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In Vivo Regulation of the *Escherichia coli araC* Promoter†

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The *ara* p_C promoter is known to be derepressed about fivefold for 20 to 30 min after the addition of arabinose. This transient derepression was studied by using *araC*::Mu *lac* insertions and *araC-lacZ* gene fusions. In strains containing increased levels of *araC* protein, the p_C promoter became progressively less derepressible, but the *ara* p_{BAD} promoter remained normally inducible. Repression of p_C was reestablished 20 min after induction in *araB* mutants, but did not occur in arabinose-transport-deficient mutants. Finally, mutant $araC^c$ proteins which normally do not repress p_C did so in the presence of arabinose.

Expression of the *araCBAD* gene complex of *Escherichia coli* is regulated by the *araC* gene product, which functions both positively and negatively to regulate expression of the *araBAD* operon (2, 3, 13). *araC* protein also acts negatively on expression of the *araC* promoter, p_C. Using *araC-lacZ* gene fusions, Casadaban (1) found that in steady state *araC* protein represses its own synthesis in the presence or absence of the inducer arabinose, and that expression of p_C is stimulated by the cyclic AMP (cAMP) receptor protein-cAMP complex (CRP-cAMP). Haggerty (Ph.D. thesis, Brandeis University, Waltham, Mass., 1977) and Ogden et al. (9) later found that p_C is derepressed by the addition of arabinose, but 20 to 30 min after arabinose addition p_C is repressed back to its former rate of expression.

In light of the overlapping binding sites for RNA polymerase and *araC* protein at p_C (7) (Fig. 1), an arabinose-induced decrease in the occupancy of the *araO* site would allow more frequent initiations by RNA polymerase at p_C. Indeed, such a proposal has been made (9). Although previous in vitro experiments are not consistent with this interpretation (7) as discussed below, they were not done under conditions which accurately measured the affinity of *araC* protein for *araO*.

In the work reported here, we studied the transient derepression of the *araC* promoter in vivo more extensively with the objective of learning more about the phenomena of the initial derepression and the subsequent reestablishment of repression. One interpretation of our results is that low intracellular arabinose concentrations decrease the affinity of *araC* protein for *araO* and higher arabinose concentrations restore it to near its initial value. We also show that in the presence of arabinose, repression of p_C can occur independently of p_{BAD} induction.

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MATERIALS AND METHODS

Media, chemicals, and strains. The composition of YT broth, minimal salts medium for plates, and M10 medium was described previously (11). Sugars were added to 0.2%. Thiamine was added to all media to 0.001%. Amino acids were added where required to 0.005%, streptomycin to 200 μg/ml, and other antibiotics to 15 μg/ml. All biochemicals were purchased from either Sigma Chemical Co. or Fisher Scientific. All strains used were derivatives of *E. coli* K-12 and are listed in Table 1.

Enzyme assays. β-Galactosidase and arabinose isomerase were assayed as previously described (11).

Genetic methods. P1 transductions and matings were done by the method of Schleif and Wensink (11). Matings with temperature-sensitive Mu *lac*-containing strains were done at 33°C for 2.5 h. Mu *lac*-containing episomes were mated into strains lysogenic for phage Mu to prevent zygotic induction. All other manipulations with these strains were carried out at 30°C.

Isolation of *araC-lacZ* fusion *araB*$^-$*A*$^-$*D*$^-$ strains. P1 grown on strain DMH90 (*leu araC-lacZ* fusion *araB*$^+$*A*$^+$*D*$^-$) was used to transduce a strain deleted of *lac* and containing an *araB* polar mutation to Lac$^+$ Leu$^-$. These Lac$^+$ transductants were screened for a functional *araB* gene by complementation. About 1% of the Lac$^+$ transductants were *araC-lacZ* fusion *araB*$^-$*A*$^-$*D*$^-$.

