JOURNAL OF BACTERIOLOGY, Feb. 1974, p. 417-421
Vol. 117, No. 2

Mutations in the L-Arabinose Operon of *Escherichia coli* B/r That Result in Hypersensitivity to Catabolite Repression

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Received for publication 6 November 1973

Two independent mutants resistant to L-arabinose inhibition only in the presence of D-glucose were isolated from an L-arabinose-sensitive strain containing the *araD139* mutation. Preliminary mapping studies indicate that these mutations are closely linked to the *araIOC* region. Addition of D-glucose to growing cultures of these mutants results in a 95 to 98% repression of *ara* operon expression, as compared to a 50% repression of the parental control. Since cultures of both mutant and parental strains undergo a 50% repression of *lac* operon expression upon addition of glucose, the hypersensitivity to catabolite repression exhibited by these mutants is specific for the *ara* operon. Addition of cyclic adenosine monophosphate reverses the catabolite repression of the *ara* operon in both mutant and parent strains to 70 to 80% of the control. It is suggested that in these mutants the affinity of the *ara* operon initiator region for the cAMP-catabolite-activator protein complex may have been altered.

Several specific components are essential for induction of the *ara* operon: (i) conversion of the *araC* gene product from repressor to activator form (6, 7, 15) must take place as a consequence of direct interaction with the inducer, L-arabinose (2, 8, 20, 21; see Fig. 1); (ii) the catabolite activator protein-cyclic adenosine monophosphate (CAP-cAMP) complex must be available for interaction with the genome (presumably to facilitate attachment of ribonucleic acid polymerase to *ara* deoxyribonucleic acid [5, 9, 13, 14, 21, 22]). When the intracellular level of catabolites is high, cAMP is excreted from the cell. As a consequence of the reduced level of CAP-cAMP complex, a corresponding reduction in operon expression takes place (2, 9, 13). This phenomenon is referred to as catabolite repression (11, 13).

The controlling regions of the *ara* operon that interact with the repressor and activator forms of the *araC* gene product have been reasonably well established as the operator and initiator sites, respectively (6, 7, 15). Although the site of action of CAP-cAMP complex has not been established directly for the *ara* operon, it has been shown that the region between the operator and the proximal region of gene *araB* (presumably the initiator region) must be intact for catabolite repression to occur (5).

In the *lac* operon, mutants have been isolated that carry lesions in the promoter region (a region in many ways comparable to the initiator region of the *ara* operon [4, 15]) and are insensitive to catabolite repression (12, 17-19). This has been taken as evidence that the promoter region is the site of action of the CAP-cAMP complex in the *lac* operon. In an attempt to further define and characterize the control elements in the *ara* operon that interact with CAP-cAMP complex, we isolated two mutants that exhibit hypersensitivity to catabolite repression of the *ara* operon. This report describes some of their genetic and physiological properties.

MATERIALS AND METHODS

Media. In addition to those previously described (2, 4, 15), the following media were employed: mineral base plus 0.2% L-arabinose, 0.2% D-glucose. and 0.2% glycerol, as described (M-Ara-Glu-Glc); EMB plus 1% L-arabinose and 1% D-glucose (EMB-Ara-Glu). L-Threonine was added to the mineral base to a final concentration of 0.04%, as described.

Bacterial strains. Table 1 lists all of the strains used in this study. The relative genetic positions of certain strains are indicated in Fig. 1.

Isolation of mutants. A culture of SB5003 (*araD139*) was grown to a titer of 4×10^9 cells per ml in L-broth on a New Brunswick Rollardrum at 37 C. The culture was diluted in sterile saline, and a large number of sterile 1.0-ml L-broth cultures were inoculated with 100 cells each. After standing incubation for 24 h at 37 C, each broth culture (approximate

TABLE 1. *Bacterial strains*

Strain	Genotype	Source or reference
ME1453	*ara*Δ*BIOC768*	Ref. 4
RG0602	*thr-1 araD139 ara-9602*	SB5003
RG0603	*thr-1 araD⁺ ara-9602*	RG0602
RG0828	*thr-1 araD139 ara-9802*	SB5003
RG0829	*thr-1 araD⁺ ara-9802*	RG0828
RG1400	*thr-1 araD⁺*	SB5003
SB1604	*thr-1 araD139 ara*Δ*C, leu*Δ*DCB1204*	Ref. 10
SB5003	*thr-1 araD139*	Ref. 15

