JOURNAL OF BACTERIOLOGY, June 1995, p. 3438–3442
0021-9193/95/$04.00+0

Vol. 177, No. 12

In Vivo Induction Kinetics of the Arabinose Promoters in *Escherichia coli*

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Received 25 October 1994/Accepted 23 March 1995

In *Escherichia coli*, the AraC protein represses transcription from its own promoter, p_C, and when associated with arabinose, activates transcription from three other promoters, p_{BAD}, p_E, and p_{FGH}. Expression from all four of these promoters is also regulated by cyclic AMP-catabolite activator protein; however, the arrangement of the protein binding sites is not identical for each promoter. We are interested in determining how the AraC protein is able to activate p_{BAD}, p_E, and p_{FGH} despite their differences. We have characterized the induction response of the wild-type arabinose operons from their native chromosomal locations by primer extension analysis. In this analysis, mRNA from the four arabinose operons plus an internal standard could all be assayed in the RNA obtained from a single sample of cells. We found that each of the operons shows a rapid, within 15 to 30 s, response to arabinose. We also found that the expression of *araFGH* is more sensitive to catabolite repression but not to arabinose concentration than are *araE* and *araBAD*. Finally, we have determined the relative levels of inducibility in wild-type cells of *araBAD*, *araFGH*, and *araE* to be 6.5, 5, and 1, respectively. These results provide a basis for subsequent studies to determine the mechanism(s) by which AraC protein activates transcription from the different arabinose promoters.

Arabinose utilization in *Escherichia coli* requires the expression of the metabolic operon, *araBAD* (8, 9, 33), and expression of either the low-affinity transport operon, *araE* (22), or the high-affinity transport operon, *araFGH* (3, 12, 15). Induction of each of these operons normally requires the AraC protein (5) complexed with arabinose and the catabolite activator protein (CAP) complexed with cyclic AMP (cAMP). The regulatory protein binding sites and the transcription start sites at each of the arabinose-responsive promoters have been determined (10, 17, 18, 23, 31, 32). Studies of the *araBAD* promoter, p_{BAD}, show that to activate transcription the AraC protein binding site must overlap the −35 region of the promoter by 4 bp (25) (Fig. 1). Further, the two half-sites recognized by the dimeric AraC protein must be in the same direct repeat orientation (4, 21) to activate transcription. These facts suggest that specific contacts are made between the AraC protein and RNA polymerase at p_{BAD}. Providing further support for this theory is the almost identical arrangement of the protein binding sites for *araBAD*, *araE*, and *araJ*, a weak, arabinose-inducible promoter whose gene product is nonessential (24). Surprisingly, the *araFGH* promoter, p_{FGH}, possesses a radically different structure (Fig. 1). In p_{FGH} the CAP site, rather than the AraC site, overlaps the −35 recognition sequence of RNA polymerase. Additionally, the AraC sites in *araFGH* are arranged in the opposite direct repeat orientation (Fig. 1).

This work is a first step in studying the mechanism(s) by which AraC regulates transcription at the *araE* promoter, p_E, and at p_{FGH}. In the present study, we have measured the induction kinetics and RNA levels of the repressible promoter, p_C, and the three activated promoters, p_{BAD}, p_E, and p_{FGH}, from their native chromosomal locations by direct primer extension analysis of mRNA. The mRNA from the four arabinose operons plus an added internal standard could all be assayed with the RNA obtained from a single extraction, thus allowing quantitative comparisons of the different promoter activities. We have found that each of the operons shows a rapid response, within 15 to 30 s, to arabinose and have determined the relative induced levels of *araBAD*, *araFGH*, and *araE* under wild-type conditions. We have also found that the kinetics depend on catabolite repression, with regard to both the intensity and the duration of expression. *araFGH* has been found to be much more catabolite sensitive than the other operons. Finally, we have found that all of the operons are immediately inducible by concentrations of arabinose as low as 0.53 mM in the growth medium.