Growth of cells for assays of β-galactosidase. Cells from overnight growth in YT broth were used to inoculate flasks containing M10 medium, 0.2% glycerol (CRP$^+$ strains) or glucose (CRP$^-$ strains), and inducers when indicated. Cells were grown for at least four generations to a density of about 5×10^7 cells per ml, with growth monitored turbidometrically. Portions of the cultures were harvested and assayed for β-galactosidase. For induction experiments, 0.1-ml sam-

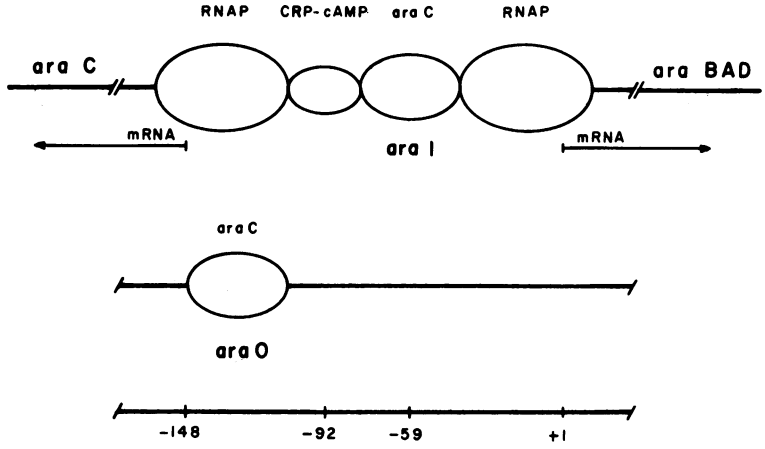

FIG. 1. *araCBAD* regulatory region. Location of the binding sites for *araC* protein, CRP-cAMP, and RNA polymerase (RNAP) as determined by DNase I footprinting and methylation protection. The numbers give the position (in base pairs) relative to the start of the *araBAD* message. CRP-cAMP also bound with low affinity at position −212 to −146, but this binding was not shown to be physiologically relevant. *araI* and *araO* refer to the *araC* protein-binding sites.

ples were removed at the indicated times with an automatic pipette. Mu *lac*-containing strains were grown at 30°C, and all other strains were grown at 35°C. For the experiments shown in Fig. 2 through 5, cells were grown for five generations in M10 medium with glycerol (and tetracycline [15 μg/ml] where required) before 0.2% arabinose was added.

RESULTS

***araC-lacZ* fusions.** *araC-lacZ* fusions were generated in one step by the insertion of Mu *lac* phage into *araC*-containing episomes (6). The properties of one representative insertion are shown in Table 2. The activity of the p_C promot-

TABLE 1. Bacterial strains

Strain	Genotype/phenotype	Comments/reference
DMH90	F⁻ *araC-lacZ* fusion, *araB⁺A⁺D⁻ leu* Str^r *thi*	*leu* derivative of strain FC-17 (1)
DJK287	F′ *araC*::Mu *lac-287 araB⁺A⁺D⁻ leu⁺ thr⁺*/Δ(*ara-leu*)*498* Δ*lac-74 thi*	Mu *lac* insertion in *araC* (6)
RFS720	F′ *leu⁺ thr⁺ ara(CBAD)⁺*/Δ(*ara-leu*)*498 thi*	10
RFS F′102	F′ *leu⁺ thr⁺ araC102 ara(BAD)⁺*/ Δ(*ara-leu*)*498 thi*	10
SH121	F′ *leu⁺ araC⁺ araB53*/Δ(*araC-leu*)*1022 ara(BAD)⁺ thi*	*araB* polar mutation (10)
SH123-146	F′ *leu⁺ araC^c araB53*/Δ(*araC-leu*)*1022 ara(BAD)⁺ thi*	Independent fucose-resistant isolates of strain SH121
SH172	F⁻ *thr leu* Δ*lac-74* Str^r *thi* Mu *c*(Ts)	Strain RFS1070 (10) lysogenic for Mu
SH173	F⁻ *thr leu* Δ*lac-74* Δ*crp* Str^r *thi* Mu *c*(Ts)	Strain SH172 P1-transduced to Δ*crp*
SH204	F⁻ Δ(*araC-leu*)*1022 ara(BAD)⁺* Δ*lac-74* Str^r *thi* Mu *c*(Ts)	Strain RFS1070 (10) deleted for *araC* and lysogenic for Mu
SH206	F⁻ Δ(*araC-leu*)*1022* Δ*lac-74* Δ*crp* Str^r *thi* Mu *c*(Ts)	Strain SH204 P1 transduced to Δ*crp*
SH242	F⁻ *araC-lacZ* fusion *araB180 leu thr* Δ*lac-74* Str^r *thi*	*araC-lacZ* fusion of strain FC-17 (1) P1 transduced to strain RFS1070 containing an *araB* polar mutation
SH271	F⁻ *araFG51 araE78* Δ*lac-74 leu* Str^r *thi* Mu *c*(Ts)	Derivative of strain DJK126 (D. Kolodrubetz, Ph.D. thesis, Brandeis University, Waltham, Mass., 1981)
SH281	F⁻ *araFG51* Δ*lac-74 leu* Str^r *thi* Mu *c*(Ts)	Strain SH271 P1 transduced to *araE⁺*
SH284	F⁻ *araC-lacZ* fusion *araB180 araE78 araFG51* Δ*lac-74 leu* Str^r *thi*	*araC-lacZ* fusion of strain SH242 P1 transduced to strain SH271
SH285	F⁻ *araC-lacZ* fusion *araB180 araFG51* Δ*lac-74 leu* Str^r *thi*	Strain SH284 P1 transduced to *araE⁺*

