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Hyperinducibility as a Result of Mutation in Structural Genes and Self-Catabolite Repression in the *ara* Operon

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Mutations in gene *araB* producing an L-arabinose-negative phenotype cause either an increase (hyperinducible), decrease (polar), or have no effect at all on the inducible rate of expression of the L-arabinose operon. Fourteen *araB* gene mutants exhibiting such effects were shown to be the result of: nonsense, frameshift, or missense mutations. All missense mutants were hyperinducible, exhibiting approximately a twofold increase in rate of L-arabinose isomerase production. All frameshift and most nonsense mutants exhibited polar effect. One nonsense mutant was hyperinducible. The *cis*-dominant polar effect of nonsense and frameshift mutants (as compared to induced wild type) were more pronounced in arabinose-utilizing merodiploids and in *araBaraC*^{c} double mutants where inducible and constitutive enzyme levels are respectively determined. On the other hand, in arabinose-utilizing merodiploids, missense mutations no longer exhibited hyperinducibility but displayed a wild-type level of operon expression. Increases in the wild type-inducible rate of expression of the operon were found when growth rate was dependent on the concentration of L-arabinose. Cyclic 3′,5′-adenosine monophosphate also stimulated expression of the operon with the wild type in a mineral L-arabinose medium. These observations are explained on the basis that the steady-state expression of the L-arabinose operon *OIBAD* is dependent on the concentration of (i) L-arabinose, the effector of this system, which stimulates the expression of the operon, and (ii) catabolite repressors, produced from L-arabinose, which dampen the expression of the operon. We have termed the latter phenomenon "self-catabolite" repression.

Effects of mutation in a structural gene upon the level of enzymes produced by adjacent genes in an operon were first described by Englesberg and Killeen (Genetics **44**:508, 1959) and Englesberg (12) in the L-arabinose system in *Escherichia coli* B/r and subsequently amplified by Lee and Englesberg (23, 24). Specifically it was shown by these workers that mutation in the *araB* gene, the structural gene for L-ribulokinase (EC 2.7.1.16) caused either a coordinate decrease, increase, or had no effect at all (in comparison to the wild type) on the inducible rate of synthesis of the enzymes coded for by the adjacent gene *araA* (L-arabinose isomerase, EC 5.3.1.4). Mutants in gene *araA*, on the other hand, all produced increased levels of L-ribulokinase. The effect of these mutations on levels of L-ribulose-5-phosphate-4-epimerase (EC 5.1.3.4) coded for by gene *araD* was not extensively studied (4, 13). In this paper we shall be concerned with the elucidation of the nature of these effects, primarily produced by mutation in gene *araB*. (See Fig. 1 for a description of the *ara* operon *OIBAD*.).

It has been previously shown that nonsense mutations in structural genes, in general, lower the rate of synthesis of gene products of adjacent structural genes in an operon at the distal end of the nonsense codon (31, 34, 50). These mutations cause a premature termination of translation within a cistron, probably by labilizing the polycistronic messenger ribonucleic acid to nucleolytic attack (33). The rate of destruction of the message together with the efficiency of reinitiation of the translation process at the adjacent distal cistron determines the extent of the polar effect observed. Polar effects of frameshift and deletion mutations have also been observed in several operons (29, 30, 42, 44). We shall show that all mutations in gene *araB* causing a decrease in rate of expression of adjacent structural genes in the operon are the result of nonsense mutations.

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Mutations in structural genes causing an elevation in rate of synthesis of gene products of adjacent structural genes have also been reported for several operons (7, 40, 41, 45). Although a number of hypotheses have been proposed to explain this phenomenon, none has previously been subjected to experimental test. Evidence presented in this paper demonstrates that the hyperinducible effects in the L-arabinose operon *OIBAD* can best be explained on the basis that the steady-state level of expression of the operon depends on the production of catabolite repressors formed from the metabolism of the inducer itself, L-arabinose, as well as on the concentration of inducer within the cell, as has been shown by others. Thus, all mutations in gene *araB* in blocking the production of catabolite repressors at the stage of ribulose have the potential of increasing the initial rate of expression of the *ara OIBAD* operon. Whether a hyperinducible phenotype is produced, however, depends on the type of mutations in gene *araB*. All missense mutations produce a hyperinducible phenotype, whereas nonsense mutations, depending upon their position in the *araB* gene, reduce in varying degrees the stimulatory effect of the block in self-catabolite repression. Thus, some hyperinducers are also produced by nonsense mutations.

Since the usage of the term catabolite repression has become synonymous with the effect of glucose and other compounds on *different* inducible, catabolite operons (a heterologous effect) and therefore obscured the self-regulatory or homologous aspect of this phenomenon, we propose to employ the term "self-catabolite repression" to accentuate the latter aspect of this phenomenon. Self-catabolite repression appears to be a general phenomenon in all inducible catabolic operons that have been analyzed. A preliminary report of this work has appeared elsewhere (L. Katz and E. Englesberg, Bacteriol. Proc., p. 50, 1968).

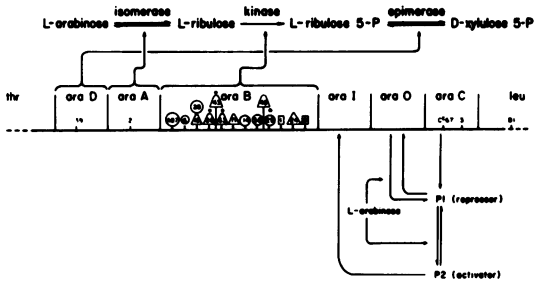

FIG. 1. *L-Arabinose operon OIBAD. Structural genes: araB, L-ribulokinase; araA, L-arabinose isomerase; araD, L-ribulose 5-phosphate 4-epimerase. Controlling sites: araI, initiator; araO, operator. Regulator gene: araC. The numbers represent mutants employed or referred to in this study. The indicated position of the mutant site has been previously determined (9). C^c67 is a constitutive mutant. The others are Ara^- mutants. Gene araB is shown in detail. Mutations encircled are nonsense mutations: those enclosed in a triangle are presumed to be frameshift. Mutation A87 has been identified as nonsense by C. Squires and N. Lee (personal communication). Mutant sites with an asterisk above the number result in kinase CRM formation (24). In the absence of the inducer, the araC gene product is mainly in the form of P_1 (repressor) bound to araO, free repressor, and relatively small amounts of P_2 (activator). The addition of the inducer removes the repressor from the operator site and shifts the equilibrium to activator. Activator functions at the initiator site and facilitates the induction of the operon (14-16).*

MATERIALS AND METHODS

Bacteria. A representative group of 14 ultraviolet (UV) or X-ray induced *araB* gene mutants, previously characterized (9, 20) and available in a *leu*$^-$ and *thr*$^-$ *leu*$^-$ genetic background, were chosen for this study (Fig. 1 and Table 1).

Mutants *araB23* and *araB24* have residual L-ribulokinase activity which is no greater than 0.6% of the activity of the fully induced wild type. Mutants *araB23*, *araB26*, *araB29*, *araB43*, and *araB63* produce kinase cross-reacting material (CRM) which is coordinate with the level of isomerase produced by each of the strains, respectively. *AraB6*, *araB14*, *araB16*, and *araB28* are non-CRM formers (24).

E. coli K-12, KH600 *leu*$^-$ (λ) and derivatives of this strain (Table 3) have the entire *thr-ara-leu* region replaced by that of B/r (21), but have the K-12 character of restriction and modification. Ochre and amber suppressor strains, SBO-22 (λ) and C600 (λ), respectively, were obtained from E. Orias. The homogenotes employed (*F'ara*$^{-x}$/*ara*$^{-x}$) were originally constructed by D. Sheppard and E. Englesberg (43). Periodic reisolation or reconstruction was performed by the method of Sheppard and Englesberg. Nomenclature employed for the *ara* mutants is the same as that described previously (43).

Media. L broth, mineral base, and complex eosin methylene blue L-arabinose (EMB-ara) have been previously described (43). Tryptone media contained 1.0% tryptone, 0.5% NaCl, and thiamine (1 μg/ml) with or without 1.0% Difco agar. Mineral base was supplemented with 0.2% sugar, 0.04% amino acids, and 0.5% Difco agar as required. In the determination of the initial rates of synthesis of L-arabinose isomerase, $MnCl_2$ at 2×10^{-5} M was added to the mineral base to insure maximal activity of the enzyme. Where D-fucose was employed, it was added in final concentration of 0.3%. Casein hydrolysate medium (CH) contained mineral base plus 1.0% Casamino Acids (Difco). In some experiments, 0.2% or 0.4% L-arabinose was added. Nomenclature for media used is as follows: M, mineral base; G, D-glucose; Gly, D-glycerol; A, L-arabinose; F, D-fucose; L, L-leucine; T, L-threonine; Met, L-methionine.

Phage. Phage P1bt was used for all transduction experiments. Propagation, storage, and transduction with the phage have all been described previously (20).

Stocks of various T7 amber (UAG) and T4rII mutants, including deletion, amber, and ochre (UAA) mutants, were generously supplied by E. Orias and T. K. Gartner. The T7 mutants employed in this study carried mutations which mapped in one of five separate cistrons. T4rII UGA mutants were obtained from S. Brenner. These were propagated on *E. coli* B/r by methods previously described (18).

Chemicals. 2-Aminopurine (2-AP) and 5-bromodeoxyuridine (5-BU) were purchased from Calbiochem Company, Los Angeles, Calif.; diethylsulfate (DES) and ethylmethanesulfonate (EMS) were obtained from Eastman Chemical Company, Rochester, N.Y.; and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (NTG) from K & K Laboratories, Plainview, N.Y. ICR compounds, 170 and 191, previously described (2), were a generous gift of Hugh J. Creech, Institute for Cancer Research, Fox Chase, Pa. Cyclic 3',5'-adenosine monophosphate (AMP) was purchased from Calbiochem Company, Los Angeles, Calif.

Construction of merodiploids. Merodiploids of the type F' araA$^-$B$^+$/araA$^+$B$^-$ or F'araA$^+$B$^-$/araA$^-$B$^+$ were constructed after the general procedure as that described previously (43). Where the episome complemented the endogenote (see following paragraph), direct selection for the complementing merodiploid was performed on MA. In cases where the episome was known not to complement the endogenote, selection was first performed for Leu$^+$ on MG employing as a donor a Leu$^+$ Ara$^-$ homogenote and a Leu$^-$ T6-resistant F$^-$ recipient. Counterselection of the male strain was performed by using T6 phage.