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MATERIALS AND METHODS

Oligonucleotides. Oligonucleotides for primer extensions and for cloning were synthesized on an Applied Biosystems 381A synthesizer, deprotected (28), and purified (7) as previously described. The oligonucleotides used as primers for primer extension reactions were as follows: CJ825, which hybridizes to positions +52 to +73 of *araE* mRNA (5′-CCGCAAAGAACGTGGCGTTAA-3′); CJ826, which hybridizes to +75 to +95 of *araFGH* mRNA (5′-TGTCGTTTTGTGT AGGGCAAA-3′); CJ919, which hybridizes to +167 to +187 of *araC* mRNA (5′-GGATCATTTTGCGCTTCAGCC-3′); and CJ1053, which hybridizes to +35 to +60 of *araBAD* mRNA (5′-ACTGCCAAAATCGAGGCCAATTGCA-3′).

Construction of internal standard plasmid pCJIS1. Two complementary oligonucleotides were synthesized that, when hybridized, would produce an insert with sticky-end overhangs for *Nco*I and *Bst*BI. The sequence of the sense strand is 5′-CCATGGTCGTGCAATTGGCCTCGATTTTGGCAGTTTAACG CCACGTTCTTTGCGGTTTGCCCTACACAAAAGCACAGGCTGAAGCG CAAAATGATCCTTCGAA-3′. After treatment with kinase and hybridization, the oligonucleotides were ligated into the *Nco*I and *Bst*BI sites of plasmid pSE380 (Invitrogen, San Diego, Calif.), a derivative of pTrc99A (1) which carries a tightly regulated *trp-lac* promoter. Under the control of this promoter, the insert produced a single mRNA strand containing the *araC*, *araBAD*, *araE*, and *araFGH* sequences complementary to each of the oligonucleotides used in the primer extension assay. The general cloning methods used were as described previously (27, 29).

Bacterial strains. The *E. coli* strains (with only relevant markers listed) used were ECL116 (F^- Δ*lacU169 endA hsdR thi*) (2), with a doubling time of 95 min in minimal media, and RFS1581 (F^- Δ*lacRV thr leu isoI* Su^- Sm^r $AraC^+B^-A^+D^+$ from JTL28) (19) and CMJ1 ($AraC^+B^+A^+D^+$ revertant from RFS1581), with a doubling time of 80 min in minimal media.

Media and culture methods. Cells were grown in M10 salts (29) plus 0.4% glycerol, and 50 μM thiamine. When required, threonine, leucine, and isoleucine were added to a final concentration of 20 μg/ml. Exogenous cAMP, when added, was at a final concentration of 5 mM. Single colonies of cells were picked from yeast extract-tryptone (YT) plates and grown in 5-ml overnight cultures of M10

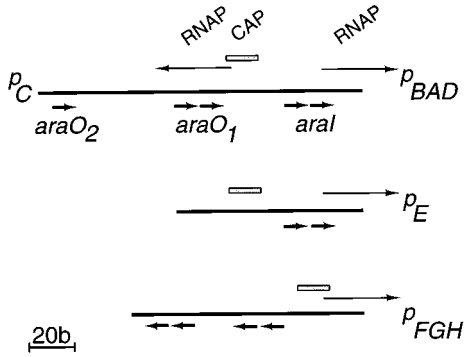

FIG. 1. Regulatory protein binding sites of the arabinose promoters in *E. coli* (drawn to scale). Small and large arrows represent AraC protein half-site sequences and RNA polymerase (RNAP) binding sites, respectively. The shaded rectangles represent CAP binding sites. b, bases.

