcGaatta
araFG1: taCAGtgagaacgTgCATAAatttaGCgGgaaaagacataa
araFG2: caCAGCAgatTaATCCATAAgattaGCctggaaatccttgtt
Underlining signifies the consensus regions included in inverted repeat symmetry. (B) Purine and phosphate contacts of the phage λ CI protein with the phage λ O_R1 site (redrawn from ref. 1).

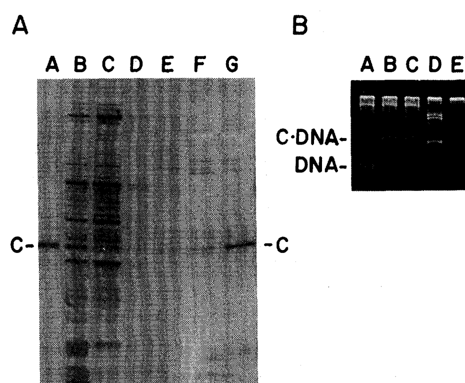

FIG. 3. Gel binding assay for stoichiometry of AraC bound to *araI*. (A) Micropurification of AraC protein. A 1-ml culture of the AraC-overproducing strain was lysed, nucleic acids were precipitated with polyethyleneimine, and the supernatant was loaded directly on a Bio-Rex-70 column. The protein in fractions that was eluted from the column with increasing concentrations of KCl was analyzed on a silver-stained (27) 10% $NaDodSO_4$/polyacrylamide gel. Labeled and unlabeled proteins were prepared in parallel. Lanes: A, AraC protein standard; B, polyethyleneimine supernatant; C–G, protein eluted in buffer containing 0–0.4 M KCl. C, position of AraC protein. (B) The indicated AraC protein and DNA preparations were incubated, and protein–DNA complexes were separated from free DNA by gel electrophoresis. Lanes A, ^{32}P-labeled *araI* DNA and unlabeled DNA fragment; B, ^{32}P-labeled DNA, unlabeled *araI* DNA, and unlabeled AraC protein; C, unlabeled *araI* DNA and ^{35}S-labeled AraC protein; D, pBR322 DNA fragments and ^{35}S-labeled AraC protein; E, ^{35}S-labeled AraC protein with no DNA fragment. After electrophoresis the gel was stained with ethidium bromide and photographed. DNA at the top of the gel lanes is whole plasmid, added to reduce nonspecific binding. Gel slices were cut out at the positions of DNA bands, and the radioactivity was determined.

with protein–protein interactions involved in dimerization occurring over the central minor groove. The symmetry of the protein is reflected in the dyad symmetry of the DNA sequence that is also centered over the minor groove. AraC protein clearly contacts DNA differently.

A protein that contacts only two major groove regions of the DNA could appear to contact three adjacent major grooves if the population of complexes contain protein bound to either of two overlapping sites. In such a case, the intensity of bands as seen in Fig. 1 would be altered no more than 2-fold at the two outer major groove contact regions since only half of the DNA molecules would have protein bound to these regions at any instant. For *araI*, strong enrichments were seen at each of the three major groove regions, indicating that the entire site on each DNA molecule is fully occupied with protein.

Table 1. A dimer of AraC binds the *araI* and $araO_1$ sites

DNA fragment	Input DNA,* fmol	DNA in AraC–DNA band,† fmol	^{35}S, cpm			AraC monomer,¶ fmol	Ratio fmol of AraC monomer/fmol of DNA	
			araC band	Non-*araC* band‡	Specific binding§		1 Exp.‖	Average**
araI	181	161	65,200	8,900	56,300	336	2.1	1.9 ± 0.1
$araO_1$	184	169	73,200	20,500	52,700	315	1.9	2.1 ± 0.1

*Total of 0.45 μg of 3800-base-pair *araI* and 3,900-base-pair $araO_1$ plasmids, with the *ara* fragments released by restriction enzyme digestion.
† Calculated from ^{32}P-labeled DNA complexed with unlabeled AraC protein (Fig. 3B, lane B).
‡ Nonspecific ^{35}S recovered from pBR322 DNA band in the same position of the gel as specific complexes (Fig. 3B, lane D). Similar backgrounds were obtained from this position of the gel with samples lacking DNA fragments (Fig. 3B, lane E).
§ Binding in *araC* band minus that in non-*araC* band.
¶ The amount of $^{35}SO_4$ added to the growth medium was determined by scintillation counting by assuming 98% counting efficiency relative to a ^{14}C sealed standard; a specific activity of 8.95×10^3 Ci/mol in the culture medium × nine sulfur-containing amino acids per protein monomer = 8.06×10^4 Ci/mol ^{35}S-labeled AraC protein. Since the counting efficiency of samples recovered from gels was 94.4%, 167.4 cpm = 1 fmol of AraC protein.
‖ Calculated from values in columns three and seven.
**Averages of four independent measurements using different cell cultures and media.

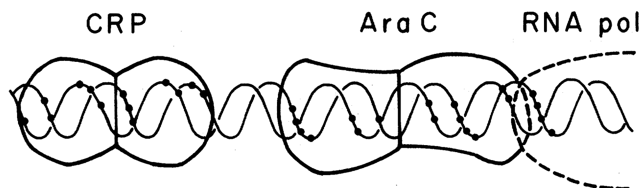

FIG. 4. Schematic of proteins bound at the *araBAD* promoter. The position of cAMP receptor protein (CRP) on the *araBAD* promoter has been inferred from methylation-protection data (10), from the contact sites determined for the *lac* and *araE* promoters (ref. 3; unpublished data) and from the CRP binding consensus DNA sequence (28). The location for AraC protein binding is based on the contact site data and on the size of the protein relative to CRP. The RNA polymerase was drawn by analogy with the *lac* UV5 and phage T7 promoter contact data (2, 18) by assuming that the contacts in the −35 region of *araBAD* will be similar.