TABLE 2. CRP-cAMP and *araC* protein dependence of p_C[a]

F⁻ strain	Chromosomal genotype	β-Galactosidase (monomers/cell)
SH172	*ara(CBAD)*$^+$	2,250
SH172	*ara(CBAD)*$^{+b}$	2,070
SH204	*araC ara(BAD)*$^+$	14,700
SH173	*ara(CBAD)*$^+$ Δ*crp*	780
SH206	*araC ara(BAD)*$^+$ Δ*crp*	2,280

[a] Steady-state β-galactosidase levels were measured in strains containing episome F′ *araC*::Mu *lac-287* *araB*$^+$*A*$^+$*D*$^-$ from strain DJK287.
[b] Arabinose added to 0.2%.

er in these strains showed the same regulatory phenomena as *araC-lacZ* fusions constructed in a different manner (1). p_C activity was repressed about sevenfold by the presence of *araC* protein, and in the absence of *araC* protein it was stimulated about sixfold by CRP-cAMP. In the strains constructed for these experiments, *lacZ* expression was independent of the insertion location of Mu *lac* in *araC*, and the various hybrid *araC-lacZ* proteins were stable in vivo, all leading us to the conclusion that p_C expression can be adequately monitored by measuring β-galactosidase production.

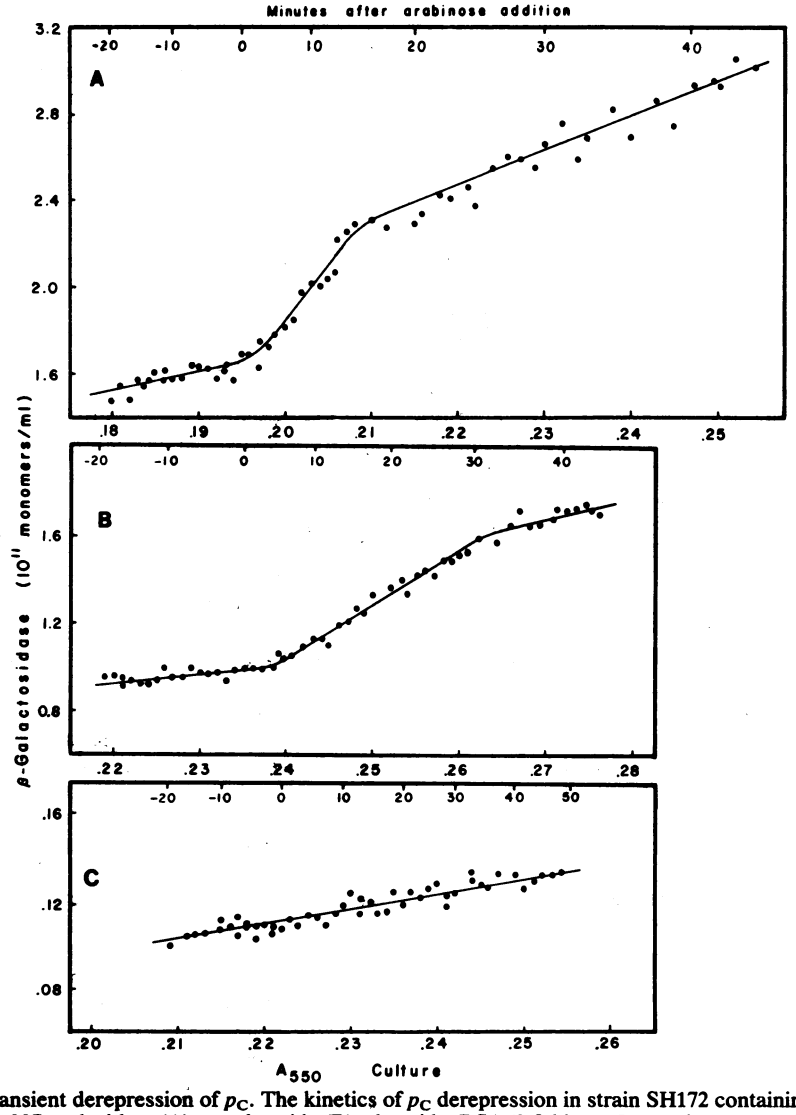

FIG. 2. Transient derepression of p_C. The kinetics of p_C derepression in strain SH172 containing episome F′ *araC*::Mu *lac-287* and either (A) no plasmid, (B) plasmid pDS4 (2-fold *araC* protein overproducer), or (C) plasmid pDS6 (60-fold *araC* protein overproducer). For each panel, the top scale indicates time after arabinose addition (minutes) and the bottom scale indicates absorbance at 550 nm (A_{550}). In panel A the cell doubling time was 170 min before and 100 min after arabinose addition.

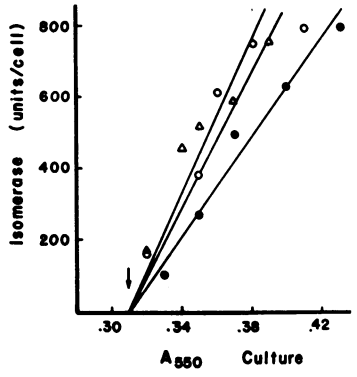

FIG. 3. Repression of p_C does not affect p_{BAD} induction. The induction of arabinose isomerase is shown in strain SH172 containing the episome F' *araC*::Mu *lac-287*. Shown are the results with (●) no plasmid, (○) plasmid pDS4, (2-fold *araC* protein overproducer), and (△) plasmid pDS6 (60-fold *araC* protein overproducer). The arrow marks the time of arabinose addition. A_{550}, Absorbance at 550 nm.

Effects of varying the level of *araC* protein. Dissociation-controlled repression effects might be revealed by varying the concentration of *araC* protein in cells. Therefore, the activity of both p_C and p_{BAD} was characterized in strains containing plasmids (12) which result in 2- or 60-fold overproduction of the *araC* protein. Both the steady-state and the derepressed activity of p_C decreased with increasing intracellular levels of *araC* protein (Fig. 2). p_C derepression was decreased 2-fold in the 2-fold overproducer strain and undetectable above the basal level of β-galactosidase in the 60-fold overproducer strain.

