Proc. Natl. Acad. Sci. USA
Vol. 75, No. 10, pp. 4724–4728, October, 1978
Biochemistry

DNA sequence of the *araBAD* promoter in *Escherichia coli* B/r

(positive control/L-arabinose operon/gene regulation)

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Communicated by Katherine Esau, July 10, 1978

ABSTRACT **The L-arabinose operon in *Escherichia coli* is a model system for the study of the control of gene expression. Maximal expression of the *araBAD* operon requires two positive control components: the *araC* protein–L-arabinose complex and the cyclic AMP receptor protein–cyclic AMP complex. Both *araC* protein and cyclic AMP receptor protein are required for the initiation of transcription of *araBAD* mRNA. We have used the plasmid pBR322 as a vector for cloning DNA fragments that contain the *araBAD* promoter. The cloned *ara* fragments were identified by both physical and genetic tests. A restriction map was constructed and the DNA sequence of the promoter was determined. The promoter contains a site that is similar to the RNA polymerase recognition sites in the galactose and lactose operons. It also contains a region similar to the known cyclic AMP receptor protein binding sites in the galactose and lactose operons.**

The L-arabinose operon in *Escherichia coli* is composed of three structural genes, *araB*, *araA*, and *araD*, that are involved in the initial steps of L-arabinose catabolism. The controlling elements of the operon are adjacent to *araB*. The operon is inducible and controlled by the *araC* gene which is closely linked to the operon but transcribed in the opposite direction (1). The *araBAD* and *araC* promoters are 150–170 base pairs apart (2) (unpublished data).

The product of the regulatory gene *araC* is a protein that, in the absence of L-arabinose, interacts with an operator site (*araO*) to decrease operon expression to $\frac{1}{30}$th–$\frac{1}{20}$th. In the presence of L-arabinose, *araC* protein is removed from *araO* and is converted into an activator that interacts at *araI* to allow maximal expression of the operon (1).

The *araBAD* promoter is defined on one side by deletions (e.g., *araΔ719*) and on the other side by the *araB* gene. The *araΔ719* deletion removes *araO* functionally but has no apparent effect on the promoter site (3, 4). Within the *araBAD* promoter there are potentially three distinct sites at which proteins may interact: these are sites for RNA polymerase, *araC* protein, and cyclic AMP receptor protein (CRP), respectively (1). The *araI* site has been defined genetically by a class of *cis*-dominant, *trans*-recessive mutations isolated as "revertants" of *araC* deletion strains and is the region where *araC* activator interacts (5, 6). The CRP site is inferred from the sensitivity of the operon to catabolite repression (7) and may be more precisely defined by mutations in the promoter that make the operon less sensitive to catabolite repression (8). Mutations in the RNA polymerase interaction site could be difficult to distinguish genetically from mutations in the *araC* activator site or in the CRP binding site. However, there is a class of mutations that produce very low levels of *araBAD* expression and are candidates for mutants in the RNA polymerase recognition site (9).

In this paper we describe the determination of the DNA sequence of the *araBAD* promoter. The promoter has some similarities to the lactose and galactose promoters, and the RNA polymerase recognition site is similar in some respects to other bacterial and phage promoters. The sequence suggests that there are separable regions involved in the interactions with RNA polymerase, CRP, and *araC* activator protein.

MATERIALS AND METHODS

Phage and Bacterial Strains. The λ*h80dara* transducing phages have been described (10). The following strains, all derivatives of *E. coli* K-12 strain RR1 (11) containing the *ara* region from *E. coli* B/r, were used as recipients for the transformations: LA1, F⁺/F⁻*lac gal leu pro thi hsdR hsdM*; LA3, F⁺/F⁻*araΔ719 lac gal pro thi hsdR hsdM*; LA4, F⁺/F⁻*araΔ735 lac gal pro thi hsdR hsdM*; LA5, F⁺/F⁻*araΔ744 lac gal pro thi hsdR hsdM*; LA6, F⁺/F⁻*araΔ766 lac gal pro thi hsdR hsdM*; and LA7, F⁺/F⁻*araB24 lac gal pro thi hsdR hsdM*. The plasmid pBR322 has been described (11).