salts. These cultures were diluted 1/500 into 150 ml of medium and grown overnight. Finally, these cultures were diluted 1/500 into a 300-ml culture and grown for approximately 5 h. This growth regimen was found to be necessary for obtaining reproducible results during the following manipulations. Optical density was monitored to ensure that growth was exponential. Cell density at the time of the assay was between 2.9×10^7 and 5.8×10^7 cells per ml. The 5-ml cultures were grown in test tubes in a rotary 37°C incubator. Both the 150- and 300-ml cultures were grown at 37°C in flasks that were no more than 1/10th full and that were shaken vigorously at approximately 280 rpm. For the generation of the internal standard mRNA, ECL116 cells with pCJIS1 were grown in YT medium (27) in the presence of 200 μg of ampicillin per ml. The arabinose-responsive mRNAs and the standard mRNA were induced with 13.3 mM arabinose and 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside), respectively, unless otherwise indicated.

Isolation of cellular RNA. For the induction kinetics assay, cells were grown to a density of 2.9×10^7 cells per ml and then were induced with 13.3 mM arabinose. If exogenous cAMP was added, cells were allowed to grow in its presence for 20 min before arabinose was added. At various time points, 10-ml samples were added to an equal volume of M10 salts containing 10 mM sodium azide, chloramphenicol (125 μg/ml), and rifampin (600 μg/ml), on ice. Samples were concentrated by centrifugation and then frozen for at least 5 h at −70°C. For the measurements of the response of the *ara* promoters to various concentrations of arabinose, cells were grown to approximately 4.0×10^7 cells per ml. When cAMP was added, cells were allowed to grow for 10 min. Ten-milliliter samples were transferred with pipettes into sterile 125-ml flasks, already shaking at 37°C. After a second 10-min incubation, so that cells remained in the presence of cAMP for a total of 20 min, cells were induced by the addition of 1 ml of arabinose at 10 times the desired final concentration. The 10-ml samples were then poured from the flasks into an equal volume of the ice-cold sodium azide mixture and processed as described above.

As a control, uninduced cells were added to the sodium azide mixture containing 133 mM arabinose. RNA extracted from those cells was assayed as described below for arabinose-specific mRNA. The absence of a detectable signal indicated that once added to this mixture, cells were no longer able to transcribe.

Frozen samples were allowed to thaw at room temperature and were then resuspended in 725 μl of 50 mM Tris (pH 8.0). After addition of 275 μl of cells containing the internal standard plasmid (also resuspended in 50 mM Tris) to each tube, the samples were then treated with 30 mM EDTA and lysozyme (2 mg/ml) for 30 min on ice. Four milliliters of RNAzol (4 M guanidine thiocyanate, 1% 2-mercaptoethanol, phenol) from Cinna/Biotecx (Friendswood, Tex.) (6) was added, and the samples were vortexed twice for 30 s, with a 15-s pause, between pulses. After the addition of 1 ml of chloroform and vigorous mixing, the samples were allowed to sit for at least 30 min on ice. The samples were then centrifuged for 30 min at 27,000 × *g* at 4°C. The aqueous phase (approximately 2.5 ml) was collected, and total RNA was precipitated by the addition of a 5-ml solution of 4 M LiCl, 20 mM Tris (pH 7.6), and 10 mM EDTA for not less than 4 h at −20°C. After centrifugation at 27,000 × *g* for 15 min in the cold, pellets were resuspended in 400 μl of water, transferred to 1.5-ml microcentrifuge tubes, and precipitated for 2 h in 1 ml of 100% ethanol (without additional salt). Samples were centrifuged at 12,000 × *g* at 4°C for 15 min, dried by lyophilization, and resuspended in 30 μl of water. This double precipitation step was absolutely necessary for preparing the RNA for use in the enzymatic reactions. The use of LiCl rather than isopropanol gave us mRNA without inhibitors of reverse transcriptase. This method provides quantities of mRNA adequate for multiple in vivo transcription assays.