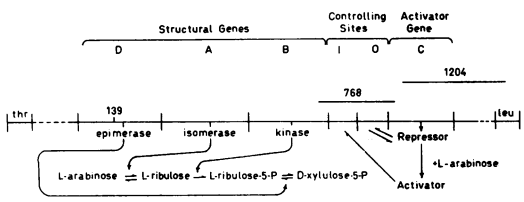

FIG. 1. *L-Arabinose operon.*

titer, 3×10^8 cells per ml) was streaked onto an EMB-Ara-Glu plate. After 24 h of incubation at 37 C, large, pink L-arabinose-resistant clones (3) were picked to EMB-Ara plates. Clones resistant to L-arabinose inhibition in the presence of D-glucose, but sensitive in its absence, were subjected to single-colony isolation. Two independently isolated mutants are the subject of this report. Since these strains were still sensitive to L-arabinose (in the absence of D-glucose), it was not possible to study the kinetics of induction of the *ara* operon without first removing the *araD139* mutation. This was accomplished by selecting for Ara⁺ revertants of the *araD139* marker on M-Ara-Thr medium. AraD⁺ revertants of the two mutant strains and the parent strain were used for all enzymatic studies.

Preparation of cell extracts and assays. Culture samples (1.0 ml) were collected on 0.45-μm membrane filters (Millipore Corp.) and prepared for assay of L-arabinose isomerase activity (EC 5.3.1.4) as described previously (4), except that each sample was frozen and thawed after toluenization. This modification resulted in reproducible attainment of the high specific activities routinely observed after sonic oscillation (2). Specific activity measurements were made on cultures that had grown for at least four generations under the specified conditions described in each figure legend. Induction kinetics were determined by taking duplicate 1-ml samples at regular intervals during exponential growth.

Mapping studies. Two attempts were made to determine the genetic location of the mutations causing hypersensitivity to catabolite repression. (i) P1 transducing phage prepared on strains RG1400 (*ara⁺*), RG0829 (*ara-9802*), and RG0603 (*ara-9602*) were used to transduce ME1453 (*araBIOC768*) (a deletion that covers a portion of the *araB* and *araC* genes as well as the entire initiator and operator regions [4] to Ara⁺). Transductants were subjected to one single-colony isolation and assayed for L-arabinose isomerase activity in the presence of D-glucose. Sample size of the L-arabinose isomerase colorimetric assay was adjusted to permit a visual distinction between transductants that exhibited a normal or hypersensitive response to catabolite repression of the *ara* operon (see Fig. 2). Appearance of transductants that exhibit normal response to catabolite repression in this cross would indicate that the site for hypersensitivity lies outside *ara*Δ*BIOC768*. (ii) $P1_{bc}$ transducing phage prepared on strains SB5003 (*araD139*), RG0828 (*araD139 ara-9802*), and RG0602 (*araD139 ara-9602*) was used to transduce SB1604 to Leu⁺. Since this recipient carries the *araD139* mutation and a deletion mutation that encompasses a region beginning in the *araC* gene and extending into gene *leuB* (10), all Leu⁺ transductants must contain those *ara-leu* region genes carried by the donor phage that correspond to the deleted region in SB1604. Each Leu⁺ transductant was subjected to one single-colony isolation and then streaked onto EMB-Ara-Glu and EMB-Ara medium. Leu⁺ transductants that also carry the mutation resulting in hypersensitivity to catabolite repression will appear resistant to L-arabinose inhibition only on EMB-Ara-Glu medium. If the site of the mutation giving rise to hypersensitivity to catabolite repression lies outside the region encompassed by the deletion mutation carried by SB1604, then transductants of the type *araD139 ara-9602⁺* and *araD139 ara-9802⁺* should occur and would be identified by their sensitivity to L-arabinose inhibition on EMB-Ara-Glu medium.

RESULTS

Effect of glucose on ara operon expression. Table 2 demonstrates the increased sensitivity of the *ara* operon to catabolite repression in mutant strains RG0603 and RG0829. Whereas the parental strain RG1400 is repressed by glucose to approximately 50% of the control

TABLE 2. *Sensitivity of the ara operon to catabolite repression*[a]

Strain	L-Arabinose isomerase sp act	
	No addition	Glucose at 5×10^{-3} to 6×10^{-3} M
RG1400	65.8 ± 3.2	35.5 ± 1.9
RG0603	31.8 ± 1.6	1.2 ± 0.4
RG0829	49.4 ± 0.9	2.3 ± 0.1

[a] Cultures were grown for at least four generations in the presence of 2.2×10^{-2} M L-arabinose, with or without 5×10^{-3} to 6×10^{-3} M D-glucose in M-Thr-Glc medium. Each value is the mean of at least eight independently grown cultures each of which was assayed for L-arabinose isomerase activity in duplicate as a measure of *ara* operon expression.

level, the two mutant strains are repressed to approximately 5% of their control levels. Furthermore, in the absence of glucose repression, both mutant strains exhibit significantly reduced levels of *ara* operon expression, an effect that could be attributed to increased sensitivity to self catabolite repression (2, 9, 16). Figure 2 also demonstrates the increased sensitivity of the *ara* operon to catabolite repression in mutant strains RG0603 and Rg0829, as compared to the wild-type control, RG1400. Addition of 2.2×10^{-2} M glucose to an exponentially growing culture of RG1400 results in an immediate transient repression (11, 13) followed by a slightly reduced differential rate of *ara* operon expression. By contrast, strains RG0603 and RG0829 are immediately repressed to approximately 95% of the control rate of synthesis for at least one cell generation after the addition of glucose.