Preparation of DNA. Bacteriophage DNA was prepared as described by Wilcox *et al.* (12). Plasmid DNA was isolated as described by Clewell and Helinski (13) after chloramphenicol amplification (14).

Enzymes. Restriction endonucleases were purified by conventional procedures or purchased from Bethesda Research Laboratories (Rockville, MD). DNA ligase (T4-infected *E. coli*) was from Miles.

Construction and Screening of Recombinant Plasmids. λ*h80dara* DNA (12.5 μg), 2 μg of pBR322 DNA, and 5 units of restriction enzyme were incubated in 50 μl of restriction buffer (100 mM Tris·HCl, pH 7.5/50 mM NaCl/5 mM MgCl) at 37° for 3 hr. The DNA mixture was heated to 65° for 10 min to inactivate the restriction enzyme and then placed on ice. The solution was diluted 1:2 by the addition of 50 μl of 66 mM Tris·HCl pH 7.5/5 mM MgCl. Then, 5 μl of 800 mM ATP, 5 μl of 200 mM dithiothreitol, and 0.2 μl of DNA ligase (1000 units ml) were added and the solution was incubated for 16 hr at 15°. Transformation was carried out as described by Cohen *et al.* (15). Ligated DNA (5–25 μl) was transformed into 0.2 ml of competent cells and plated on selective media.

The single *Pst* I site on pBR322 is located within the ampicillin-resistance gene (11). Therefore, insertion of DNA into the *Pst* I site results in an ampicillin-sensitive (Ap^s) phenotype. λ*h80dara*, λ*h80daraΔ718*, and λ*h80daraΔ735* DNAs and pBR322 DNA digested with *Pst* I were ligated and transformed into strain LA6 and plated on EMB/L-arabinose/tetracycline agar plates. Ara^+ colonies were picked and replica-plated to TYE/tetracycline (20 μg/ml)/ampicillin (100 μg/ml) and TYE/tetracycline plates. Ap^s tetracycline-resistant (Tc^r) clones were saved and screened as described below. λ*h80daraΔ766* and λ*h80daraΔ719* chimeras were transformed into strain LA4. Small Ara^- colonies were replica-plated and Ap^sTc^r clones were saved for screening.

Purified clones to be screened were grown in 1 ml of TYE

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Abbreviations: CRP, cyclic AMP receptor protein; Ap^s, ampicillin-sensitive; Tc^r, tetracycline-resistant.