Primer extension mapping and quantitation. Primer extension reactions were performed as previously described (25) with the following modifications. One-fifth of each total cellular RNA extraction was mixed with 20 ng of [γ-^{32}P]ATP-labeled primer, CJ825, CJ826, CJ919, or CJ1053 and with 1 μl of 5× hybridization buffer {5× buffer: 2 M NaCl, 0.2 M PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)]-HCl (pH 6.5), 5 mM EDTA} to yield a total volume of 15 μl. The samples were heated to 90°C for 5 min and allowed to cool to 37°C for 30 min. Ten microliters of reaction mixture was added to give final concentrations of 1 mM in each deoxynucleoside triphosphate, 50 mM Tris HCl (pH 8.3), 10 mM dithiothreitol, 75 mM KCl, 3 mM $MgCl_2$, and 100 U of Moloney murine leukemia virus reverse transcriptase. The reaction mixtures were incubated in a 37°C air incubator for 30 min and then were precipitated with ethanol. After drying, samples were resuspended in 5 to 25 μl of a 1:1 (vol/vol) mixture of Tris-EDTA (TE) buffer and stop solution (95% formamide, 25 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol FF). cDNAs were then separated by electrophoresis on a 6 or 10% polyacrylamide–urea gel. Sequencing reactions with DNA from pCJIS1 were used to generate size standards. The relative band intensities of the cDNAs were quantified with a Molecular Dynamics PhosphorImager (13, 26). The band intensity was then used to calculate the relative numbers of mRNA molecules per cell, based on sample cell density and primer specific activity. The specific activity of each primer was determined after a known amount (assuming that an optical density at 260 nm of 1 is equivalent to 20 μg/ml) of radioactive primer was separated from the unincorporated label by gel electrophoresis. The band intensity generated by the primer was then quantitated with a Molecular Dynamics PhosphorImager. The data presented here are averages of at least three separate experiments, except for the single measurement of the induction kinetics of p_{FGH} in an arabinose metabolism-deficient strain, *araB*, with exogenous cAMP.

RESULTS

Internal standard plasmid pCJIS1. We have measured the induction response of each of the *E. coli* arabinose promoters over the first 70 min following arabinose addition. At the various time points, total cellular RNA was extracted and the *ara*-specific messengers were transcribed to DNA and quantitated.

To compensate for any nonspecific loss of mRNA, either during the extraction step or during the precipitation step after the reverse transcription reaction, an internal standard was constructed such that each *ara*-specific oligonucleotide was able to hybridize both to the *ara*-specific messenger in question and to a control RNA that was added to all samples. This control RNA contained sequences complementary to all four *ara* promoters studied in this work (see Materials and Methods). A plasmid coding for this standardizing RNA, under control of a *trp-lac* promoter, pCJIS1 (Fig. 2A), was transformed into wild-type strain ECL116. Cells containing this plasmid were induced with IPTG for 15 min. At the time of RNA extraction, an equal number of cells (from a single pellet) containing pCJIS1 were added to each sample of arabinose-induced cells. RNA was then extracted, and primer extension reactions were performed. Bands representing standard and arabinose-specific cDNAs were identified on urea-polyacrylamide sequencing gels (Fig. 2B) and quantitated with a Molecular Dynamics PhosphorImager. The signal intensities for the standard were used to normalize the individual *ara* mRNA bands.

For reasons we could not explain or remedy, the primer extension reactions produced a high-level background in the gel lane (Fig. 2B). This interference obscured low-level signals. Fortunately, we were able to detect an arabinose-specific signal above those background levels as early as 30 s after arabinose was added.