Spot test for complementation. Approximately 2.5×10^8 cells from an overnight L-broth culture of an F$^-$ strain were plated onto MA or MALTMet. Cells (2×10^6 to 4×10^6) of an exponentially growing culture of a fertile homogenote were subsequently spotted onto the bacterial lawn. The plates were incubated at 37 C. If complementation occurred, a patch of growth could be detected in 18 hr at the location where the homogenote was deposited. Distinct colonies in the spot, appearing only after 24 hr, indicated that recombination, but not complementation, had occurred.

Merodiploid genotype analysis. A sample of the culture used to determine enzyme levels was plated onto EMB-ara. In the case of a complementing merodiploid, if greater than 5% of the colonies appearing on the plate was Ara$^-$, the culture was discarded and the merodiploid was reisolated. Where less than 5% segregation to Ara$^-$ was observed, 10 Ara$^+$ colonies were picked and grown for a period of 24 hr in L broth and then plated onto EMB-ara. Ara$^-$ segregants (20 to 200) of each of the 10 cultures were tested for the presence of the *araA2* or *araB*$^-$ markers, or both, by the spot complementation test. In the assays reported, the presence of the pertinent genetic marker was confirmed.

Construction of araB$^-$ C^{c}67 double mutants. Phage P1bt was used to transduce the *araC^{c}67* mutation into various *thr$^-$araB$^-$leu$^-$* recipients by first selecting Leu$^+$ transductants on MGT and testing for the inability to utilize arabinose (*araB*$^-$) and presence of the *araC^{c}67* allele. The latter was determined by the spot complementation test with *F'araC3/araC3* on MATF. *AraC^{c}67* is a constitutive mutant isolated as resistant to D-fucose inhibition on MAF agar plate (14). *C^{c}67* is dominant to the *C*$^-$ allele (inducible and fucose sensitive). Isogenic *araB$^-$C^{c}67* double mutants were made by growing phage on the constructed strains and subsequently transducing the entire *araB$^-$C^{c}67* region into strain UP1002 (*ara$^+$leu$^-$*) by the method described above. In a similar fashion, *araB*$^-$ and *araB$^-$C^{c}67* mutations were inserted into strain KH600 *leu*$^-$ (λ).

Isolation of Ara$^+$ revertants. Spontaneous and chemically induced Ara$^+$ revertants were isolated from *araB*$^-$ and *araB$^-$C^{c}67* strains by the identical procedure of Cribbs (8). To eliminate the possibility of losing a temperature-sensitive class among the revertants, selection was performed at 30 C for a period up to 4 days. Where ICR compounds were used to induce reversions, the plates were covered and kept in the dark.

Test for nonsense mutations. To determine whether any of the *araB*$^-$ mutants was the result of nonsense mutations, we isolated Ara$^+$ revertants (spontaneous or DES induced) of each *araB*$^-$ mutant and screened each revertant to see whether it would suppress a group of nonsense phage mutants. A set of amber mutants in phage T7, restricted in *E. coli* B/r, was employed. In addition, also available were a large number of amber, as well as ochre and UGA rII mutants of phage T4. Since *E. coli* B/r does not restrict the growth of these mutants but *E. coli* K-12 (λ) does, it was necessary for us to transfer each of the *araB*$^-$ mutants in *E. coli* B/r to K-12 to make use of these mutant strains in the screening process. Accordingly, each *araB*$^-$ mutation was individually transduced into *E. coli* K-12 KH600 (λ) *leu*$^-$, and Ara$^+$ revertants were isolated as described above.

The Ara$^+$ revertants were tested for nonsense mutations in the following manner. Ara$^+$ revertants were grown for 18 to 24 hr in 1 ml of tryptone broth, after which time approximately 5×10^7 cells were added to 2.5 ml of semisolid tryptone agar (0.6% agar kept at 45 C). The contents were poured over the surface of a tryptone agar plate. As soon as the agar hardened, 0.01 ml, containing 2×10^5 to 5×10^5 particles of various strains of T7 or T4 phage, was spotted onto the surface of the plate (20 spots per plate). The plates were incubated at room temperature (for the T7 mutants) or at 30 or 37 C (for the T4 mutants) for up to 12 hr. Where suppression had occurred, a clearing of the bacterial lawn was detected in the zone where the phage had been deposited.

To test large numbers of revertants for ability to supress nonsense mutations in phage, the following modified procedure was employed. All the T7 amber mutants were mixed together in equal concentrations. Each single revertant was spotted onto a tryptone plate (20 revertants per plate). When the spots had dried, one drop of the phage mixture was deposited onto each bacterial spot, and the plates were incubated. If the revertant strain could suppress any one of the phage in the mixture, it would grow and subsequently cause a detectable clearing in the bacterial spot. Nonsuppressing strains appeared as smooth patches of growth after incubation. The phage mixture was appropriately diluted to ensure that lysis of a bacterial spot was not the result of phage complementation. In a similar manner, T4rIIA and rIIB cistron mutants were tested on revertants in the KH600 (λ) strains. Where suppres-

sion was evidenced, the revertants were purified and tested with each individual phage mutant for suppressor patterns. At least 100 independently isolated Ara$^+$ revertants of each *araB* mutation were tested for nonsense phage suppression.

Mapping of the revertant site. Phage P1bt, grown on the Leu$^+$ derivatives of the revertant strains was crossed with strain SB1076 (full genotype *araD139Δ714 leuB1*). Deletion 714 excises most, if not all, of the *ara* operon *O1BAD*. Ara$^+$ transductants were selected by plating the transduction mixture on MAL. This cross tested for linkage between the revertant site and the *thr-ara-leu* region. (*Leu* cotransduces with *ara* over 50% of the time and 4% of the time with *thr*). For Ara$^+$ transductants to occur, both the revertant site and the *ara* region had to be cotransduced. A portion of the transduction mixture was also plated onto MG for selection of Leu$^+$ transductants. Failure of appearance of Ara$^+$ transductants in a sample of the transduction mixture that gave 250 Leu$^+$ transductants was employed as an indication that the revertant site mapped outside of the *thr-ara-leu* region.

Growth of cells in batch culture. For single determinations of induced enzyme levels, overnight casein hydrolysate cultures were diluted into 150 ml of fresh homologous medium in 1-liter Erlenmeyer flasks to a density of 15 units recorded on the Klett-Summerson colorimeter (blue filter). The flasks were incubated at 37 C with aeration achieved by a rotary shaker. After a period of one doubling, L-arabinose was added to a final concentration of 0.4%, and the cultures were allowed to grow for another 2.5 generations before harvesting.

For single determinations of constitutive isomerase levels in batch culture, cells were diluted from an overnight MGly culture into 100 ml of homologous medium to a turbidity of 25 Klett units. After 3.5 doublings, the cells were diluted to 100 Klett units in the same volume with prewarmed fresh medium and allowed to grow for 1.5 more generations before harvesting. At least four generations of exponential growth had taken place before harvesting.

Determinations of differential rates of synthesis of the enzymes of the *ara* operon were performed by the methods described previously (14) with slight modifications. Overnight cultures of cells were diluted into 1.5 liters of homologous media in 2-liter Kluyver flasks to a turbidity of 25 Klett Units; the flasks were incubated at 37 C with aeration. After 2.5 generations, L-arabinose, in the desired amount, was added. Where cyclic 3′,5′-AMP was used, it was added in the desired quantity directly to the culture. Samples containing at least 100 ml of the culture were taken every 5 min after the addition of the inducer for a period up to at least 30 min. Growth was stopped by addition of chloramphenicol (50 μg/ml) and by chilling the sample quickly. The turbidity was recorded, and the samples were left in the cold until the end of the experiment.

Growth of cells in a chemostat. Overnight cultures of cells, grown in either mineral arabinose or mineral glucose (0.05%) were added to the growth chamber of a chemostat (200 ml) similar to that described by Kessler and Englesberg (22). When the culture reached maximal density (130 Klett Units), fresh media was allowed to flow into the growth chamber at a controlled rate. Samples of the outflow were collected only after at least four doublings of the population of cells had occurred for any given doubling time.

Preparation of cell-free extracts. Samples collected for enzyme assay were harvested and washed once with 10^{-3} M ethylenediaminetetraacetic acid (EDTA), *p*H 7.6. The cell pellet was suspended into 1.5 ml of extraction buffer (10^{-3} M EDTA; 10^{-3} M reduced glutathione, *p*H 7.6), sonically oscillated, and centrifuged by the method described previously (14).

Enzyme assays. Assays for L-arabinose isomerase, L-ribulokinase, and L-ribulose-5-phosphate-4-epimerase activities were performed by the method previously described (14). Isomerase assays were performed at 30 or 37 C as indicated, the kinase and epimerase at 37 C.

Protein determination. Protein determination was performed by the method of Lowry et al. (26).

RESULTS

Reversion analysis. *AraB1* and *araB8* are reverted to Ara$^+$ by ICR 170 and ICR 191 (Table 1). Both mutants also revert to Ara$^+$ spontaneously. Neither is reverted by NTG or the base analogues, 2-AP, or 5-BU. (*AraB8* is also reverted by DES and EMS). On the basis of these results, *araB1* and *araB8* may be classified as frameshift mutants (2, 3, 36).

Mutants *araB6*, *araB24*, *araB43*, *araB46*, *araB55*, *araB63*, and *araB71* are reverted to Ara$^+$ by 2-AP and are not reverted by the two ICR compounds employed. All these strains revert spontaneously and can also be induced to revert by DES and EMS. Some are reverted by NTG. On the basis of reversion induced by 2-AP these mutations are probably the result of base-pair substitutions (transitions; references 4 and 51) and therefore could be nonsense or missense mutations.

AraB14, *araB16*, *araB26*, and *araB29* are not revertible by the ICR compounds and the transition-causing chemicals, 2-AP and 5-BU. However, they revert spontaneously and are revertible by alkylating agents. They would therefore appear to be the result of base-pair substitutions, presumably transversions, and could therefore also be either nonsense or missense mutations.

Determination of mutation type by analysis for nonsense suppression. The *araB* mutants which originated from base-pair substitutions could contain either nonsense or missense mutations. Although both types of mutations can be reverted by extragenic suppressors (17, 49), we are able to identify the suppressors of nonsense mutations by their ability to suppress nonsense mutations in phage T4 and T7.