The data (Fig. 2) suggest that increasing levels of *araC* protein lead to increasing degrees of occupancy of the *araO* site (see below). Since genetic and physiological data implicate the *araO* site in repression of p_{BAD} (2), it was of great interest to determine whether increasing the intracellular levels of the *araC* protein also interfered with the induction of p_{BAD}. p_{BAD} remained fully inducible despite the high levels of *araC* protein (Fig. 3).

Reestablishment of p_C repression. The p_C promoter is known to be repressed back to its steady-state activity 20 to 30 min after the addition of arabinose (9). Our present understanding of bacterial physiology suggests three possibilities for the generation of this repression: catabolite repression generated by the metabolism of arabinose (4), the accumulation of high intracellular arabinose concentrations as a result of induction of the arabinose transport systems, and the accumulation of elevated levels of *araC* protein resulting from p_C derepression.

The effect of catabolite repression was examined in an *araB* mutant which was unable to metabolize arabinose. The derepression and subsequent reestablishment of repression were virtually identical in otherwise isogenic AraB$^+$ and AraB$^-$ strains, excluding catabolite repression as the cause of reestablished repression (Fig. 4).

The kinetics of p_C derepression were also measured in a pair of isogenic strains which differed only in their ability to actively transport arabinose. p_C is derepressed normally in both strains, but the repression which ordinarily begins after about 20 min did not occur (Fig. 5). However, at longer times partial repression did occur, and in steady state p_C was expressed at about twice the rate measured in wild-type cells (Table 3). These results suggest that accumulation of high intracellular arbinose levels is the major cause of the reestablishment of p_C repression. The high intracellular arabinose concentrations could affect p_C activity via the *araC* or some other protein or by altering the cell physiology. The possibility of such an indirect effect was examined by using the *lac* operon. The expression of β-galactosidase was not affected by the addition of arabinose (not shown), suggesting that the effect of arabinose is specific to the p_C promoter and is probably mediated by the *araC* protein.