Analysis of the conserved sequences of six AraC protein binding sites suggests that AraC binds an imperfect inverted repeat centered in the middle major groove. The right-most region is less well conserved; three of five positions are highly conserved compared with five of five on the left. Although AraC protein binds as a dimer and the consensus binding site possesses a dominant 2-fold axis of symmetry at its center, it is clear from the pattern of phosphate contacts that the two subunits of the protein do not make identical contacts.

Binding of RNA polymerase at the *araBAD* promoter requires the presence of both AraC protein and cAMP receptor protein (21). The relative positions of the proteins on the DNA can be determined by comparing our data for AraC with sequence and contact data previously determined for cAMP receptor protein and RNA polymerase (2, 3, 10, 18, 28). A model of contact sites and positions of the proteins is shown in Fig. 4. Comparing this model with those similarly derived for the phage λ P_{RM} and phage P22 P_{RM} promoters (29), we see that the orientation between the positive activator and RNA polymerase is different for the *araBAD* promotor. The activators of the phage promoters have contacts along the same DNA major groove adjacent to, and possibly overlapping, the assumed RNA polymerase sites. AraC protein binds the major groove further upstream, separated by the minor groove from the nearest assumed polymerase contacts. However, AraC and RNA polymerase are still sufficiently close on the DNA that protein–protein contacts between them could occur. The spacing of cAMP receptor protein and AraC protein, one full turn of the helix apart, indicates that if interactions occur between the two positive activators, they might be relatively distant from the DNA-binding domains of the proteins and could involve bending of the DNA. The stoichiometry measurements show that AraC binds as a dimer to the DNA. One model that would accommodate the data is shown in Fig. 4. A protein monomer may have a bidentate binding domain that contacts bases in two major grooves of the DNA, and the monomers jointly contact the central major groove region. Although the helix-turn-helix motif could be involved in DNA binding by AraC protein, the model suggests that some other structure is used for at least part of the AraC–DNA contacts.

This work was supported by grant number GM18277 to R.S. from the National Institutes of Health.

1. Lewis, M., Jeffery, A., Wang, J., Ladner, R., Ptashne, M. & Pabo, C. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 435–440.
2. Siebenlist, U., Simpson, R. & Gilbert, W. (1980) *Cell* **20**, 269–281.
3. Majors, J. (1975) Dissertation (Harvard Univ., Cambridge, MA).
4. Sauer, R., Yocum, R., Doolittle, R., Lewis, M. & Pabo, C. (1982) *Nature (London)* **298**, 447–451.
5. Steitz, T., Weber, I. & Matthew, J. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 419–426.
6. Takeda, Y., Ohlendorf, D., Anderson, W. & Matthews, B. (1983) *Science* **221**, 1020–1026.
7. Ptashne, M., Jeffrey, A., Johnson, A., Maurer, R., Meyer, B., Pabo, C., Roberts, T. & Sauer, R. (1980) *Cell* **19**, 1–11.
8. Pabo, C. & Sauer, R. (1984) *Annu. Rev. Biochem.* **53**, 293–321.
9. Ogden, S., Haggerty, D., Stoner, C., Kolodrubetz, D. & Schleif, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3346–3350.
10. Lee, N., Gielow, W. & Wallace, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 752–756.
11. Sheppard, D. & Englesberg, E. (1967) *J. Mol. Biol.* **25**, 443–454.
12. Englesberg, E., Squires, C. & Meronk, F. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1100–1107.
13. Greenblatt, J. & Schleif, R. (1971) *Nature (London) New Biol.* **223**, 166–170.
14. Wilcox, G., Meuris, P., Bass, R. & Englesberg, E. (1974) *J. Biol. Chem.* **249**, 2946–2952.
15. Dunn, T., Hahn, S. & Schleif, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5017–5020.
16. Hahn, S., Dunn, T. & Schleif, R. (1984) *J. Mol. Biol.* **180**, 61–72.
17. Stoner, C. & Schleif, R. (1982) *J. Mol. Biol.* **154**, 649–652.
18. Siebenlist, U. & Gilbert, W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 122–126.
19. Schleif, R. & Wensink, P. (1981) *Practical Methods in Molecular Biology* (Springer, New York).
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Hendrickson, W. & Schleif, R. (1984) *J. Mol. Biol.* **178**, 611–628.
22. Bencini, D., O'Donovan, G. & Wild, J. (1984) *Biotechniques* **2**, 4–5.
23. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
24. Steffen, D. & Schleif, R. (1977) *Mol. Gen. Genet.* **157**, 333–339.
25. Fried, M. & Crothers, D. (1983) *Nucleic Acids Res.* **11**, 141–158.
26. Garner, M. & Revzin, A. (1982) *Biochemistry* **21**, 6032–6036.
27. Wray, W., Boulidas, T., Wray, V. & Hancock, R. (1981) *Anal. Biochem.* **118**, 197–203.
28. Ebright, R. (1982) in *Molecular Structure and Biological Activity*, eds. Griffen, J. & Duax, W. (Elsevier/North-Holland, New York), pp. 91–100.
29. Hochschild, A., Irwin, N. & Ptashne, M. (1983) *Cell* **32**, 319–325.
30. Stoner, C. & Schleif, R. (1983) *J. Mol. Biol.* **171**, 369–381.