The results of this screening for nonsense suppressors are shown in Table 2. Only revertants of *araB6*, *araB14*, *araB28*, *araB29* and *araB55* were found to suppress nonsense mutations in T4 and T7. Where suppression was not evidenced, at

TABLE 1. *Reversion response of AraB⁻ mutations*[a]

Parental strain	Spontaneous[b]	2-AP	5-BU	DES	EMS	NTG	ICR170	ICR191	Presumptive class[c]
araB1	+	−	−	−	−	−	+	+	f.s.
araB6	+	+	−	+++	++	+	−	−	b.p.s.
araB8	+	−	−	+	+	−	+++	+++	f.s.
araB14	+	−	−	+++	+++	++	−	−	(b.p.s.)
araB16	+	−	−	++	++	−	−	−	(b.p.s.)
araB24	+	++	−	++	++	++	−	−	b.p.s.
araB26	+	−	−	+++	+++	++	−	−	(b.p.s.)
araB28	+	+	−	++	+++	++	−	−	b.p.s.
araB29	+	−	−	++	+++	−	−	−	(b.p.s.)
araB43	+	++	−	+++	+++	+++	−	−	b.p.s.
araB46	+	+	−	++	+++	−	−	−	b.p.s.
araB55	+	+	−	+	+	−	−	−	b.p.s.
araB63	+	+	−	++	++	−	−	−	b.p.s.
araB71	+	+	−	++	++	++	−	−	b.p.s.

[a] All strains carried the *araC^c67* mutation in the B/r genetic background. The strains were grown overnight in nutrient broth, washed, and added to the top layer of MA (mineral base, L-arabinose) plus 1% nutrient broth double layer plates. Mutagens were added to the plates as described in Materials and Methods. The plates were incubated at 30 C for 4 days. The responses indicated are as follows: −, 0 to <5 colonies above number on plate with no mutagen; +, 5 to 10 colonies above number on plate with no mutagen; ++, 11 to 50 colonies above number on plate with no mutagen; +++, more than 50 colonies above number on plate with no mutagen. Abbreviations: 2-AP, 2-aminopurine; 5-BU, 5-bromo-deoxyuridine; DES, diethylsulfate; EMS, ethylmethanesulfonate; NTG, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; f.s., frameshift mutation; b.p.s., base-pair substitution mutation; (b.p.s.), probably base-pair substitution mutation.

[b] Spontaneous reversion to Ara⁺ indicated by appearance of colonies on plate with no mutagen.

[c] The presumptive class of mutation was determined as described in the text.

TABLE 2. *Suppression tests for identification of nonsense mutations*[a]

Parental strain[b]	Genetic background	Total no. of revertants tested	No. of revertants permissive for				Nonsense mutation
			T7 amber	T4 rII amber	T4 rII ochre	T4 rII UGA	
araB1	B/r	44	0	—[c]	—	—	No
araB1	KH[d]	70	0	0	0	0	
araB6	B/r	10	1	—	—	—	Yes
araB6	KH	14	4	4	0	0	
araB8	B/r	95	0	—	—	—	No
araB14	B/r	10	4	—	—	—	Yes
araB14	KH	12	8	8	0	0	
araB16	B/r	60	0	—	—	—	No
araB16	KH	30	0	0	0	0	
araB24	B/r	90	0	—	—	—	No
araB24	KH	107	0	0	0	0	
araB26	B/r	10	0	—	—	—	No
araB26	KH	63	0	0	0	0	
araB28	B/r	106	38	—	—	—	Yes
araB28	KH	25	6	6	0	0	
araB29	B/r	80	7	—	—	—	Yes
araB29	KH	20	10	10	0	0	
araB43	B/r	117	0	—	—	—	No
araB43	KH	42	0	0	0	0	
araB46	B/r	56	0	—	—	—	No
araB55	B/r	40	1	—	—	—	Yes
araB55	KH	20	0	0	0	0	
araB63	B/r	105	0	—	—	—	No
araB63	KH	45	0	0	0	0	
araB71	B/r	35	0	—	—	—	No

[a] Revertants were grown in tryptone broth and spotted onto a tryptone agar plate. Phage mixtures containing T7 amber, T4 rII amber, T4 rII ochre, or T4 rII UGA mutants were deposited onto the bacterial spots. Clearing of the bacterial spot after 24 hr indicated permissiveness of the revertant for at least one phage in the mixture.

[b] All strains carried the *araC^c67* allele along with the *araB⁻* mutation.

[c] Strains in the B/r genetic background were not tested with the T4 phage mutants.

[d] Abbreviated from KH600 (λ).

least 50 revertants of independent origin to each mutation were screened before the analysis was terminated. The suppression pattern of some nonsense suppressors is shown in Table 3. Only amber mutations were found to be suppressed. (The differences in suppressor pattern do not necessarily indicate the presence of different nonsense suppressors. These differences could be due to different efficiencies of activity of the same suppressor present in different strains.)

Determination of missense mutations. Of the five *araB* gene mutants which could not be identified as containing a nonsense or frameshift mutation, the possibility still exists that they may be the result of nonsense mutations. The failure to observe suppression in these cases could be the result of the failure of these strains to yield extragenic suppressors. Hence, all of the revertants in these cases would be carrying intragenic mutations. Alternatively, these strains may possess missense mutations. In this case, neither intragenic nor extragenic revertants of these *araB*$^-$ mutants could permit the growth of nonsense phage mutants. The demonstration that Ara$^+$ revertants, which failed to suppress the nonsense phage mutants, did possess an extragenic suppressor would constitute indirect evidence that the parental *araB*$^-$ was a missense mutant. Accordingly, we analyzed at least 8 to 10 of the spontaneous and DES-induced revertants of *araB16*, *araB24*, *araB43*, *araB46*, and *araB63* for extragenic suppressors by determining if any of the revertant mutant sites were unlinked to the *ara* region of the chromosome (*see above*). It was found that, in at least 20% of the revertants so screened, the revertant site was not linked to the *ara* region (Table 4). Since these extragenic revertants were not able to suppress any of the 28 different nonsense phage mutants, it is likely that the five *araB* mutants so analyzed are the result of missense mutations.

Although the revertants of *araB26* and *araB71*, all of which fail to suppress phage nonsense mutations, were not analyzed for extragenic suppressors, it seems likely that these two mutants are also the result of missense mutations. First of all, *araB26* has been previously shown to map in the middle region of the *araB* gene (9) and produces hyperinducible amounts of isomerase as well as kinase CRM. *AraB71* is believed to be the result of a missense mutation on the basis of its similarity to the other hyperinducers particularly with regard to the results of merodiploid analysis (*see below*).

Induced L-arabinose isomerase levels in araB mutants. The *araB* mutants were reassayed for L-arabinose isomerase activity by using a slightly modified procedure from that previously employed (see Table 5 and above). The spread of isomerase activity found is similar to that previously reported (9). Activities range from 14 times (*B14*) less than to six times (*B26*) greater than wild-type activity. If one were to order the *araB* mutants with respect to their isomerase activities between these two extremes, the order would differ somewhat from that based on previous work. However, there is complete agreement with regard to the grouping of the mutants into the categories of hyperinducers and hypoinducers or polar mutants as we shall call the latter group.

A comparison of the isomerase levels of the fourteen strains (Table 5) reveals that all mutants that produce lower than wild-type levels of this enzyme are nonsense or frameshift mutants. The nonsense and frameshift mutants are placed together in group A in order of their map position (see also Fig. 1). It can be seen that a gradient of polarity for the nonsense mutations is not evident. The mutant *araB14* is more polar than the nonsense mutants *araB55* and *araB29* which map close to the operator end of the *araB* gene. Similarly, the most distal nonsense mutation employed in this study, *araB6*, exhibits a stronger polar effect than does the nonsense mutation *araB28* which maps operator-proximal to *araB6*. In fact, mutant *araB28* is not polar but hyperinducible with respect to the isomerase level in the wild type. The fact that mutant *araB28*, produced by a nonsense mutation, is a hyperinducer is indicative of the fact that more is involved here than a simple polarity effect caused by a nonsense codon. Two frameshift mutations, *araB8* and *araB1*, which map at the operator end of the *araB* gene, also show polar effect. The polarity exhibited is less than the polarity shown by three adjacent, operator-distal nonsense mutations.

Of the strains that produce higher than wild-type levels of isomerase, one is a nonsense mutant (*B28*) discussed above, and the remainder are probably missense mutations as we have indicated.

The missense mutants are placed in group B in Table 5 in order of their map position (Fig. 1). No correlation between the map position and the isomerase levels of these mutants can be observed.

Test for cis dominance of the araB mutations. Two hypotheses can account for the levels of isomerase observed in the *araB* gene mutants. The levels may be the result of a direct, *cis*-dominant effect of the *araB*$^-$ mutation on translation or, alternatively, an indirect effect of the *araB*$^-$ mutation on the rate of expression of the *ara* operon such as that resulting from the inability to metabolize L-arabinose.

If the isomerase levels in the *araB* mutants

TABLE 3. *Nonsense phage suppressor patterns of revertants of AraB gene mutants*[a]

Host	Genotype	Phage mutants																										
		T7 Amber											T4 rII															
													Amber							Ochre								UGA		
		T7+	OSS9	OSS11	OSS14	OSS15	OSS20	SUS15	SUS18	SUS19	SUS22	SUS30	T4+	r638	rHB118	rN11	rN24A	rB94	rGU63	rAP53A	rN21	rUV375	rN24	rN17	rN7	r609	rSD160	rUV199	rAP53	rN65	rX655	rX665
K-12(λ)	C600[b]	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
K-12(λ)	SB022[b]	+	+	+	+	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	−	−	−
K-12(λ)	CAJ64[b]	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
KH600(λ)	*araB6C[c]67su⁺6E1*	+	+	+	+	−	+	−	+	+	−	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
B/r	*araB6C[c]67su⁺6*	+	+	+	+	−	+	−	+	+	+	+	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KH600(λ)	*araB14C[c]67su⁺14E1*	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
B/r	*araB14C[c]67su⁺14D*	+	+	+	+	−	+	−	+	+	+	+	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KH600(λ)	*araB28C[c]67su⁺28E1*	+	+	+	+	−	+	−	+	+	−	+	+	−	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
B/r	*araB28C[c]67su⁺28D*	+	+	+	+	−	+	−	+	+	+	+	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
KH600(λ)	*araB29C[c]67su⁺29E1*	+	+	+	+	−	+	−	+	+	+	+	+	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
B/r	*araB29C[c]67su⁺29D*	+	+	+	+	−	+	−	+	+	+	+	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
B/r	*araB55C[c]67su⁺55D*	+	+	+	+	−	+	−	+	+	+	+	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[a] Suppression was tested by spotting phage mutants onto plates containing bacteria. Symbols: +, clearing of bacteria on spot: suppression; −, no suppression; X, not tested.