Induction of p_C is not dependent on arabinose metabolism. In the wild-type strain, the maximum levels of *araC* mRNA were detected within 45 s after the beginning of induction with arabinose. In that time, the *araC* message increased from less than 1.7 molecules per cell prior to induction to approximately 3.9 molecules per cell. After 5 min, however, the messenger levels decreased and by 30 min, the level of *araC* mRNA had fallen to below our background level (Fig. 3). The half-life of

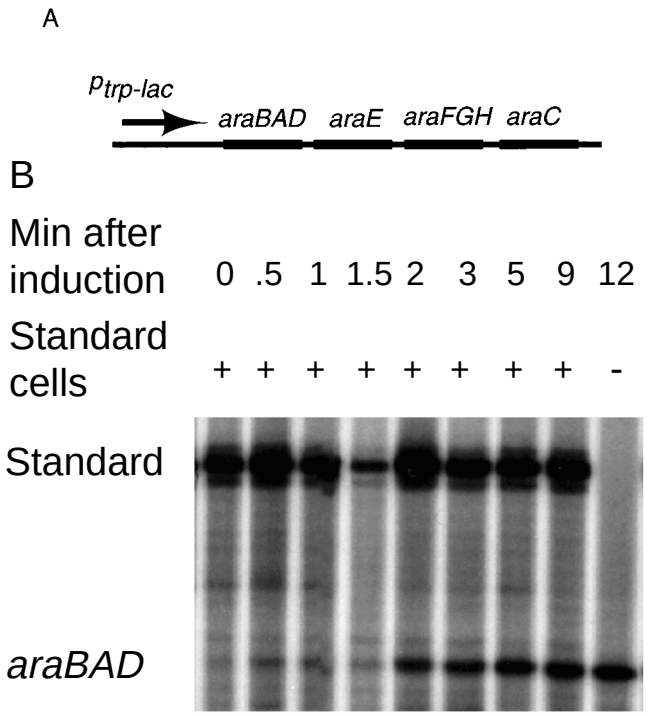

FIG. 2. Summary of standardizing mRNA structure. (A) Diagram of sequences cloned into a vector with a tightly regulated promoter that, when transcribed, produced a single-stranded mRNA with sites complementary to each of the oligonucleotides used for primer extensions. (B) Gel electrophoresis results. Each oligonucleotide produced cDNAs of two different sizes, one representing the standard mRNA and the other representing arabinose-specific mRNA. The position of *araBAD* is depicted here. The gel was scanned into Adobe Photoshop, version 2.5.

the mRNA, estimated from the kinetics of the approach to steady-state levels, was approximately 1 min.

Repeating the measurements with a strain which was unable to metabolize arabinose, RFS1581, indicated similar kinetics (Fig. 3) and messenger half-life.

Induction of AraC-activated promoters in wild-type *E. coli*. As early as 30 s after induction, p_{BAD} gave a level of messenger of approximately 10 molecules per cell. After 5 min, the *ara BAD* mRNA reached maximum levels of approximately 30 molecules per cell. The levels of mRNA began to decrease after 17 min, and by 70 min the *araBAD* message level had fallen very close to the background levels in our assay (Fig. 4).

The p_E promoter also showed a rapid response to arabinose. By 1 min *araE* mRNA reached 50% of its maximum levels. After 5 min, there were about three molecules of p_E mRNA

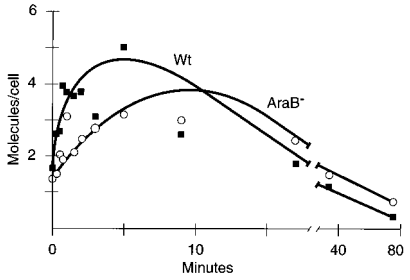

FIG. 3. Induction kinetics of p_C as a function of time in wild-type (Wt) ECL116 and RFS1581 cells (solid squares) and AraB$^-$ CMJ1 cells (open circles).

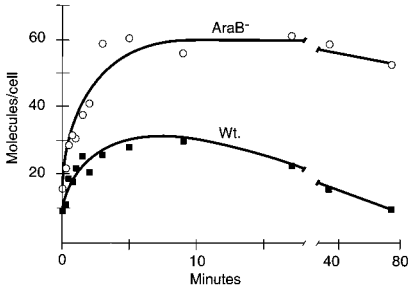

FIG. 4. Induction kinetics of p_{BAD} as a function of time in wild-type (Wt.) ECL116 and RFS1581 cells (solid squares) and AraB$^-$ CMJ1 cells (open circles).

per cell. Similarly to the results with *araBAD* mRNA, this message declined after 30 min and by 70 min, its level was only one-fourth its level at the maximum (Fig. 5).