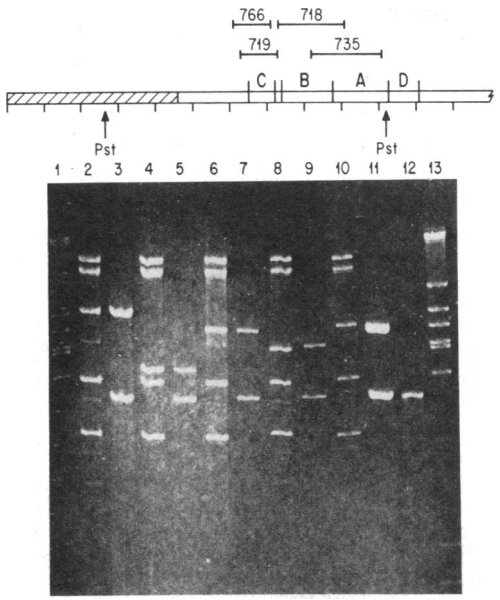

FIG. 1. Agarose gel electrophoresis of phage and plasmid DNAs digested with *Pst* I. DNA samples (10 μg) were restricted with 5 units of enzyme in a total volume of 50 μl for 18 hr at 37°. Lanes 1 and 13 contained λ DNA standards digested separately with *Eco*RI and *Hin*dIII and then mixed. All other lanes contained DNA digested with *Pst* I. Lanes: 2, λ*h80dara*; 3, pBR322-*ara*; 4, λ*h80dara*$^+$Δ*718*; 5, pBR322-*ara*Δ*718*; 6, λ*h80dara*Δ*719*; 7, pBR322-*ara*Δ*719*; 8, λ*h80dara*Δ*735*; 9, pBR322-*ara*Δ*735*; 10, λ*h80dara*Δ*766*; 11, pBR322-*ara*Δ*766*. Above the gel is a physical map of the left end of the λ*h80dara* bacteriophage. The arrows indicate the *Pst* I restriction sites; the numbers represent the deletions, and the bars represent the extent of the deletions. The letters represent the *ara* genes. The double horizontal line is DNA: ▨, Ø80 DNA; □, bacterial DNA.

overnight. An additional 10 ml of TYE was added to the overnight cultures. After 1 hr, 2 mg of chloramphenicol was added and, after 10–16 hr of further incubation, the cells were centrifuged at 8000 rpm for 7 min in a Sorvall SS-34 rotor at 4°. The pellets were resuspended in 0.6 ml of 50 mM Tris·HCl, pH 8.0/25% (wt/vol) sucrose and kept on ice. A spatula spot of lysozyme was added; after 15 min, 30 μl of 0.25 M EDTA (pH 8.0) was added. After 15 additional min, 30 μl of 20% (wt/vol) sodium dodecyl sulfate was added, followed by 0.17 ml of 5 M NaCl. After 1 hr, the lysed cells were centrifuged at 17,000 RPM in a Sorvall SS-34 rotor for 40 min at 4°. One-tenth milliliter of the supernatant was dialyzed for 75 min against restriction buffer; then, to 20 μl of the dialyzate was added 1 unit of restriction enzyme. After 1 hr at 37°, samples were electrophoresed on 1% agarose gels.

Containment. These experiments were performed under EK1, P1 conditions.

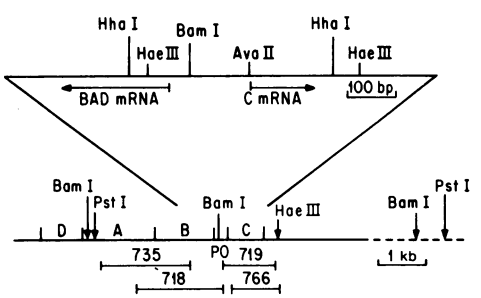

FIG. 2. Restriction map of the *ara* region. Restriction endonuclease sites used in the cloning and sequencing are indicated. The *Bam* I and *Pst* I restriction sites on the far right are located in the Ø80 DNA (---). All other restriction sites are on *E. coli* DNA (—). The mRNA start sites are indicated by the horizontal bars. kb, kilobase; bp, base pairs.

Table 1. Genetic complementation tests with hybrid plasmids containing *ara* restriction fragments generated by *Pst* I.

Recipient	Hybrid plasmid DNA used in transformation				
	ara$^+$	*ara*Δ*718*	*ara*Δ*719*	*ara*Δ*735*	*ara*Δ*766*
LA1(*ara*$^+$)	+	+	+	+	+
LA2(*ara*Δ*718*)	−	−	−	−	−
LA3(*ara*Δ*719*)	+	+	−	+	−
LA4(*ara*Δ*735*)	−	−	−	−	−
LA5(*ara*Δ*744*)	−	−	−	−	−
LA6(*ara*Δ*766*)	+	+	−	+	−
LA7(*araB24*)	+	−	+	−	+

The indicated strains (LA1 to LA7) were transformed with plasmid DNA and Tc^r clones were selected. A culture of each purified Tc^r clone was grown overnight in TYE tetracycline medium and serially diluted; 0.1 ml of each dilution was plated on a minimal supplemented L-arabinose tetracycline (20 μg/ml) agar plate and a TYE tetracycline plate. If complementation occurred, the number of Ara$^+$ colonies equaled the number of Tc colonies in the overnight culture. +, Complementation; −, lack of complementation.