[b] These strains do not have *araB* mutations but contain nonsense suppressors: C600, amber suppressor; SB022, ochre suppressor; CAJ64, UGA suppressor.

were solely the result of a *cis*-dominant effect upon translation of the *ara* operon, in Ara$^+$ merodiploids of the type *F'araA*$^-$*B*$^+$/*araA*$^+$*B*$^-$, the isomerase levels should remain the same as the levels observed in the respective haploid *araB*$^-$ strains. Ara$^+$ merodiploids of this type were constructed and assayed for induced levels of isomerase in the same manner as described for the haploids.

The results obtained for the merodiploids containing nonsense or frameshift mutations (group A mutants) are shown in Table 6. It can be observed that, although the polar effect of this group of mutants is *cis* dominant, the isomerase levels in all the merodiploids, except those containing the two most polar mutations, *araB14* and *araB55*, are lower than the levels found in the respective haploids. The most notable case is *araB28* which in the haploid strain was observed to be hyperinducible. The merodiploid containing this nonsense mutation exhibits a polar effect.

It is clear, from these results, that the polar isomerase levels observed in the *araB*$^-$ haploid strains cannot be explained *solely* on the basis of a *cis*-dominant effect of the mutation upon translation.

The results obtained for the Ara$^+$ merodiploids containing the mutations shown in group B of Table 5 (five hyperinducible and presumably missense) are shown in Table 7. It can be observed that, in all cases, the hyperinducible effect is recessive; the isomerase levels are no longer elevated four- to sixfold over the respective level in the wild type but, in fact, are very close to the wild-type level. (The variation in levels among the different Ara$^+$ merodiploids may be due to the presence of a low frequency of Ara$^-$ segregants in a merodiploid population.)

A *trans* effect of the *araB*$^-$ mutation was also examined in two cases. L-Ribulokinase levels were determined in the wild type and in the merodipoids *F'araA2B*$^+$/*AraA*$^+$*B6* and *F'araA2B*$^+$/*araA*$^+$*B24*. It was observed that the kinase levels were very similar in these three strains.

The finding that the isomerase levels in the Ara$^+$ merodiploids of the hyperinducible *B*$^-$ mutants are very similar to the isomerase level in the wild type indicates that these *B*$^-$ mutations have no direct effect upon transcription or translation.

Thus, in addition to a *cis*-dominant polar effect caused by some nonsense mutations, the rate of expression of the *ara* operon as compared to the wild type may be indirectly affected by a mutation to *araB*$^-$. Inability of an *araB*$^-$ strain to utilize the inducer, L-arabinose, may result in an increase in rate of expression of the *ara* operon over the rate in an Ara$^+$ strain (merodiploid) which carries the same *araB*$^-$ marker.

TABLE 4. *Linkage analysis of the revertant sites*[a]

Donor	Recipient	Ara$^+$ transductants
araB$^+$	*ara-714 leu*$^-$	+
araB16	*ara-714 leu*$^-$	–
araB16R1, R7, R30, R35, R40, R43	*ara-714 leu*$^-$	+
araB16R49, R50, R60	*ara-714 leu*$^-$	–
araB24	*ara-714 leu*$^-$	–
araB24R2, R6, R33	*ara-714 leu*$^-$	+
araB24R42, R50, R58, R66, R73	*ara-714 leu*$^-$	–
araB43	*ara-714 leu*$^-$	–
araB43R55, R66, R72, R79, R88, R98, R117	*ara-714 leu*$^-$	+
araB43R48, R78, R82, R87, R93, R116	*ara-714 leu*$^-$	–
araB46	*ara-714 leu*$^-$	–
araB46R15	*ara-714 leu*$^-$	+
araB46R24, R31, R40, R43, R46, R50, R52, R56	*ara-714 leu*$^-$	–
araB63	*ara-714 leu*$^-$	–
araB63R37, R50, R51, R63, R74, R75, R83, R96	*ara-714 leu*$^-$	+
araB63R120, R122	*ara-714 leu*$^-$	–

[a] Donor phage carried the *araC*^{c}*67* allele and the genes for leucine independence. Selection for Ara$^+$ was performed on MAL (mineral base, L-arabinose, L-leucine) agar. An equal volume of the transduction mixture was plated onto MG (mineral base, D-glucose) for selection of Leu$^+$ transductants. Each transduction mixture yielded at least 250 Leu$^+$ transductants/ml.

Polarity in constitutive strains. If the inability to metabolize L-arabinose by the *araB*$^-$ mutants is the complicating feature in determining the true polarity of the nonsense and frameshift mutations (measured with respect to wild type activity), as suggested in the merodiploid analysis, a comparison of the wild-type activity and that of the various *araB*$^-$ nonsense and frameshift mutants in constitutive strains grown in the absence of L-arabinose should eliminate this complication.

Accordingly, a set of *araB*$^-$*C*^{c}*67* and *araB*$^+$*C*^{c}*67*, isogenic except for the *araB*$^-$ nonsense or frameshift mutations, was analyzed for constitutive isomerase activity. All *araB* nonsense and frameshift mutants are observed to be polar (Table 8). The constitutive levels of isomerase range from 2% of the *araB*$^+$*C*^{c}*67* level in *araB14C*^{c}*67* to 61% in *araB28C*^{c}*67*. As was observed in the case of the induced isomerase levels (Table 5), no gradient of polarity is found when the constitutive isomerase levels are determined. *AraB14* is still the most polar mutant. Further-

TABLE 5. *Induced L-arabinose isomerase levels in AraB gene mutants*[a]

Strain	Group	L-Arabinose genotype	Specific activity of L-arabinose isomerase						Mutation type[b]
			1	2	3	4	Avg	Corrected	
UP1002		Wild type	21.5	29.2	22.8	—[c]	24.5	100	
UP1015	A	*araB8*	9.6	13.6	8.2	10.4	10.7	43	f.s.
UP1008	A	*araB1*	11.0	16.0	10.3	16.9	13.3	54	f.s.
UP1032	A	*araB29*	9.1	7.5	6.9	6.5	7.5	31	n.s.
UP1055	A	*araB55*	2.7	5.6	5.5	—	4.6	19	n.s.
UP1019	A	*araB14*	1.6	1.4	1.8	—	1.6	7	n.s.
UP1031	A	*araB28*	36.5	36.5	—	—	36.5	150	n.s.
UP1013	A	*araB6*	18.5	20.9	—	—	19.8	81	n.s.
UP1027	B	*araB24*	95.0	100	—	—	97.5	398	m.s.
UP1046	B	*araB46*	111.2	98.6	—	—	104.9	427	m.s.
UP1064	B	*araB71*	95.3	103	96.3	—	98.2	400	(m.s.)
UP1060	B	*araB63*	115	103	—	—	109	445	m.s.
UP1043	B	*araB43*	93.2	89.5	—	—	91.4	373	m.s.
UP1029	B	*araB26*	150	144	—	—	147	600	(m.s.)
UP1021	B	*araB16*	97.5	—	—	—	97.5	398	m.s.

[a] Cultures were grown at 37 C in 1% casein hydrolysate plus 0.4% L-arabinose for 2.5 generations and harvested in the exponential phase of growth. Isomerase assays were performed at 30 C as previously described (14). Columns 1 to 4 represent assays from different extracts. L-Arabinose isomerase specific activity is expressed as micromoles of ribulose produced per hour per milligram of protein. The column labeled "corrected" gives the relative isomerase values adjusted to the wild-type level of 100. Mutants in groups A and B are listed in order of their map position (9; see Fig. 1).

[b] Abbreviations: f.s., frame shift; n.s., nonsense mutation; m.s., missense mutation; (m.s.), probably a missense mutation.

[c] —, no determination was performed.

TABLE 6. *Cis-dominance of the polar mutations*[a]

Strain	L-Arabinose isomerase				L-arabinose isomerase		
	1	2	Avg	Corrected	Strain	Avg	Corrected
					ara$^+$	24.5	100
F'*araA2B*$^+$/*araA*$^+$*B8 leu*$^-$	5.0	5.4	5.2	21	*araB8*	10.7	43
F'*araA2B*$^+$/*araA*$^+$*B1*	7.2	6.9	7.1	29	*araB1*	13.3	54
F'*araA2B*$^+$/*araA*$^+$*B29*	4.9	5.0	5.0	20	*araB29*	7.5	31
F'*araA*$^+$*B55*/*araA2B*$^+$*leu*$^-$*Sm*^{r}	4.7	—[b]	4.7	19	*araB55*	4.6	19
F'*araA2B*$^+$/*araA*$^+$*B14leu*$^-$*T6*^{r}	2.1	2.9	2.5	10	*araB14*	1.6	7
F'*araA2B*$^+$/*araA*$^+$*B68leu*$^-$*T6*^{r}	6.6	—	6.6	27	*araB68*	16.3	67
F'*araA2B*$^+$/*araA*$^+$*B28*	20.2	19.5	19.8	81	*araB28*	36.5	150
F'*araA2B*$^+$/*araA*$^+$*B6leu*$^-$	6.6	6.6	6.6	27	*araB6*	19.8	81

[a] Cultures were grown and assayed for isomerase as described in Table 5. Columns 1 and 2 stand for independent determinations of isomerase specific activity. The corrected values were calculated as described in Table 5 by using the average specific value of isomerase in the wild type as a comparison. The average and corrected values for the haploid strains are shown in the right hand side of this table as a comparison and are taken from Table 5.

[b] No determination was performed.

TABLE 7. *Loss of hyperinducibility in Ara*$^+$ *merodiploids*[a]

Strain	L-Arabinose isomerase					L-Arabinose isomerase		
	1	2	3	Avg	Corrected	Strain	Avg	Corrected
						ara$^+$	24.5	100
F'*araA2B*$^+$/*ara*$^+$*B24*	23.0	24.3	21.7	23.0	94	*araB24*	97.5	398
F'*araA2B*$^+$/*araA*$^+$*B63*	22.6	21.0	—[b]	21.8	89	*araB63*	109	445
F'*araA2B*$^+$/*araA*$^+$*B26*	27.8	31.2	—	29.0	118	*araB26*	147	600
F'*araA2B*$^+$/*araA*$^+$*B71*	19.0	—	—	19.0	77	*araB71*	98.2	400
F'*araA2B*$^+$/*araA*$^+$*B16*	24.3	—	—	24.3	99	*araB16*	97.5	398

[a] Cultures were grown and assayed for isomerase as described in Table 5. Isomerase is expressed as specific activity (*see* Table 5). Columns 1 to 3 indicate independent determinations. The corrected values were calculated as described in Table 5 by using the average specific activity in the wild type as a comparison. The average and corrected values for the haploid strain are taken from Table 5.