As is explained more fully below, one mechanism suggested for p_C derepression is that the addition of arabinose causes a decrease in the affinity of *araC* protein for *araO*. Since the *araC* protein represses its own synthesis, the initial derepression of p_C would result in its overproduction, which would eventually repress p_C but at a new and higher steady-state level of expression. As discussed in the appendix, it is possible to predict the induction kinetics resulting from a decrease in the affinity of the *araC* protein, assuming noncooperative binding of *araC* protein to the operator. The analysis presented there shows that the measured kinetics of derepression and final steady-state levels of p_C expression closely fit those predicted (Fig. 5A).

AraC^c proteins repress p_C only in the presence of arabinose. In addition to causing p_C derepression, arabinose also interacts with the *araC* protein to induce the p_{BAD} promoter. The result, that reestablishment of p_C repression 20 to 30 min after arabinose addition has no effect on p_{BAD} induction, suggests that the inducing form of *araC* protein (i.e., the form responsible for the induction of p_{BAD}) can exist in two substates, which differ in their ability to repress p_C. We therefore tested whether analogous behavior would be displayed by a mutant *araC* protein which induces p_{BAD} in the absence of arabinose. Most *araC^c* proteins do not repress p_C, although

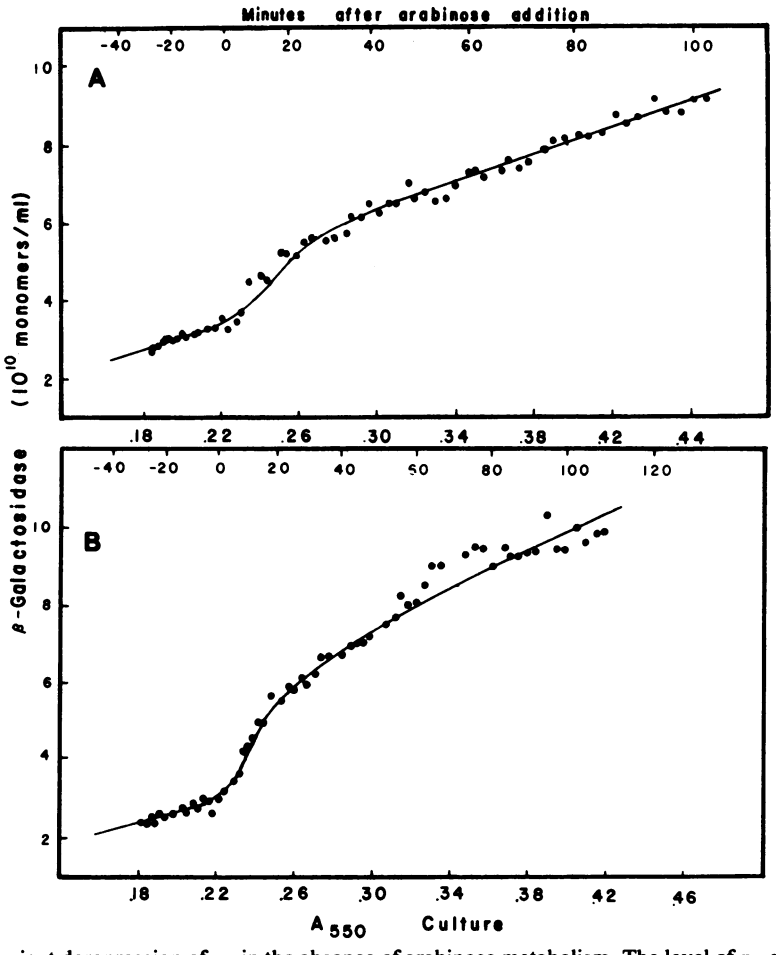

FIG. 4. Transient derepression of p_C in the absence of arabinose metabolism. The level of p_C expression was measured in strain SH285 (F^- *araC-lacZ* fusion *araB*) containing the episome from (A) strain RFS720 (metabolism$^+$) or (B) strain SH121 (metabolism$^-$). For each panel, the top scale indicates time after arabinose addition (minutes) and the bottom scale indicates absorbance at 550 nm (A_{550}). In panel A, the addition of arabinose decreased the doubling time from 110 to 90 min.

in the presence or absence of arabinose they are in the inducing state for the promoter p_{BAD} (1; Haggerty, thesis). The addition of arabinose caused repression in all *araC*^{c} mutants tested (Table 4). This shows that *araC*^{c} protein as well as the wild-type *araC* protein can exist in a state which induces p_{BAD} but possesses two substates that differ in their ability to repress p_C.