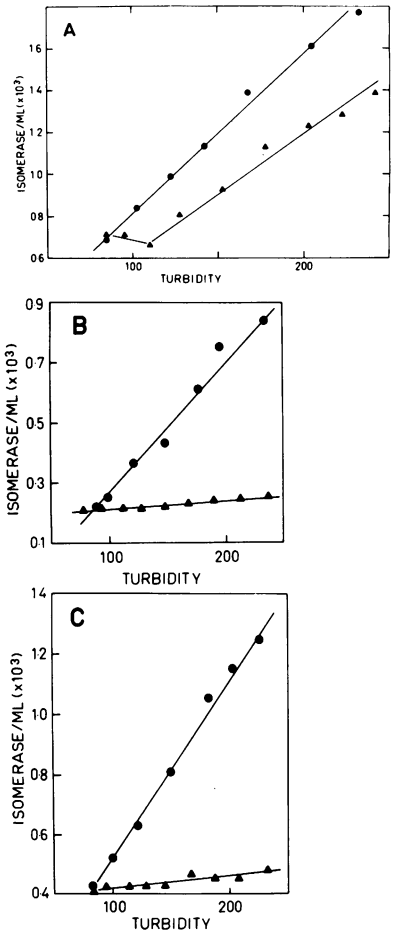

FIG. 2. *Effect of glucose on expression of the ara operon. Each culture was allowed to grow for about two cell generations in 200 ml of M-Ara-Glc-Thr medium. The cultures were then split, and* D*-glucose was added to the first flask to a final concentration of* 2.2×10^{-2} *M (▲). No addition (●) was made to the second flask.* L*-Arabinose isomerase activity, as described in Materials and Methods, was used as a measure of ara operon expression. A, RG1400; B, RG0603; C, RG0829.*

Sensitivity of the lac operon to catabolite repression. Figure 3 describes the effect of various concentrations of glucose upon the expression of the *lac* operon. In both mutant and wild-type strains, the *lac* operon is repressed to between 44 and 58% of the control. Furthermore, no difference in degree of repression between the strains can be detected over a wide range of glucose concentrations. Thus, hypersensitivity to catabolite repression in strains RG0603 and RG0829 is specific for the *ara* operon and does not appear to be related to ability to concentrate glucose or to an alteration in some other component universally required for catabolite repression (13).

Effect of cAMP on catabolite repression Figure 4 demonstrates the ability of cAMP to reverse partially the effects of catabolite repression. Both the parent strain, RG1400, and the hypersensitive mutant strains, RG0603 and RG0829, respond to cAMP to achieve 70 to 80% of the control rates of *ara* operon expression. This indicates that the CAP-cAMP complex is still employed in the induction of the *ara* operon in the hypersensitive mutants.

Mapping studies. It has not yet been possi-

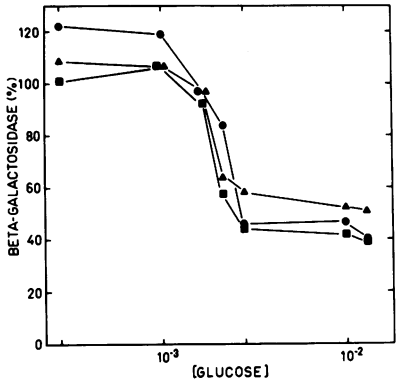

FIG. 3. *Sensitivity of the lac operon to catabolite repression. Control cultures were grown in M-Glc-Thr medium containing* 10^{-3} *M IPTG (13) for at least four cell generations. Various final concentrations of* D*-glucose were added to the experimental cultures. β-Galactosidase activity (13) was used as a measure of lac operon expression. Symbols: ●, RG1400; ▲, RG0603; ■, RG0829.*