[b] Determinations not performed.

TABLE 8. *Constitutive L-arabinose isomerase levels in AraB polar mutants*[a]

Strain	L-Arabinose isomerase				Corrected polarity value				
	1	2	3	4	1	2	3	4	Avg
araB$^+$*C*^{c}*67*	100	73.5	87.0	86.5	100	100	100	100	100
araB8C^c*67*	30.8	20.2	—[b]	—	31	27	—	—	29
araB1C^c*67*	17.1	11.6	15.2	—	17	16	17	—	17
araB29C^c*67*	—	2.5	3.4	—	—	3	4	—	4
araB55C^c*67*	—	3.6	4.5	4.8	—	5	5	6	5
araB14C^c*67*	—	0.7	1.1	2.0	—	1	1	2	2
araB28C^c*67*	59.5	—	56.4	51.0	60	—	65	59	61
araB6C^c*67*	—	18.4	21.5	25.0	—	25	25	29	26

[a] Cultures were grown in 0.2% mineral glycerol. Isomerase assays were performed at 30 C (*see* Table 5). Columns 1 to 4 refer to enzyme activities determined from cell-free extract prepared on different days. Because of the variation in enzyme levels experienced from day to day, the isomerase levels of the B$^-$ mutant were corrected to that obtained with *araB*$^+$*C*^{c}*67* for each experiment. These corrected values, based on setting the wild-type activity to 100, are so indicated. The last column shows the average of the calculated values. The mutants are listed in order of their position in the *araB* gene beginning with *araB8* closest to the operator (*see* Fig. 1).

[b] Determinations not performed.

more, when compared to the respective Ara$^+$ strain, the constitutive levels of isomerase of the *B*$^-$*C*^{c} strains are "more polar" than the inducible levels in *araB*$^-$*C*$^+$ strains.

These results confirm the findings with merodiploids that all of the nonsense and frameshift mutations examined exhibit polar effects. The actual polar effect, however, may be masked when inducible isomerase levels in the *araB* gene mutants, such as *araB28* or *araB8*, etc., are compared to the inducible isomerase level in the wild type. An accurate estimate of the polar effect of the nonsense or frameshift mutation would seem to depend, therefore, on the exclusion of the indirect effects of the mutation.

Differential rate of synthesis of L-arabinose isomerase by the missense mutants. The differential rate of synthesis of L-arabinose isomerase was determined in the wild type and the five isogenic missense mutants, *araB16*, *araB24*, *araB43*, *araB46*, and *araB63*. The results are shown in Fig. 2. The rates were determined in exponentially grown cells at 0 to 30 min after the addition of L-arabinose as the inducer. The rates with

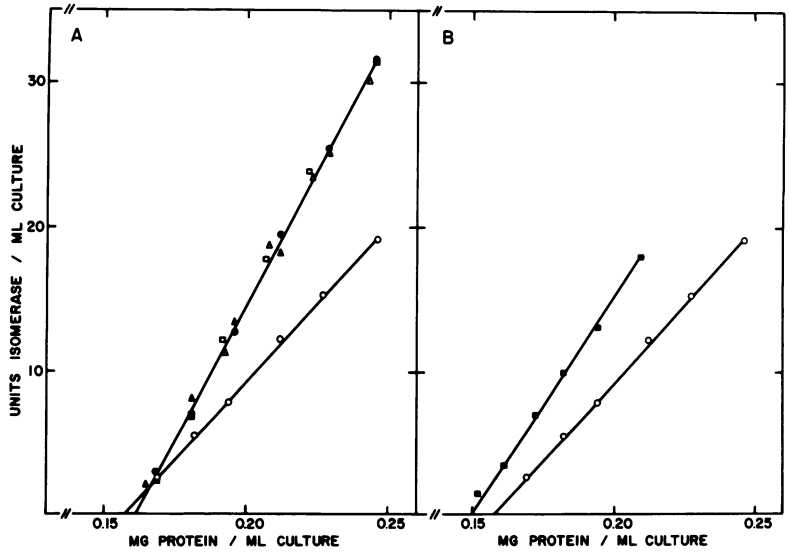

FIG. 2. *Initial differential rate of isomerase synthesis in araB missense mutants. The cultures were grown in casein hydrolysate medium with L-arabinose as inducer. Samples were taken at 5-min intervals for up to 35 min after addition of arabinose. Isomerase assays at 37 C and protein measurement were performed as described in Materials and Methods. The lines were fitted by the method of least squares. The slope is the differential rate of synthesis. Symbols: ○, wild type; ●, araB46; △, araB43; ▲, araB63; □, araB24; ■, araB16.*

araB24, *araB43*, *araB46*, and *araB63* are not distinguishable from each other and are twofold greater than the rate as determined in the wild-type strain. The differential rate with *araB16*, which is 1.5-fold greater than the wild-type rate, is based on an average of three independent experiments with a 5% error and is significantly lower than the rates determined with the other mutants examined.

Although there was a maximum of a twofold difference between four of the hyperinducible mutants from that of the wild type, single determinations of isomerase level in cells harvested two or three generations after the addition of arabinose yielded levels in *araB* missense mutants four- to sixfold higher than the respective levels in the wild type as shown in Table 5. To reconcile these differences, the differential rate of isomerase synthesis in *araB24* was determined for a period of 2.5 hr after the addition of the inducer. The results of this experiment are shown in Fig. 3. Here again it can be observed that the initial rate of synthesis of L-arabinose isomerase

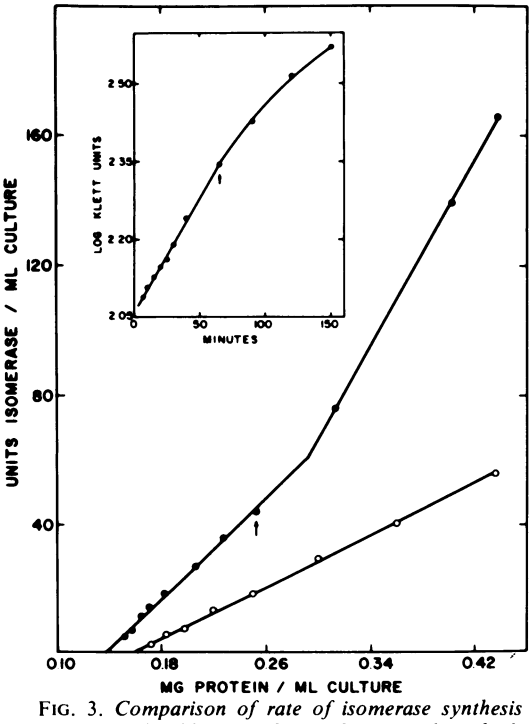

FIG. 3. *Comparison of rate of isomerase synthesis in araB24 and wild type. The conditions and methods of determinations are the same as those described in the legend to Fig. 2 except that the determinations were conducted for a period of up to 150 min after addition of inducer. Symbols: ○, wild type; ●, araB24. The insert is a plot of growth of araB24 after addition of arabinose. The arrow represents the corresponding points in growth and enzyme synthesis. The lines were fitted by the method of least squares.*

in *B24* is approximately twofold greater than that observed with the wild type. However, at the point in growth corresponding to 1 hr after the addition of inducer, there is an abrupt change in rate of isomerase synthesis with *B24* corresponding to a further twofold increase in rate of synthesis as compared to the wild type. The wild type is observed to synthesize isomerase at a constant rate for a period of 2 hr after the addition of inducer.

The insert to Fig. 3 indicates the growth rate of the mutant after the addition of arabinose to the casein hydrolysate culture. It can be observed that the growth of the mutant is no longer exponential after 1 hr and that the onset of the decrease in growth rate corresponds roughly to the point where the differential rate of isomerase synthesis increases. The growth rate of the wild type was constant for the duration of the experiment.

Gross and Englesberg (20), in studying the growth of Ara$^-$ strains, found that *araB* mutants were slightly inhibited in both growth rate and growth yield upon addition of arabinose to casein hydrolysate cultures. From the results of many experiments employing the five *araB* mutants used in the above study, growth ceases to be exponential and begins to become inhibited approximately 40 to 60 min after the addition of arabinose, independent of the cell density at which the inducer was added. These inhibitions of growth were always found to be associated with an increase in the rate of synthesis of isomerase. The secondary rates of synthesis of isomerase were not found to be identical in the different strains, nor were they linear in all cases.

Because of the difficulty of interpreting the results of the kinetics of induction determined during the phase of L-arabinose inhibition and the great variability of results found during this period, we concentrated our efforts in this study on the analysis of the hyperinduction as characterized by the twofold increase in rate of enzyme synthesis which occurs during the first hour after the addition of the inducer.

It should be pointed out, however, that the test for dominance of hyperinducibility (Table 7) demonstrates that the entire range of hyperinducibility (two- to sixfold increase in isomerase activity) is recessive in merodiploids.

Analysis of the hyperinducible effect: models considered. The following models have been considered to explain the indirect (*cis* and *trans* recessive) hyperinducible effect of mutations in the *araB* gene. We shall be concerned with the hyperinducible effect as demonstrated by the approximate twofold increase in the initial rate of synthesis of L-arabinose isomerase.

Model 1: accumulation of high concentration of effector. Mutations in gene *araB* lead to the accumulation of larger concentrations of L-arabinose and L-ribulose than found in the Ara$^+$ (wild type). Either the higher concentrations of L arabinose or ribulose, or both, converts more of the *araC* gene product into activator than in the wild type, thus stimulating further expression of the *araOIBAD* operon. Missense mutations in gene *araB* would be hyperinducible; however, nonsense mutations, because of their possible polarity effect, might obscure to varying degrees the potential increase in level of expression of the distal structural genes in the operon.

Model 2: self-catabolite repression. L-Arabinose is a source of catabolite repressors. In the wild type, the steady-state level of expression of the L-arabinose operon *araOIBAD* is determined not only by the internal concentration of L-arabinose, the effector in this system, but also by the accumulation of products formed from L-arabinose which act to shut down the expression of the operon presumably at the level of transcription (39, 52). In the wild type, therefore, at balanced growth with L-arabinose as the inducer, the operon is not functioning at full potential. However, in mutants blocked in the metabolism of L-arabinose (*araB*$^-$ and *araA*$^-$), full expression of the operon becomes possible. In missense mutants in the *araB* gene, this full potential is realized. However, as in model 1, nonsense mutation in the *araB* gene might obscure these effects because of their potential polarity effect on the expression of the proximal genes in the operon. (Similar effects might be expected with *araD*$^-$ mutants. However, since L-arabinose is extremely inhibitory to growth of these mutants, it is difficult to achieve conditions that would produce full induction).