DISCUSSION

In this paper, we present the results of a number of experiments investigating the regulation of the promoter for the *araC* gene, p_C. These results provide physiological data against which any proposed mechanism and direct biochemical experiments must be compared.

After arabinose addition, p_C was derepressed about fivefold. There are several possible mechanisms which could cause this derepression. First, derepression might be the result of either translational control or activation of transcription at a promoter other than p_C. However, nuclease S1 mapping of in vivo transcripts has shown that transcription is initiated at p_C with or without arabinose and increases after arabinose addition (C. M. Stoner, Ph.D. thesis, Brandeis University, Waltham, Mass., 1982). Second, derepression might be the sole result of a conformational change of *araC* protein bound at *araO*, which allows concurrent binding of RNA polymerase and *araC* protein at p_C. With this mechanism, derepression would not be sensitive to the level of *araC* protein in vivo. However, our results show that derepression is almost inversely proportional to the level of *araC* protein. In

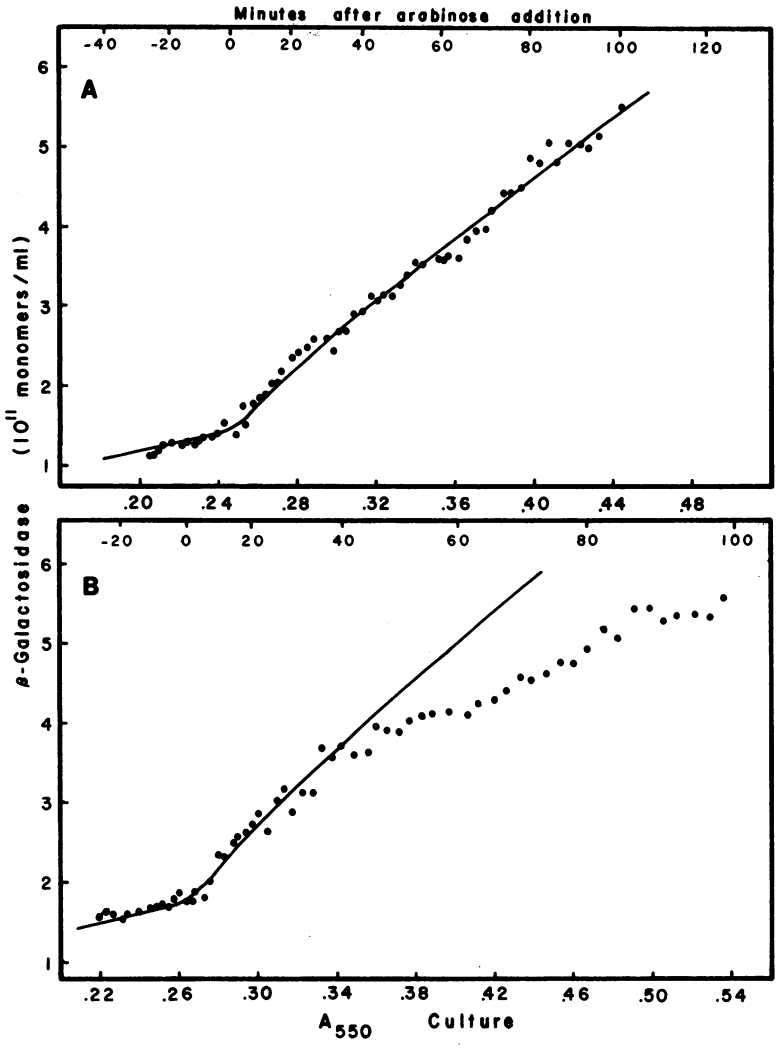

FIG. 5. p_C remains permanently derepressed in transport-deficient mutants. The level of p_C expression (●) was measured in (A) strain SH271 (transport$^-$) and (B) strain SH281 (transport$^+$), both containing the episome F' *araC*::Mu *lac-287*. For each panel, the top scale indicates time after arabinose addition (minutes) and the bottom scale indicates absorbance at 550 nm (A_{550}). In panel B, the doubling time decreased from 115 to 90 min after the addition of arabinose. The line drawn is that predicted for about a 15-fold arabinose-induced decrease in the apparent affinity of *araC* protein for the operator (see the text and the appendix).

light of these results, the simplest explanation for p_C derepression is that arabinose addition causes a decrease in the apparent affinity of *araC* protein for *araO*.