DNA Sequence Analysis. Purified plasmid DNA was restricted and terminally labeled with [γ-^{32}P]ATP as described (16) with the following modifications: (*i*) [γ-^{32}P]ATP was prepared and used immediately; (*ii*) the DNA fragments were dialyzed against the appropriate buffer to remove salt prior to treatment with the bacterial alkaline phosphatase and polynucleotide kinase. Restriction fragments were isolated by preparative electrophoresis and subjected to each of the base-specific cleavage reactions developed by Maxam and Gilbert (17). The products of hydrolysis were separated by electrophoresis in a 0.15 × 20 × 40 cm slab gel of 20% acrylamide containing 7 M urea. All gels were pre-electrophoresed for at least 8 hr at 1000 V.

RESULTS

***Pst* I Restriction Analysis of λ*h80dara* DNA.** Each λ*h80dara* DNA was digested with *Pst* I and electrophoresed on a 1% agarose gel. The mobility of the third band (Fig. 1) was greater in the DNA from the *ara* deletion phages (lanes 4, 6, 8, and 10) than in that from the *ara*$^+$ phage (lane 2), and no other fragments had an altered mobility. The difference between the molecular weight of the third band in the *ara*$^+$ DNA and that of each *ara* deletion DNA corresponds exactly to the molecular weight of the deletions as determined by heteroduplex analysis. Therefore, the third band contains all of *araB* and *araC*, and the *Pst* I restriction sites in the λ*h80dara* DNA must be to the left of *ara*Δ*766* and to the right of *ara*Δ735. The precise location of the *Pst* I restriction sites is shown in Fig. 1.

Cloning of *Pst* I Restriction Fragments. It is possible to select, by complementation, clones containing *ara* genes, because the *Pst* I restriction fragments of the different *ara* phages contain at least *araC* or *araB* or both. Because the *Pst* I fragments of *ara*$^+$, *ara*Δ*718*, and *ara*Δ735 all contain an intact *araC* gene, they were cloned into strain LA6 which contains a deletion of the *araC* gene. For each λ*h80dara* DNA, Ara$^+$ colonies were observed on EMB/L-arabinose/tetracycline plates. All of the Ara$^+$ clones tested had an Ap^{s}Tc^r phenotype. When plasmid DNA from these clones was restricted with *Pst* I and analyzed by gel electrophoresis, in every case it was found to contain the expected *ara* fragment and a band corresponding to pBR322 (Fig. 1, lanes 3, 5, and 9).

Because the *Pst* I fragments from *ara*Δ*719* and *ara*Δ*766* contain the *araB* gene and perhaps all of *araA*, they were cloned into strain LA4 containing an *araAB* deletion. However, no Ara$^+$ colonies appeared on the EMB/L-arabinose/tetracycline