Is ribulose accumulation in araB$^-$ mutants responsible for the hyperinducible effects? The accumulation of high concentrations of effector model states that the *araB* mutants exhibit elevated rates of synthesis of isomerase as a result of accumulation of L-arabinose or ribulose, or both, which serve to "hyperinduce" the operon. Early experiments have shown that L-arabinose is probably the effector in the L-arabinose system (12): (i) L-arabinose is the inducer in L-arabinose isomeraseless mutants. (ii) L-Arabinose is the only compound that could be found to be accumulated within isomeraseless strains (35). The possibility was not eliminated, however, that ribulose might also be an effector and that the accumulation of ribulose might be responsible for the hyperinducible effect of missense *B*$^-$ mutants. A direct test of this possibility was not possible since externally given ribulose is not accumulated by these strains; it cannot be used for growth by the wild type, and it is not an inducer of the system. However, it had been previously shown that *araA*$^-$ mutants are all hyperinducers for the kinase. It would thus appear that hyperinducibility does not depend on ribulose. It was decided, however, to provide a more rigorous test of the possibility that ribulose produced internally might be contributing a fraction of the hyperinducibility of *B*$^-$ mutants by measuring the rate of synthesis of epimerase and isomerase (where applicable) in *B*$^+$*A*$^+$, *B*$^-$*A*$^+$, and *B*$^-$*A*$^-$ double mutants that were otherwise isogenic. As seen in Table 9, *araB23* is a hyperinducer of both isomerase and epimerase, synthesizing these enzymes at approximately two times the respective rates in the wild type. Both the isomeraseless mutant, *araA13*, and the isomeraseless, kinaseless double mutant, *araA13B23*, however, still synthesize epimerase at approximately two times the wild-type rate in spite of the fact that they do not produce ribulose. These results indicate that the accumulation of ribulose is not significant in determining hyperinducibility.

The possibility that the increased concentration of L-arabinose as a result of the break in L-arabinose metabolism may be responsible for the hyperinducible effect cannot be eliminated at this point and shall be referred to subsequently.

Self-catabolite repression of the arabinose OIBAD operon. The second model to explain the hyperinducible effect states that the utilization of arabinose itself results in the production of catabolites which repress the expression of the *ara* operon. McFall and Mandelstan (32), in a study of catabolite repression resulting from the metabolism of glucose (the glucose effect), were able to show that this effect could be severely reduced if glucose was fed to the cells under conditions in which it limited the growth rate, such conditions as are obtainable in the chemostat. Apparently, under such conditions the pool of catabolites produced from glucose is depleted,

TABLE 9. *Hyperinducible effect in AraA and AraA AraB double gene mutants*[a]

Strain	Arabinose genotype	Differential rate of synthesis	
		Isomerase	Epimerase
K-12 KH600	*ara*$^+$	200	16
K-12 KH600	*araB23*	470	46
K-12 KH600	*araA13B23*	<0.5	36
K-12 KH600	*araA13*	<0.5	42

[a] Cultures were grown as described in legend to Fig. 2. The rates of synthesis were determined graphically.

thereby reducing catabolite repression. Accordingly, to determine whether L-arabinose is a source of catabolite repressors, we measured the level of L-arabinose isomerase under conditions where the growth rate of the wild type was varied in response to arabinose limitation in a chemostat. Samples were collected and assayed for the level of L-arabinose isomerase at various generation times, between 70 and 120 min. In addition, as a control, specific activity of L-arabinose isomerase of the same wild-type cells growing in a mineral arabinose medium in which arabinose was in huge excess was determined. The results of these experiments are presented in Fig. 4. It can be observed that, as the generation time of the culture increases, L-arabinose isomerase activity increases as well. Between the limits set in this experiment, 45- to 120-min doubling time, a twofold linear increase in enzyme level was found. These results indicate that the rate of expression of the *ara* operon is regulated by the rate of utilization of the inducer, L-arabinose. As the pool of catabolites produced from arabinose is depleted by the increasingly slower feeding of arabinose to the cells, a proportional increase in level of expression of the *ara* operon ensues. These experiments suggest that arabinose, through its metabolism, is a source for catabolite repressors of the *ara* operon.

Comparison to glucose mediated catabolite repression. Although it has been reported that the activity of L-arabinose permease is sensitive to a glucose effect (35; K. Sweadner and E. Englesberg, *unpublished data*), only indirect evidence has been presented suggesting that the *ara* operon is sensitive to the classical glucose-mediated catabolite repression (13). To explore the latter phenomenon directly, L-arabinose isomerase levels were determined with *araB*$^+$*C*^{c}*67* growing in a chemostat with glucose as limiting carbon source. Three different exogenous concentrations of glucose were used, and the doubling times were adjusted to 60, 90 and 260 min. The *ara* operon is extremely sensitive to glucose-mediated catabolite repression (Table 10). As the doubling time was raised from 60 to 260 min, greater than an eightfold increase in isomerase level took place. This increase was not linear over the entire range of doubling times, however. A fivefold increase occurred as the doubling time was increased from 60 to 90 min, whereas only a 1.5-fold further increase was seen as the doubling time was raised from 90 to 260 min.

Effect of cyclic AMP. Since it has been shown that cyclic AMP can reverse the catabolite repression produced by glucose on the *lac* operon (10, 48), it was of interest to determine whether this compound could reverse the self-catabolite repression produced by arabinose. Accordingly, the differential rate of synthesis of L-arabinose isomerase in the wild-type strain was examined in mineral arabinose medium with or without cyclic AMP. It can be seen (Fig. 5) that addition

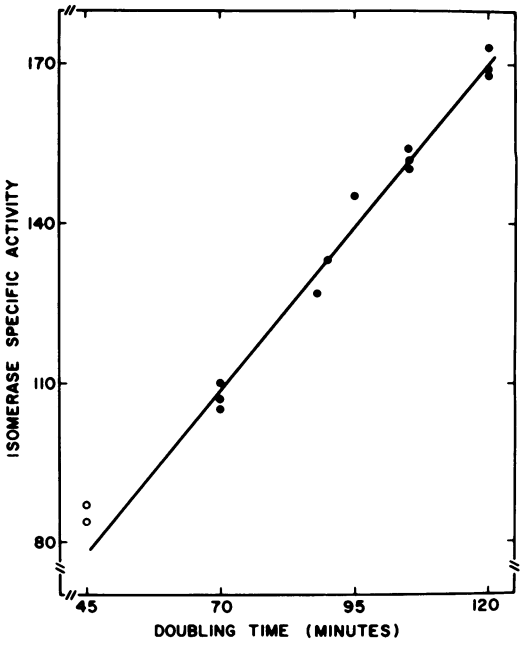

FIG. 4. *Effect of arabinose metabolism on isomerase production. The wild-type strain was grown in mineral arabinose medium in a batch culture or a chemostat. Isomerase assays were performed at 37 C. Symbols:* ●, *isomerase specific activity in samples removed from the chemostat (average of four independent determinations);* ○, *isomerase specific activity of batch culture growing in mineral* L-*arabinose medium with* L-*arabinose in large excess. The line was fitted by the method of least squares.*

TABLE 10. *Effect of glucose starvation in a chemostat upon the constitutive production of* L-*arabinose isomerase*[a]

Glucose concn (%)	Cell density × no. of cells/ml	Isomerase specific activity doubling time (min)		
		60	90	260
0.02	6×10^7		48	71
0.05	3×10^8	8.2	45	68
0.10	7×10^8		47	74

[a] Strain *araB*$^+$*C*^{c}*67* was grown in a chemostat in mineral glucose at the reservoir glucose concentrations and doubling time indicated. Isomerase specific activity was determined for at least four independent samples of the culture after at least four doublings had occurred under each set of conditions. Enzyme assays were performed at 30 C. The values shown are the averages of four independent determinations.

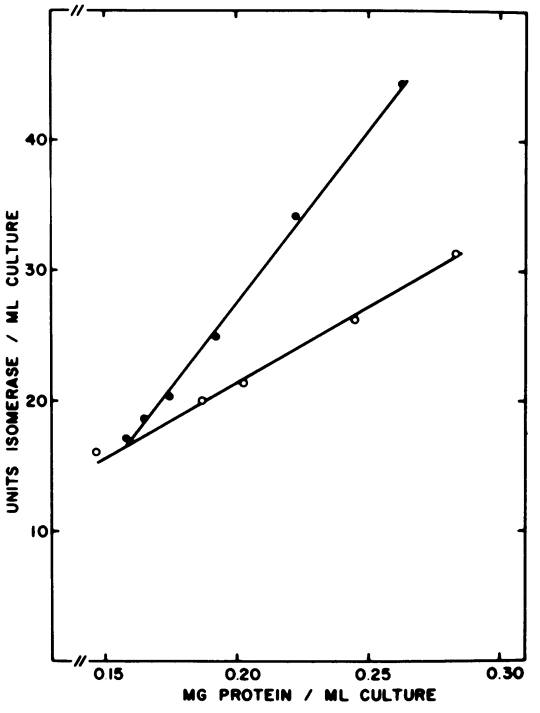

FIG. 5. *Effect of cyclic AMP on inducible rate of isomerase synthesis. An exponentially growing culture of the wild-type strain in mineral arabinose medium was divided into two portions. To one portion, 3',5'-cyclic AMP was added in final concentration of 2 × 10^{-3} M. The differential rate of isomerase synthesis was determined in both portions by the procedure described in the legend to Fig. 2. Symbols: O, no cyclic AMP; ●, plus cyclic AMP. The lines were fitted by the method of least squares.*

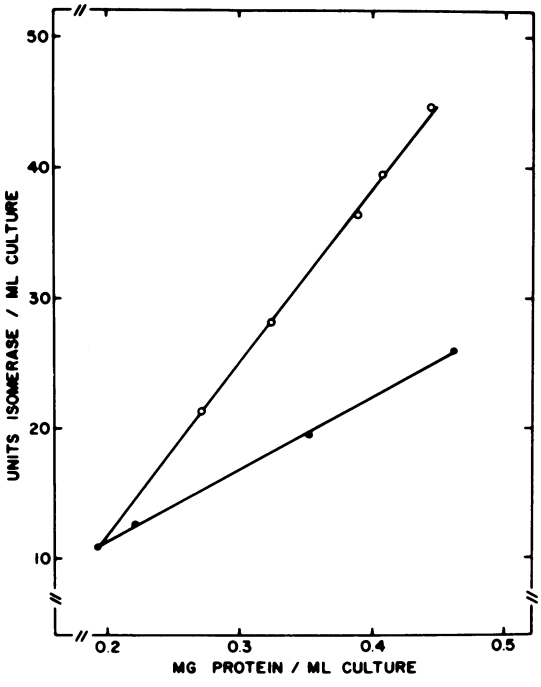

FIG. 6. *Effect of cyclic AMP on the constitutive rate of isomerase synthesis in glucose medium. The procedure was identical to that described for Fig. 5 except that the araB⁺C^c67 strain was employed and mineral glucose was substituted for mineral arabinose. Symbols: ●, no cyclic AMP; O, plus cyclic AMP. The lines were fitted by the method of least squares.*

of cyclic AMP resulted in a 2.2-fold increase in rate of isomerase synthesis over the rate observed in the control. At the concentration of cyclic AMP employed (2 × 10^{-3} M), there was no effect on the growth rate of the organism although there was a 20% reduction in growth yield.