One minute after arabinose addition, p_{FGH} reached a level of approximately 10 molecules of mRNA per cell, which further increased to approximately 25 molecules per cell by 9 min (Fig. 6). Unlike the gradual reduction in the *araE*, *araBAD*, and *araC* mRNA levels, the fall in *araFGH* mRNA levels was more rapid: by 17 min the levels were half that at maximum, and by 70 min only 1.5 molecules per cell were detected (Fig. 6).

The kinetics of induction of the four promoters did not significantly differ when cells were induced with 133 mM arabinose (data not shown) rather than 13.3 mM arabinose, showing that the turndown in promoter activity was not due to insufficient levels of arabinose in the medium.

The half-life of each mRNA was estimated from the kinetics of the approach to steady-state levels. All of the mRNAs had half-lives of 1 to 2 min. Since the messenger half-lives were comparable and short on the time scales covered in these experiments, the activities of the promoters closely paralleled the messenger levels measured. At the time of maximum activity, approximately 3 min after arabinose addition, the p_{FGH} and p_{BAD} promoters were, respectively, 5 and 6.5 times as active as the p_E promoter.

Because *araFGH* specifies the high-affinity arabinose uptake system, it seems plausible for the p_{FGH} promoter to be induced by particularly low concentrations of arabinose and perhaps not to be significantly induced by high arabinose concentrations. This response would explain the need for two separate transport systems in one cell and would explain why p_{FGH} is so catabolite sensitive. We found, however, that all of the *ara* promoters are activated by concentrations of arabinose as low as 0.53 mM. We also found that the relative activities for each promoter do not significantly differ when induced with 133,

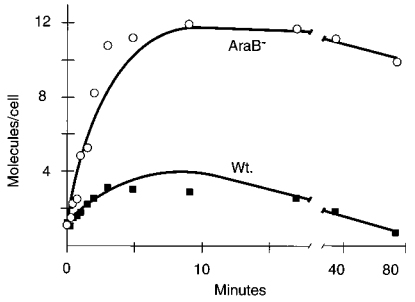

FIG. 5. Induction kinetics of p_E as a function of time in wild-type (Wt.) ECL116 and RFS1581 cells (solid squares) and AraB$^-$ CMJ1 cells (open circles).

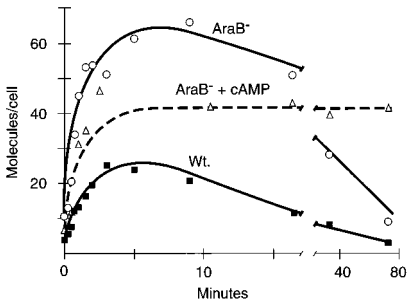

FIG. 6. Induction kinetics of p_{FGH} as a function of time in wild-type (Wt.) ECL116 and RFS1581 cells (solid squares) and in AraB$^-$ CMJ1 cells in the presence (open triangles) and absence (open circles) of exogenous cAMP.

13.3, 9.3, 5.3, 1.33, 0.93, or 0.53 mM arabinose (data not shown).