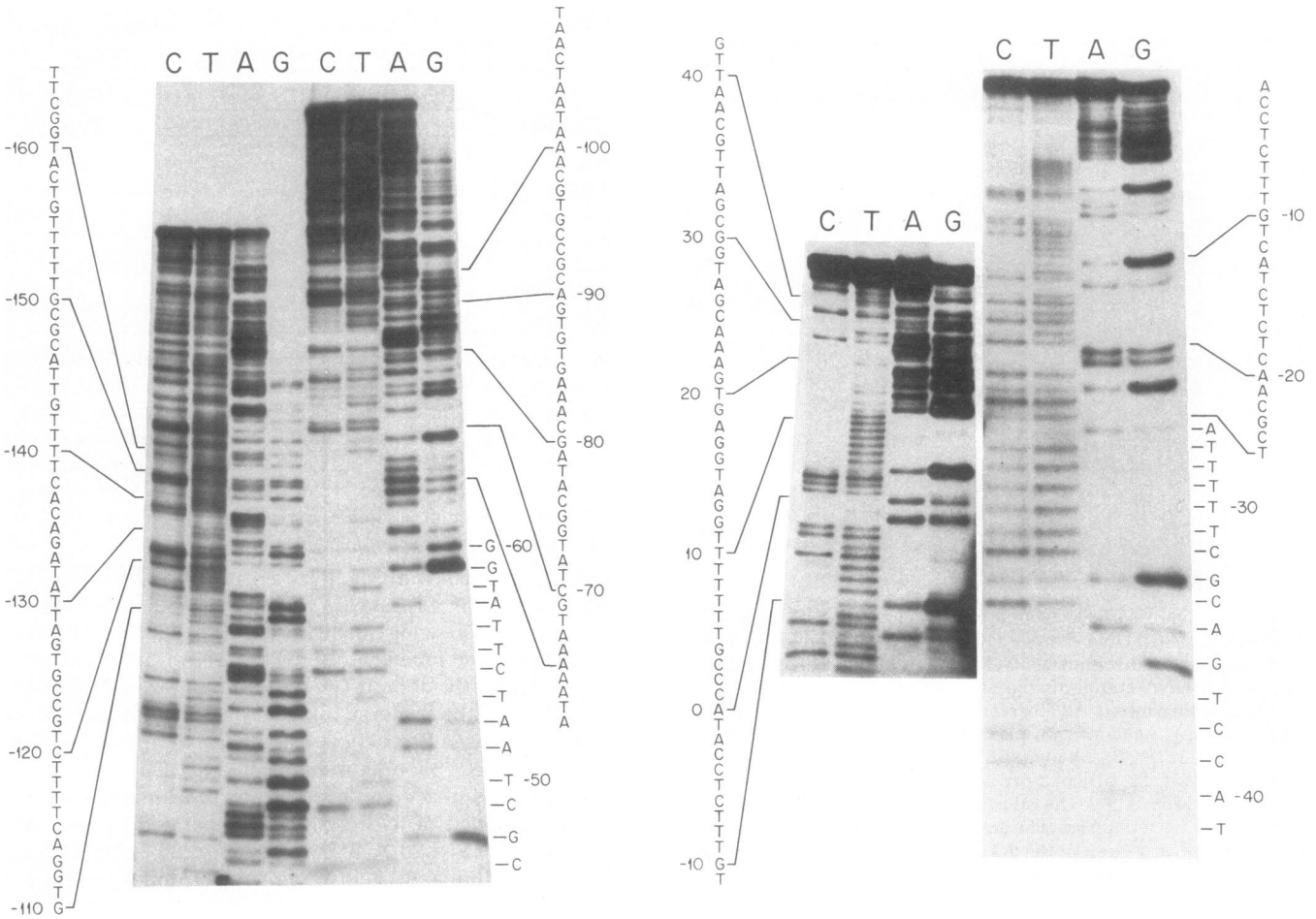

FIG. 3. Autoradiograms of 20% polyacrylamide7 M urea gels used for the nucleotide sequence determination. (*Left*) *Bam* I/*Hae* III fragment containing part of the *araB* gene; the *Bam* I end was labeled. (*Right*) *Bam* I/*Hae* III fragment containing part of the *araC* gene; the *Bam* I end was labeled. The terminally labeled products from each of the base-specific chemical reactions were loaded at 12-hr intervals. The nucleotide sequence can be read from the autoradiograms as indicated. The C, T, A, and G at the top of each lane represent the C-enriched, T- and C-specific, A-enriched, and G-specific chemical reactions, respectively.

plates, indicating that complementation had not occurred. Large and small Ara$^-$ colonies were observed. All of the small Ara$^-$ colonies tested were Ap^{s}Tc^r whereas only 25% of the large Ara$^-$ colonies were Ap^{s}Tc^r. This suggested that perhaps the small colonies contained *ara* genes. When plasmid DNAs from these small colonies were restricted with *Pst* I and analyzed by gel electrophoresis, they were found to have the DNA fragment containing *ara* genes (Fig. 1, lanes 7 and 11).