We also studied the turndown in p_C activity which occurs 20 to 30 min after induction. First, we have shown that the turndown is not a consequence of arabinose metabolism, as previously proposed (9), because the kinetics of p_C expression were similar in strains which can and can not metabolize arabinose. Second, high concentrations of arabinose appear to be required for the reestablishment of repression, since it

TABLE 3. p_C activity in arabinose-transport-negative mutants[a]

Recipient strain	Chromosomal genotype	β-Galactosidase (monomers/cell)	
		Without arabinose	With arabinose
SH281	*ara(CBAD)*$^+$ *araFG*$^-$ *araE*$^+$	2,100	2,300
SH271	*ara(CBAD)*$^+$ *araFG*$^-$ *araE*$^-$	1,950	3,850

[a] Steady-state levels of β-galactosidase were measured in strains containing episome F' *araC*::Mu *lac-287 araB*$^+$*A*$^+$*D*$^-$ from strain DJK287. Arabinose was added to 0.2%.

TABLE 4. Repression of p_C by *araC*^{c} proteins in arabinose-transport-plus and -minus strains[a]

araC allele of episome	β-Galactosidase (monomers/cell) in strain with chromosomal genotype:			
	araE$^+$ *araFG*$^-$		*araE*$^-$ *araFG*$^-$	
	With arabinose	Without arabinose	With arabinose	Without arabinose
araC$^+$ *araB53*	650	510	850	450
araC^c*123 araB53*	790	5,000	812	4,500
araC^c*129 araB53*	760	5,100	720	3,900
araC^c*146 araB53*	670	6,800	800	6,300
araC^c*124 araB53*	1,180	4,700	1,100	6,800
araC1022 ara(BAD)$^+$	5,580	4,800	5,300	5,300

[a] Steady-state β-galactosidase levels were measured in *araC-lacZ* fusion *araB* strains SH285 (transport$^+$) and SH284 (transport$^-$) containing an episome with the indicated *araC* allele. Arabinose was added to 0.2%.

does not occur in arabinose-transport-negative cells. Conceivably this is an indirect physiological effect caused by the high arabinose concentrations. However, this does not seem likely, because both the *araBAD* promoter and the *lac* promoter were unaffected by the high arabinose concentrations. Most likely, then, high concentrations of arabinose exert a specific effect on p_C. Finally, our experiments indicate that part of the turndown was the result of accumulation of higher intracellular levels of *araC* protein. After the initial derepression, the level of *araC* protein approximately doubled before repression was reestablished.

The simplest explanation consistent with the derepression and subsequent reestablishment of repression is that low arabinose concentrations decrease the affinity of *araC* protein for *araO*, but that still-higher arabinose concentrations raise its affinity to near the initial value. This view is consistent with the 20- to 30-min lag before p_C was repressed. Since expression of both the *araE* and *araFG* transport operons is induced after arabinose addition (6), the lag might be required for synthesis of the proteins and; significant arabinose transport. In vivo experiments such as those reported here are incapable of proving these hypotheses or determining whether the reestablishment of p_C repression is mediated via the *araC* protein or some other cellular component.

Quantitative in vitro protection experiments measuring arabinose-induced effects on the affinity of *araC* protein have not been reported. Published experiments (7) have not reported detailed measurements of the dissociation constants of araC protein for *araO* and *araI* since the concentration of active *araC* protein was unknown under the various conditions tested, making it impossible to interpret partial protection data. Also, the experiments reported do not directly pertain to our own experiments because the concentration of arabinose was not varied.

In the experiments reported here, we also found that increasing levels of *araC* protein repressed p_C to low levels without affecting the induction of p_{BAD}. It is likely that this repression of p_C is generated by the binding of *araC* protein to *araO*, since *araO* overlaps the p_C promoter and no other site for repression of p_C has been revealed by in vitro experiments. However, this raises a problem. Previously, the *araO* site was defined as the site of p_{BAD} repression (2, 8). One explanation for the discrepancy is that *araC* protein bound at *araO* possesses two states. In the absence of arabinose (the classical experiments), it is capable of repressing p_{BAD}, and in the presence of arabinose (our experiment), it is incapable of such repression.