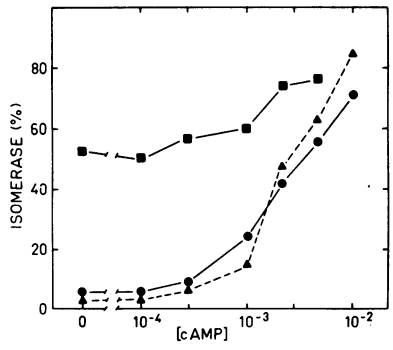

FIG. 4. *Effect of cAMP on catabolite repression. Control cultures were grown for at least four cell generations on M-Glc-Thr medium containing 2.2 × 10⁻² M L-arabinose. Experimental cultures contained, in addition, 6 × 10⁻³ M D-glucose and various concentrations of cAMP. L-Arabinose isomerase specific activity, assayed as described in Materials and Methods, was used as a measure of ara operon expression. Symbols: ■, RG1400; ▲, RG0603; ●, RG0829.*

ble to assign the catabolite-hypersensitive mutations to a specific site in the *ara* region. P1 transducing phage prepared on strains RG0603 and RG0829 was used to transduce ME1453 (*araBIOC768*) to Ara⁺. Each of 200 Ara⁺ transductants tested from each cross was found to be hypersensitive to catabolite repression of the *ara* operon. Since deletion mutation *araBIOC768* encompasses only a very small region of gene *araB* and *araC*, these results suggest that the hypersensitive mutations lie within or very close to the *araIO* region (not separable at the 0.5% recombination level). When transducing phage prepared on RG0602 and RG0828 was used to transduce SB1604, all of the 978 and 864 Leu⁺ transductants, respectively, were resistant to L-arabinose inhibition in the presence of glucose. This indicates that the catabolite hypersensitive mutations are either within or closely linked to the region encompassed by deletion mutation *araC* leuDCB1204. These results are consistent with a merodiploid analysis that places the site for catabolite repression in the *ara* operon between the operator region and the *araB* gene (5).

DISCUSSION

The mutants described in this study will facilitate the characterization of the site of action of the CAP-cAMP complex on the *ara* operon. Clearly, these mutants exhibit a striking sensitivity to catabolite repression that is specific for the *ara* operon. Although the genetic evidence indicates that these mutants lie within or are closely linked to the *araIO* controlling region, it unfortunately does not permit one to determine whether these mutations lie within the *araIO* *cis*-acting regulatory elements or whether they lie within the *trans*-acting *araC* gene. It should be possible to discriminate between these two possibilities with the construction of a merodiploid of the type, F *araA*⁻ catabolite-hypersensitive/*araA*⁺ *araC*⁻. *ara* operon expression in such a strain should be severely inhibited by glucose only if the hypersensitivity effect is *trans* active, that is, if it is acting via an altered *araC* gene product. Experiments soon to be in progress should resolve this issue.

One would predict that any mutation that increases the sensitivity of the operon to catabolite repression should also result in increased sensitivity to "self catabolite repression," a process whereby a metabolite of arabinose "feeds back" to partially repress operon expression (2, 9, 16). The observation that the two hypersensitive mutants exhibit reduced specific activity levels of *ara* operon expression (48 and 74% of the wild-type control) is consistent with this prediction. By comparison, specific activity levels of *lac* operon expression are the same in mutant and wild-type strains.

In the *lac* operon "down-promoter" mutants and revertants of promoter mutants result in strains that are insensitive to catabolite repression and, in fact, appear to function independently of any requirement for the CAP-cAMP complex (1, 17-19). Clearly the CAP-cAMP complex is involved in *ara* operon induction in the hypersensitive mutants described here, since cAMP addition reverses catabolite repression to approximately 70 to 80% of the unrepressed control.

A simple model to explain the nature of hypersensitivity to catabolite repression would be one in which some specific component of the *ara* operon (presumably the initiator region or the *araC* gene product (5) has a reduced affinity for the CAP-cAMP complex. It should be possible to test this prediction now that in vitro analysis of *ara* operon expression has become feasible (8, 20, 21). An alternative explanation is that glucose more severely blocks inducer (L-arabinose) uptake in mutant than in wild-type strains. This possibility could be tested by placing the hypersensitive mutations into a strain in which the *ara* operon in expressed constitutively, thus eliminating the need for inducer transport. If the catabolite-hypersensitive mutants prove to define the region of the initiator region where CAP-cAMP interacts,

then the initiator must contain at least three subregions, one for binding of ribonucleic acid polymerase, one for *araC* gene activator, and one for the binding of the CAP-cAMP complex. A group of *ara* mutants with the properties of down-promoter mutants have been described previously (4). The effects of these mutations and their reversions upon catabolite repression may prove to be useful in the further elucidation of the relationships between these three regions.

ACKNOWLEDGMENTS

This investigation was supported by National Science Foundation Grant GB-6439.

The technical assistance of Edith Jones is gratefully acknowledged.

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