The effect of cyclic AMP on the glucose-mediated catabolite repression of isomerase synthesis was also examined in the *araB⁺C^c67* strain. It can be observed (Fig. 6) that addition of cyclic AMP results in a 2.5-fold stimulation of constitutive isomerase production. In this case as well, no effect of cyclic AMP on growth rate was observed.

The twofold stimulation of isomerase synthesis in the wild type in arabinose medium is quantitatively similar to the difference in inducible rate of isomerase synthesis between the wild type and the *araB* mutants.

As can be seen in Fig. 7, cyclic AMP had no effect on the rate of synthesis of isomerase by *araB24* growing in a mineral glycerol L-arabinose medium. This is what would be expected if *araB24* is immune to L-arabinose self-catabolite repression and if glycerol itself is a poor catabolite repressor under these cultural conditions. The concentrations of cyclic AMP up to 5 × 10^{-3} M also had no effect on isomerase synthesis.

Transient repression effect of arabinose. We have found, as shown in Fig. 2 and in many other experiments employing minimal or complex medium, that the rate of synthesis of isomerase in the wild-type strain is linear with respect to the total protein from the onset of the induction process. This would indicate a balance between the inducing ability of arabinose and the repressing ability of its metabolites at all stages of induction. It was of interest, therefore, to examine the effect of addition of arabinose to a culture in which a high level of *ara* enzymes was already existent. Thus, arabinose was added to a mineral glycerol culture, *araB⁺C^c67*, and the rate of synthesis of isomerase was measured. An arabinose nonutilizing constitutive, *araB24C^c67*, was also examined in this way. It was found

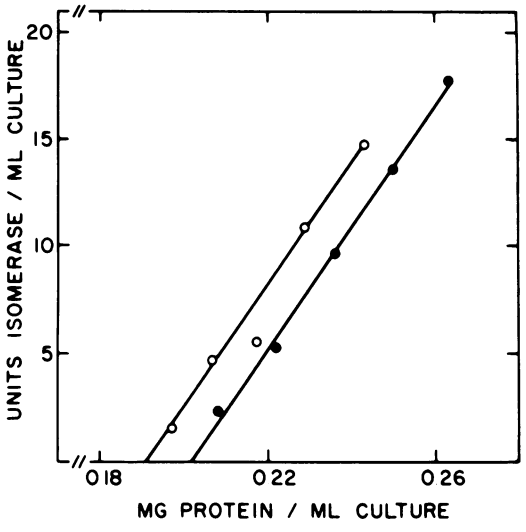

FIG. 7. *Effect of cyclic AMP on inducible isomerase synthesis in araB24. A mineral glycerol culture of araB24 was divided into two portions: to one was added* L-*arabinose (0.2%); to the other cyclic AMP (2* $\times 10^{-3}$ *M), followed 15 sec later by arabinose. The differential rate of isomerase synthesis was determined in both cultures as described in the legend to Fig. 5. The straight lines were fitted by the method of least squares. Symbols:* ●, *cyclic AMP not added;* ○, *cyclic AMP added.*

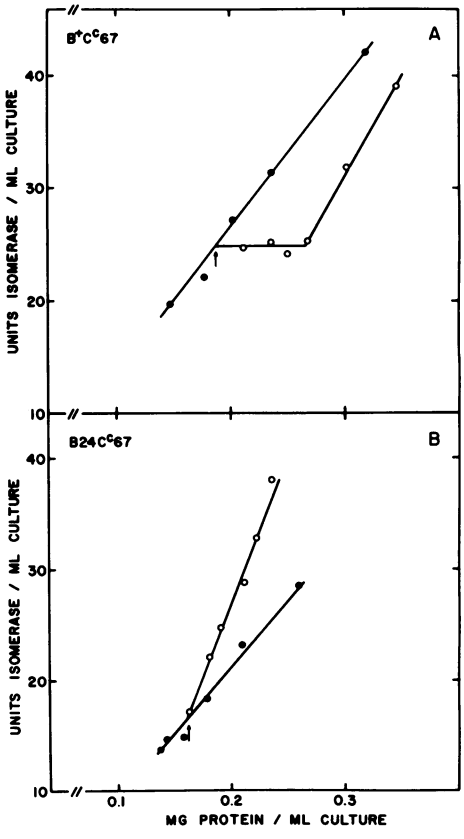

FIG. 8. *The effect of* L-*arabinose on the constitutive rate of synthesis of* L-*arabinose isomerase in glycerol-grown cultures. To portions of cultures of araB*$^+$*C*^{c}*67 (A) and araB24C*^{c}*67 (B) growing in mineral glycerol medium,* L-*arabinose was added at a final concentration of 0.2% at the points indicated by the arrows. The differential rate of isomerase synthesis in both parts of each culture was determined as in the legend to Fig. 2. The determinations were conducted for 90 min after addition of ara to A and 30 min after addition to B. Symbols:* ●, *arabinose not added;* ○, *arabinose added. The lines were fitted by the method of least squares.*

(Fig. 8B and Table 11) that when arabinose was added to the *araB24C*^{c}*67* strain an immediate rise of 2.3-fold in isomerase production occurred. This rise is due presumably to a further activating effect of arabinose on the *C*^{c}*67* gene product. In contrast to this, upon addition of arabinose to the *araB*$^+$*C*^{c}*67* strain, a short-lived, total cessation of isomerase synthesis was found. This cessation was found to last approximately one generation and was followed by isomerase synthesis at a rate 1.4-fold greater than the constitutive rate but 1.5-fold less than the arabinose-induced rate in *araB24C*^{c}*67*. The short-lived cessation of L-arabinose isomerase synthesis is a typical example of transient repression (37, 46). The subsequent rate of isomerase synthesis of 180 as compared to the rate of 280 with *araB24C*^{c}*67* no doubt reflects the L-arabinose self-catabolite repression of *araB*$^+$*C*^{c}*67* and the absence of such repression in the *araB24C*^{c}*67* double mutant.

The preceding experiment was repeated with the same two strains except that glucose was used as the initial growth substrate in place of glycerol. By comparison of Fig. 8 and Fig. 9, it can be observed that the constitutive rates of isomerase synthesis in either *araB*$^+$*C*^{c}*67* or *araB24C*^{c}*67* are approximately 2.5-fold lower in glucose than in glycerol media. Secondly, as was noted in the case where glycerol was employed (Fig. 8B), arabinose can promote a 2.5-fold increase in the rate of synthesis of isomerase in *araB24C*^{c}*67* as well (Fig. 9B). In contrast to the finding of transient repression in glycerol medium, however, addition of arabinose to *araB*$^+$*C*^{c}*67* cells growing in glucose resulted in no significant change in the rate of synthesis of isomerase from the rate in glucose alone (Fig. 9A and Table 11).

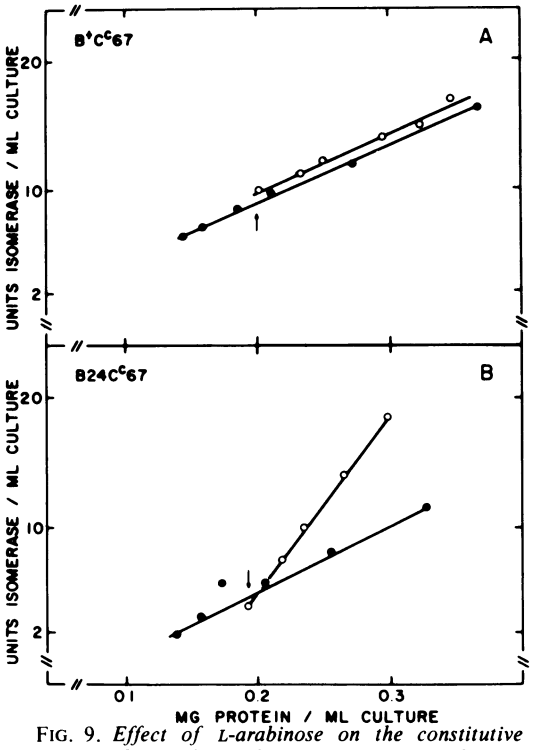

FIG. 9. *Effect of* L-*arabinose on the constitutive rate of synthesis of* L-*arabinose isomerase in glucose-grown cultures. The conditions, strains, and procedures were the same as those described for Fig. 8 except that mineral glucose medium was used in place of mineral glycerol. Symbols:* ●, *arabinose not added;* ○, *arabinose added. The lines were fitted by the method of least squares.*

TABLE 11. *Catabolite and transient repression*

Strain	Differential rate of isomerase synthesis[a]			
	Glycerol		Glucose	
	−Ara	+Ara	−Ara	+Ara
araB$^+$*C*^{c}*67*	131	180	46	51
araB24C^c*67*	120	280	50	133

[a] The rates entered are derived from Fig. 8 and 9. Initial rate for one doubling = 0.

DISCUSSION

L-Arabinose, in addition to its role as the effector of the L-arabinose operon (14) *araOIBAD*, also functions as a source of catabolite repressors which serve to dampen the rate of expression of the L-arabinose operon. This latter phenomenon we have called "self-catabolite repression." The mechanism of this L-arabinose effect is no doubt similar to the heterologous effect of glucose on the L-arabinose operon.