We are not the first to suggest that *araC* protein bound at *araO* does not affect the induction of p_{BAD}. Lee et al. (7) made a similar proposal based on more limited data which did not show the simultaneous binding of *araC* protein to *araO* or the inducibility of p_{BAD}. Indeed, they observed p_{BAD} induction only in the absence of such binding or p_C repression.

In summary, we present a series of experiments investigating the derepression and subsequent reestablishment of p_C repression after arabinose addition. That p_C repression was reestablished 20 to 30 min after arabinose addition without affecting p_{BAD} induction suggests that the inducing form of *araC* protein (i.e., the form responsible for the induction of p_{BAD}) can exist in two substates, which differ in their ability to repress p_C. We did observe two such states in a mutant *araC* protein. AraC^c mutants induced p_{BAD} with or without arabinose, but repressed p_C only in its presence.

APPENDIX

With several assumptions, it is possible to predict the induction kinetics of p_C resulting from an arabinose-induced decrease in the affinity of *araC* protein for the operator. These assumptions are that (i) the binding of *araC* protein to the operator is noncooperative, i.e., it can be described by a simple dissociation constant, and (ii) a promoter with *araC* protein bound at the operator is inactive, i.e., the activity of p_C is proportional to the fraction of operators unoccupied by repressor. This is given by the relation $O/O_T = K/(C + K)$, where O is the level of unoccupied operator, O_T is the total concentration of operators, C is the internal concentration of *araC* protein, and K is the dissociation constant of *araC* protein from operator. Wild-type

cells contain about 40 monomers of *araC* protein (5). Assuming that most of the *araC* protein exists as a dimer within cells, this corresponds to an intracellular concentration of about 2 nM.

After the addition of arabinose, p_C was derepressed fivefold. Assuming that this resulted from a decrease in the affinity of *araC* protein for operator and that the level of free *araC* protein did not change appreciably after arabinose addition, the new dissociation constant can be estimated. For a fivefold derepression, O/O_T increases fivefold and K increases from its preinduction value of 3×10^{-9}M to a new value of 5×10^{-8} M, a 17-fold increase.

With these parameters and assumptions, it is possible to predict the induction kinetics of p_C after the addition of arabinose. The rate at which *araC* protein increases in an exponentially growing culture equals its rate of synthesis minus its rate of degradation. Assuming noncooperative binding, the synthesis rate per cell ($[dC/dt]_s$) is given by $(dC/dt)_s = \alpha K/(C + K)$, where α is a constant. The rate of degradation ($[dC/dt]_d$) is given by $(dC/dt)_d = -dC$, where d is the first-order decay constant ($\ln 2/t_{1/2}$). The half-life of *araC* protein has been estimated to be about 60 min (5).

The rate of increase in *araC* protein can be written in terms of total *araC* protein in the culture:

$$\frac{dC_T}{dt} = \frac{\alpha K}{K + (C_T/BN_0\, e^{\mu t})} N_0\, e^{\mu t} - C_T\, d$$

where C_T is the total *araC* protein in the culture, B is the volume per cell, N_0 is the number of cells at time zero, and μ is the growth rate constant (ln2/doubling time). At time zero, dC_T/dt can be determined, since during exponential growth $(dC_T/dt)/C_T = \mu$.

These equations can be used to predict the derepression kinetics resulting from an increase in the dissociation constant of *araC* protein from the operator. At time zero, K is shifted to its new value and the equation is numerically iterated. In our experiments we measured β-galactosidase synthesized under the control of the p_C promoter. Its kinetics of accumulation in the culture can be calculated by the methods described above, but using equations lacking the decay terms, since β-galactosidase is stable in cells.

With this analysis, the predicted induction kinetics were plotted against the data for a wild-type *araC-lacZ* fusion strain (Fig. 4B). The theoretical curve began to deviate from the experimental curve at about 25 min after induction. When the same analysis was made for the induction kinetics found in an arabinose-transport-negative strain, the predicted curve closely matched the observed kinetics (Fig. 5A).

Our analysis of the induction kinetics of the p_C promoter assumed that *araC* protein binds to operator noncooperatively. This was not proven and must, at present, remain an assumption. *araC* protein protects about 40 bases of DNA from DNase I digestion (9). This is rather long for a dimeric protein made of two 32,000-molecular-weight monomers, and it is possible that two of the dimers bind to the operator. Thus far, however, none of the DNase footprinting experiments have revealed any evidence for cooperative binding or half-site binding of *araC* protein to DNA.

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