Induction of AraC-activated promoters in an *araB* mutant. To test whether the turndown of the various promoters which occurs after approximately 10 min was brought on by arabinose metabolism, the induction kinetics of the different *ara* promoters were also tested in a mutant unable to metabolize arabinose due to a nonpolar point mutation in the *araB* gene, RFS1581. Curiously, it took slightly longer for the promoters to reach full activation in the *araB* strain, yet full activation level was approximately two to three times higher than that of wild-type cells. This is a result consistent with the presence of self-catabolite repression (14). Both p_{BAD} and p_E maintained their maximum induction levels for at least 130 min after induction (Fig. 4 and 5). The p_{FGH} promoter was also significantly affected by the lack of arabinose metabolism (Fig. 6). The promoter was slightly more active than that in the wild-type cell, but it still lost activity by 70 min after induction. Neither the mRNA half-lives nor the relative promoter inducibilities of RSF1581 are altered significantly from those of the wild-type strain.

p_{FGH} is highly sensitive to catabolite repression but not to arabinose. It has previously been shown that p_{FGH} is more dependent on CAP than the other *ara* promoters (16). This difference is not surprising, since the placement of the CAP and AraC protein binding sites on p_{FGH} suggest that CAP, not AraC, is the primary activator interacting with RNA polymerase. We hypothesized that since p_{FGH} is more sensitive to the catabolism of arabinose than the other arabinose promoters and since RFS1581 behaved in the experiments described above as if it were leaky (p_{FGH} still shut down after induction), the addition of exogenous cAMP to the media could have an effect on the promoter. Indeed, in the presence of cAMP, p_{FGH} reached and maintained maximum levels for at least 70 min after induction (Fig. 6). When the same concentration of cAMP was added to media containing wild-type cells, the induction response of p_{FGH} to arabinose was similar to the response seen when RSF1581 was used in the absence of cAMP. The mRNA reached higher levels, for slightly longer periods, but was still completely turned off within 70 min of induction (data not shown).

DISCUSSION

The experiments described in this report provide a detailed description of the transcriptional regulation of the *araBAD*, *araE*, and *araFGH* promoters in *E. coli* in vivo. Each of our measurements was made from the same strain, with all RNAs produced at the same time in the cell. Thus, differences between promoter activities cannot be ascribed to possible differences in strains or copy numbers of plasmids. The results of this work provide us a framework with which to compare the results of further studies addressing the mechanisms by which the AraC protein regulates the different *ara* promoters.

Regulation by the AraC protein of p_{BAD} and p_C has been extensively characterized (20). For p_{BAD}, the two half-sites $araI_1$ and $araI_2$ designate the stronger polymerase-distal and the weaker polymerase-proximal sites, respectively (Fig. 1). In the absence of arabinose, one monomer of the AraC dimer occupies the $araI_1$ site, while the other occupies a half-site approximately 200 bp away, known as $araO_2$, via DNA looping (20). This looping most likely inhibits the accessibility of RNA polymerase to its binding site at p_C, thus allowing only a low level of transcription of *araC*. When arabinose is added, the AraC protein undergoes a conformational change and shifts to occupy the adjacent half-sites, $araI_1$ and $araI_2$ (20). As a result, p_{BAD} is induced. Furthermore, because the loop is opened, free access of RNA polymerase to the p_C promoter likely increases, thereby explaining this promoter's increased activity for about 10 min. Subsequently, p_C shuts down as a result of the AraC protein binding to the $araO_1$ site and blocking the access of RNA polymerase to the p_C promoter.

At p_{BAD}, moving of the AraC protein binding site further upstream or reversing of the orientation of the half-sites destroys the contacts and prevents induction (25). These results imply that specific contacts are made between the AraC protein and RNA polymerase. Thus, it is not surprising that the spacing of the AraC protein binding sites at p_E and at p_J are almost identical. At p_{FGH}, however, the CAP protein and not the AraC protein occupies the site adjacent to and partially overlapping the −35 region (Fig. 1). The AraC protein binds upstream from CAP, at two different sites, one centered at position −80 and the other centered at −154. Compared with the half-site orientations at p_{BAD}, all four half-sites at p_{FGH} are oriented in the opposite direction, yet in vitro transcription studies have shown that p_{FGH} requires the AraC protein for full activation (10). How is AraC able to activate p_{FGH} in this atypical arrangement?