Complementation Analysis. Each hybrid plasmid DNA was prepared and transformed into each of seven host strains. Transformants were selected on TYE/tetracycline plates. The complementation analysis (Table 1) showed that *ara*$^+$,

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        40        30        20        10         0        -10       -20
5'-CCAATTGCAATCGCCATCGTTTCACTCCATCCAAAAAAACGGGTATGGAGAAACAGTAGAGAGTT
3'-GGTTAACGTTAGCGGTAGCAAAGTGAGGTAGGTTTTTTTGCCCATACCTCTTTGTCATCTCTCAA

   GGUUAACGUUAGCGGUAGCAAAGUGAGGUAGGUUUUUUUGCCCAppp      araBAD mRNA


       -30       -40       -50       -60       -70       -80
GCGATAAAAAGCGTCAGGTAGGATCCGCTAATCTTATGGATAAAAATGCTATGGCATAGCAAAG
CGCTATTTTTCGCAGTCCATCCTAGGCGATTAGAATACCTATTTTTACGATACCGTATCGTTTC

   -90       -100      -110      -120      -130      -140
TGTGACGCCGTGCAAATAATCAATGTGGACTTTTCTGCCGTGATTATAGACACTTTTGTTACGC
ACACTGCGGCACGTTTATTAGTTACACCTGAAAAGACGGCACTAATATCTGTGAAAACAATGCG

-150      -160
GTTTTTGTCATGGCTT
CAAAAACAGTACCGAA
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FIG. 4. DNA sequence of the *araBAD* promoter and adjacent regions. Sequence was determined as described in the text and in Fig. 3.

*ara*Δ*719*, or *ara*Δ*766* on the plasmid complements *araB24* on the chromosome. This is consistent with the previous conclusion, based on restriction analysis of the λ*h80dara* DNA, that the *Pst* I site was outside of the *araB* gene. When the *ara*$^+$ fragment was on the plasmid and *ara*Δ*744*, which deletes *araCBA*, was on the chromosome, there was no complementation. If *ara*Δ*744* expressed the *araD* gene, an Ara$^+$ phenotype would result if L-arabinose isomerase were produced; if *ara*Δ*744* does not express *araD*, then an L-arabinose-senstive phenotype (18) would result if L-arabinose isomerase were produced by the plasmid. Thus, we conclude that the plasmid does not produce L-arabinose isomerase.

Restriction Map of the Controlling Site Region. Previous work (18) from this laboratory has identified the *Bam* I restriction endonuclease sites shown in Fig. 2. There are two *Bam* I restriction sites within the *Pst* I sites identified in Fig. 1. The restriction map was obtained by digesting each hybrid plasmid with *Bam* I, end-labeling the DNA, digesting with a second restriction endonuclease, and separating the fragments on a 2% agarose gel or a 10% polyacrylamide gel. The map shown in Fig. 2 was constructed by comparing fragments obtained from the different *ara* deletions. Of particular importance was the identification of a 120-base pair *Bam* I/*Hae* III fragment from *ara*Δ*719* which contains the distal part of the *araBAD* promoter.

DNA Sequence Analysis. The DNA sequence of the controlling site region was determined from the cloned *ara*$^+$-*Pst* I restriction fragment. The plasmid DNA was digested with *Bam* I and end-labeled with ^{32}P. After restriction with *Hae* III, the appropriate *Bam* I/*Hae* III fragments were isolated by preparative polyacrylamide gel electrophoresis. Each isolated DNA fragment was sequenced by the chemical method desdribed by Maxam and Gilbert (17). Two representative sequencing gels are shown in Fig. 3, and the DNA sequence of the *araBAD* promoter is shown in Fig. 4. That the two *Bam* I/*Hae* III fragments are adjacent was confirmed by sequencing through the *Bam* I site. The *Hae* III/*Hae* III fragment which contains the intact promoter was isolated by preparative electrophoresis on 10% polyacrylamide gels. The fragment was end-labeled and restricted with *Hha* I, and the appropriate *Hae* III-*Hha* I fragment was isolated and sequenced (data not shown).