We have shown that glucose is an effective catabolite repressor of L-arabinose isomerase (*araA* gene product) and therefore presumably of the entire positively controlled *ara* operon. [A more detailed analysis of the glucose effect in relationship to the glucose-L-arabinose diauxie will appear elsewhere (Sweadner and Englesberg, *unpublished data*).] By using a mutant constitutive for the *ara* operon, we have shown that glucose effects a 2.5-fold lowering of the rate of production of the isomerase as compared to the effect of glycerol. In addition, when the metabolism of glucose was limited through slow feeding in a chemostat, as much as an eightfold increase in isomerase levels was observed. Presumably, with glucose limiting the growth rate, there is a decrease in concentration of intermediates (catabolite repressors) formed from glucose within the cell, resulting in a concomitant alleviation of the catabolite repressive effect (27). In addition, 3′,5′-cyclic AMP has been shown to reverse the glucose-mediated catabolite repression of the *ara* operon in a constitutive mutant as manifested by a 2.5-fold increase in rate of isomerase production upon the addition of cyclic AMP to a culture of *C*^{c}*67* growing in a mineral glucose medium. Evidence from a number of sources indicates that the glucose catabolite repression is mediated through its effect on the internal concentration of 3′,5′-cyclic AMP (39). A protein (CAP) required for the expression of all inducible operons is dependent on cyclic AMP for its activity (52). Catabolite repressor(s) in lowering the effective intracellular concentrations of cyclic AMP reduce operon activity.

We have presented similar evidence demonstrating that L-arabinose is an effective catabolite repressor of the L-arabinose operon. Previous work has demonstrated that L-arabinose metabolites subsequent to ribulose-5-phosphate are effective sources of catabolite repressors for β-galactosidase and tryptophanase (11). When the metabolism of L-arabinose is limited by slow feeding of the wild type, inducible strain in a chemostat, we find there is a linear increase in L-arabinose isomerase activity with increasing generation time. At a generation time of 120 min, which was the slowest generation time in the experiment, a twofold increase in L-arabinose isomerase activity was achieved over that obtained with cultures grown in an excess of L-arabinose. The addition of cyclic AMP to a wild-type culture growing in medium containing an excess of L-arabinose resulted in a 2.2-fold stimulation of rate of isomerase production.

With the demonstration of self-catabolite repression by L-arabinose in Ara$^+$ cultures, we are now in a position to consider a possible mechanism to explain the hyperinducibility in *AraB*$^-$ cultures. We have defined hyperinducibility as

the potential for an approximately twofold increase in rate of expression of the *ara* operon over the inducible rate in the wild type as shown in strains carrying a mutation in one or more of the structural genes in the L-arabinose operon. (As indicated, this effect is observable in missense mutants in *araA* and *araB* genes. It has not been observed in mutants in gene *araD*, probably because of the difficulty of obtaining fully induced cultures of these strains as the result of the L-arabinose inhibition of growth.) This hyperinducible effect is most reasonably explained on the basis that with the Ara$^+$, wild-type strain, L-arabinose gives rise to catabolites which result in repression of the operon, and *araA*$^-$ and *araB*$^-$ strains (as the result of missense mutations) which no longer produce from L-arabinose the necessary intermediates for the catabolite repression are therefore resistant to the effect.

If this hypothesis to explain the hyperinducible effect is correct and *araB*$^-$ mutants are not subject to L-arabinose catabolite repression, cyclic AMP should have no stimulatory effect on the expression of the operon; this is what was observed (Fig. 7). In addition, with the restoration of an Ara$^+$ phenotype, it would be predicted that the hyperinducible effect elicited by L-arabinose would disappear; this is the case. With Ara$^+$ merodiploids of the type A^-B^+/A^+B^-, constructed for *B*$^-$ missense, hyperinducible mutants, the isomerase level is reduced to the haploid wild-type inducible level (Table 7).

This hypothesis of self-catabolite repression also offers an explanation for the finding that all missense mutations in gene *araB* are hyperinducible and although most nonsense mutants in gene *araB* are polar, there are among this set some hyperinducers. In strains containing missense mutations, the rate change is the consequence of the removal of the self-catabolite repression effect exerted on the operon. Thus, all missense mutants should exhibit the same degree of hyperinducibility. With the exception of one strain, *B16*, all *araB* missense mutants tested did produce isomerase at the same rate (Fig. 2). We have no explanation for this exception. In the case of the nonsense and frameshift mutants, the actual rate of expression of the *ara* operon would be a composite of two effects: removal of catabolite repression and polarity. These effects would work in opposite directions. Polarity would decrease the rate of expression, whereas absence of self-catabolite repression would tend to increase it. Since self-catabolite repression would be removed to the same extent in all polar strains, the differences in operon expression among nonsense and frameshift mutants should only be due to polarity differences. Thus, the relative polarity should be the same among nonsense mutants whether inducible or constitutive. As seen in Tables 5 and 8, this prediction was upheld.

Whether a nonsense or frameshift mutant is polar or hyperinducible would be dependent only on the extent of the polar effect. In general, nonsense mutations which map in the operator distal end of the *araB* gene (*B28* and *B6*) are less polar than those which map closer to the operator (Table 8). In mutant *araB28*, when induced, the potential for hyperinducibility is greater than the potential for polarity, thus the slightly hyperinducible rate of isomerase production. In the other nonsense and frameshift mutants, the polar effects are greater than the potential hyperinducibility.

The fact that in Ara$^+$ merodiploids containing polar mutations, the isomerase levels were decreased below the level in the haploid strains is consistent with the result one would expect if the restoration of the ability to metabolize L-arabinose past the kinase stage leads to the production of catabolite repressors.

The results presented in Table 8 indicate that a simple polarity gradient does not exist among nonsense mutations in the *araB* gene. These findings confirm the results of Sheppard and Walker (44), who, in studying many more polar mutations than reported here, also found that the most polar mutations map in the middle of the *araB* gene, near *araB14*. This suggests that gene *araB* may contain at least one internal reinitiation sequence proximal to *araB14* (19).

We have considered several alternative models to explain the hyperinducible effect. The hyperinducible effect is not the result of an alteration in the genetic code of the structural gene that results in an increased rate of translation of the operon. Such effect would be *cis*-dominant, and we have shown the hyperinducible effect is recessive. Only nonsense mutations in gene *araB* have been shown to have a *cis*-dominant effect.

Another alternative model considered was that *araB*$^-$ mutants accumulate high concentrations of inducer(s) which serve to hyperinduce the operon. Since *araA* gene mutants are as hyperinducible as *araB* gene mutants (Table 9), we can conclude that hyperinducibility is not a result of the accumulation of ribulose by *araB*$^-$ mutants.

In addition, we have shown that under conditions of L-arabinose limitations of growth of the wild type in the chemostat (Fig. 4) where intracellular accumulation of L-arabinose would be expected to fall, there is an increase in L-arabinose isomerase activity equal to that found in hyperinducible *araB*$^-$ missense mutants. Therefore, it is unlikely that the hyperinducible effect found in these mutants is the result of unusually high levels of L-arabinose accumulation. Employing D-fucose as a gratuitous inducer of a par-

tially constitutive (C^c) fucose-resistant mutant (13) Beverin et al. (*3a*) confirmed our results demonstrating that the hyperinducible effect is the result of L-arabinose self-catabolite repression.

We have used the term self-catabolite repression throughout this paper to refer to the effect of arabinose on synthesis of the enzymes involved in its metabolism. The proposal of the term self-catabolite repression is to emphasize the self-regulatory role upon gene expression which occurs upon inducer metabolism, an aspect of catabolite repression which has been overlooked by investigators in the field (27, 38). Self-catabolite repression insures that excessive expenditure of energy in the form of overproduction of enzymes through the induction process will not occur. It is the interplay of induction and this feedback mechanism that provides a means of insuring that optimal levels of enzymes are produced for the efficient metabolism of the inducer.

Self-catabolite repression is probably a general phenomenon regulating operon expression in all instances where the inducer is metabolized such as in the case of α-glycerophosphate, L-rhamnose, maltose, and galactose. In each of these operons, as in the *ara* operon, where metabolism is blocked as a result of mutation in a structural gene, hyperinduction has been found to occur (7, 40, 41, 45). Furthermore, self-catabolite repression may also serve to explain a result of Cohn and Monod (6) that was uncovered in some early studies on induction of β-galactosidase. They found that addition of lactose to a *lac* constitutive strain growing in glycerol caused a total cessation of β-galactosidase synthesis for a short period of time (first demonstration of transient repression), followed by a rate lower than the rate observed in glycerol medium alone (self-catabolite repression). At the time, the former effect was used to justify the organizer model for enzyme induction.

We have also shown that L-arabinose can effect a transient repression of the rate of synthesis of L-arabinose isomerase. Transient repression has been defined as a short-lived (usually one generation) total cessation of enzyme synthesis (37). In studies on the *lac* operon transient repression was observed to occur upon addition of glucose to glycerol-grown cells and to be followed by catabolite repression (37, 46). How this effect occurs is not entirely known; however, it is believed to arise from a rapid disappearance of cyclic AMP from the cells after glucose addition (28, 39). The transient repression by L-arabinose on the rate of synthesis of L-arabinose isomerase occurs when L-arabinose is added to mineral glycerol cultures of cells constitutive for the L-arabinose operon and which have an active L-ribulokinase (B^+C^c67). It does not occur when L-arabinose is added to a mineral glycerol culture of uninduced wild type. In this case, the addition of L-arabinose results in a maximal rate of induction of the isomerase with no greater than a 5-min delay. It does not occur in a constitutive, kinaseless strain (*B24C^{c}67*), or in cultures of *B24C*$^+$ previously growing in mineral glucose.

These experiments indicate that at least an active L-ribulokinase must be present in the culture for L-arabinose transient repression of the L-arabinose operon to occur. It is reasonable to suggest that it is the phosphorylation of L-ribulose itself to ribulose-5-phosphate and perhaps the further metabolism of this compound that is essential for transient repression. Therefore we may assume that the presence of L-arabinose permease and L-arabinose isomerase must also be essential for this repression, although we have no direct evidence as yet to support this. These results differ from the criteria required for galactose transient repression of the *lac* operon in *E. coli* strain K-12. Tyler and Magasanik (47) have shown that a galactose permease but no galactokinase must be present in addition to at least enzyme I of the phosphotransferase system for galactose to produce transient repression. We have not tested for the possible requirements of the elements of the phosphotransferase system in the L-arabinose transient repression measured here.

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