Studies aimed at the quantitative analysis of the activities of the *ara* promoters by different methods have been done before. Kolodrubetz and Schleif used Mu-*lac* fusions to study the in vivo regulation of the arabinose operons (16). They found that p_{FGH} is much more catabolite sensitive than p_{BAD}, which was found to be more catabolite sensitive than p_E. Kolodrubetz and Schleif also found that p_{BAD} is induced to levels about three times higher than those of p_{FGH} and p_E and that all three are induced at about the same concentrations of arabinose. Stoner and Schleif (30–32) and Hendrickson and others (10) used S1 nuclease to map the transcription start sites and to measure the kinetics of mRNA induction for the *ara* promoters. The Mu-*lac* fusion studies, however, and the S1 nuclease mapping both contained fewer and more widely separated time points than our study and were done on samples isolated from different strains at different times for each promoter. Further, because the total amount of enzyme present at different times was measured in the fusion studies, these measurements also were unable to detect the down regulation we have described in this paper.

We expected the arabinose promoters to remain maximally active after induction. Surprisingly, we found that the promoters are down regulated after arabinose metabolism begins. Because the turndown could be countermanded by blocking arabinose metabolism or fully eliminated by blocking arabinose metabolism and adding exogenous cAMP, it appears to be a result of catabolite repression. Because the *ara* mutant

that we used was slightly leaky in its inability to metabolize arabinose, we were able to show that p_{FGH} is more susceptible to catabolite repression than the other promoters. This difference is consistent with the previous finding that p_{FGH} is more dependent on CAP for activation than are the other promoters (16).

Although we could not reduce background levels enough to make accurate basal level determinations, our data show that p_{BAD} is more active than p_E and p_{FGH} and that all three promoters are induced by the same concentrations of arabinose.

It is interesting that p_E and p_{BAD} show such similar induction kinetics. Previous work has found no evidence for DNA looping at p_E. Initially, we had assumed that the 10-min delay in the onset of repression at p_C in the presence of arabinose reflected the time required for the AraC protein to bind to $araO_1$ after the site becomes accessible and that rapid induction at p_{BAD} (11) was due to the presence of the AraC protein at the $araI_1$ site prior to induction. Therefore, it seemed likely that p_E would not be induced until the AraC protein could find its previously unoccupied binding site and that induction at p_E would take significantly longer than it takes for the AraC protein at p_{BAD} to change conformations. On the basis of the induction kinetics we have obtained for p_E, we believe it is possible that the AraC protein is able to find its DNA binding sites much faster than originally thought or that AraC is somehow bound at p_E in the absence of arabinose.

The sensitivity of p_{FGH} to catabolite repression is not surprising. In fact, the structure of the promoter, with the CAP binding site being the more proximal to RNA polymerase, suggests that the AraC protein is required only to make the promoter arabinose specific. Although Hendrickson et al. reported activation of p_{FGH} in the absence of the AraC protein in vitro (10), we could not detect activation in vivo of p_{FGH} in *araC* mutants (data not shown). This absence and the potential for binding two dimers of the AraC protein to p_{FGH} imply that AraC protein plays a significant role in the activation of this promoter.

In summary, we have found that the *ara*-specific promoters are able to respond rapidly to arabinose and that their relative inducibilities under wild-type conditions do not change with changes in induction of arabinose concentrations. We have also found that the promoters, especially the *araFGH* promoter, are sensitive to catabolite repression.

ACKNOWLEDGMENTS

We thank Richard Gourse and Wilma Ross for suggestions on developing the standardizing mRNA, the members of our laboratory and Fred Moshiri for critical discussions and comments on the manuscript, and Carey Johnson for computer programming assistance.

This work was supported by National Institutes of Health grant GM18277 to Robert Schleif. Casonya Johnson is the recipient of predoctoral fellowship GM14202 from the National Institutes of Health.

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