DISCUSSION

Cloning of the appropriate *ara* deletion mutations into the plasmid pBR322 has facilitated the identification of DNA restriction fragments containing all or part of the *ara* controlling site region. A DNA fragment generated by the restriction endonuclease *Pst* I has been cloned and shown to contain functional *araB* and *araC* genes by complementation analysis; thus, it follows that this fragment also contains the *araBAD* promoter (see Fig. 1). A restriction map of the controlling site region has been made and two *Bam* I/*Hae* III restriction fragments were identified in the controlling sites. Both fragments were sequenced from the *Bam* I restriction site. The smaller of the two fragments is 86 base pairs in length and contains a sequence of 44 base pairs complementary to the 5′ end of the *araBAD* mRNA that was sequenced by Lee and Carbon (19). The second DNA fragment is about 350 base pairs in length and has been sequenced to a distance of 120 base pairs from the *Bam* I restriction site. Because the fragments are adjacent, a sequence of 150 base pairs preceding the *araBAD* mRNA start site has been determined. The sequence shown in Fig. 4 contains the entire *araBAD* promoter. This conclusion is based on the fact that *ara*Δ*719* leaves the promoter intact (4) and that the end point of this deletion is within 120 base pairs of the *Bam* I site (Fig. 2). The *Bam* I restriction fragment containing *araB* and part of *araA* has been cloned but it is not able to complement *araB* mutants (20). It has also been shown that a DNA template restricted with *Bam* I is not active in a purified *in vitro* transcription system (2). Thus, the *Bam* I site is within the promoter.

FIG. 5. Comparison of sequences preceding the mRNA start site in the *lac* (21), *gal* (22), and *araBAD* operons.

Several features of the sequence are interesting. The region contains 60% dA·dT base pairs. The regions that contain predominantly dA·dT base pairs alternate with regions rich in dG·dC base pairs. There are stop codons in every reading frame on both strands. Regions of dyad symmetry are centered at −75 and −59/−60. This region of the sequence is probably required for *araBAD* transcription but at present we do not know if these symmetries are binding sites for regulatory proteins.

The *ara* (1), *lac* (21), and *gal* (22), operons are sensitive to catabolite repression; they all require the cyclic AMP–CRP complex for maximal expression. A comparison of the DNA sequences preceding the mRNA start sites is shown in Fig. 5. There is some homology to the sequence TATPuATG near the start of the mRNA in *ara*, *lac*, and *gal*. This sequence has been noted for many bacterial and phage promoters (23). There is also a sequence at −35, ACACTTTT, that is similar in the *ara*, *gal*, and *lac* promoters. The region at −35 has been suggested to be involved in RNA polymerase recognition (23).

In Fig. 6, a potential binding site for the cyclic AMP–CRP complex is shown. This sequence in *ara* is similar to the known CRP binding sites in *lac* and *gal*. We cannot be certain whether this is the only site for CRP binding in the *ara* region; other binding sites not as homologous to the sequences in Fig. 6 exist, for example at −95 to −110. However, if there is only one CRP binding site in *ara*, this would be interesting for two reasons. First, the *araC* promoter is stimulated 2 to 3-fold by the cyclic AMP–CRP complex both *in vivo* (24) and *in vitro* (2) and therefore might share this site with the *araBAD* promoter. A single regulatory site shared by two promoters could explain the observation that transcriptions of *araC* and *araBAD* are interactive *in vitro* (2). Second, if the proposed CRP site regulates *araBAD*, then it places the cyclic AMP–CRP complex at a much greater distance from the presumed RNA polymerase recognition region at −35 than in the *lac* and *gal* operons.

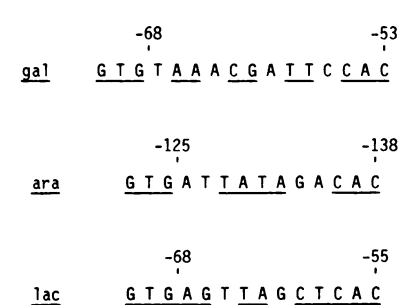

FIG. 6. Comparison of sequences in *lac* (21), *gal* (22), and *ara* that are probably involved in the interaction with the CRP.

This research was supported by National Science Foundation Grant PCM 76-80445. L.G. was supported by Training Grant CA-09056-03 from the National Cancer Institute, T.B. by the National Research Service Award training program in Genetics and Regulatory Mechanisms, and G.W. by an American Cancer Society Faculty Research Award.

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