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Constitutive Mutations in the Controlling Site Region of the *araBAD* Operon of *Escherichia coli* B/r That Decrease Sensitivity to Catabolite Repression

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Strains of *Escherichia coli* B/r containing a deletion of the regulatory gene *araC* are Ara$^-$. Slow-growing revertants of these strains were isolated and designated *araI*^{c} because they contain a second mutation in a controlling site, *araI*, that allows for a low level of constitutive expression of the *araBAD* operon (Englesberg et al., 1969). We mutagenized *araI*$^c\Delta C$ strains and selected mutants that grow faster in mineral L-arabinose medium. The new mutations, called *araX*^{c}, map very close to the original *araI*^{c} mutations and are in the controlling site region between *araB* and *araC*. The *araI*^{c}*X*$^c\Delta C$ strains have a higher constitutive level of expression of the *araBAD* operon than the *araI*$^c\Delta C$ parents. The *araX*^{c} mutations are *cis* acting and decrease the *araBAD* operon's sensitivity to catabolite repression. The *araBAD* operon is expressed equally well in *ara*ΔC and *ara*ΔC *cya crp* backgrounds. The repressor form of *araC* protein is able to repress the constitutive synthesis due to the *araX*^{c} allele.

The L-arabinose operon, a cluster of three structural genes (*araB*, *araA*, and *araD*), its associated controlling sites, and the regulatory gene *araC* lie between the threonine and leucine operons on the linkage map of *Escherichia coli* (Fig. 1). The controlling site region has been shown to be composed of two distinct sites, *araI* and *araO*, which are the sites of action of the activator and repressor, respectively. It is the removal of the repressor from *araO*, its conversion to the activator form by L-arabinose, and the subsequent action of the activator at *araI* that result in the expression of the operon (4, 6-8, 13). In addition to the two functions of repression and activation that occur in the controlling site region, it has been shown that the segment designated *araI* also contains the site for promoter function (*araP* site for ribonucleic acid [RNA] polymerase activity) and the site for catabolite repression function (*araCRP* site for cyclic adenosine 3',5'-monophosphate [cAMP]-cAMP receptor protein [CRP] function) (1, 4, 5, 12). These additional sites, *araP* and *araCRP*, could be overlapping, independent, or identical to *araI* and mainly serve to facilitate discussion.

The characterization of the *araI* site has been based upon an analysis of the map position and phenotypic effect of several deletion mutations and initiator constitutive, *araI*^{c}, mutations (6, 9, 15). The *araI*^{c} mutants were isolated as Ara$^+$ revertants of *araC* deletion mutants subsequent to mutagenesis with diethyl sulfate (6) or 2-amino purine (9). These *araI*^{c} strains have constitutive levels of expression of the *araBAD* operon ranging up to 10% of the induced wild type and are not further inducible by L-arabinose. The *araI*^{c} mutations map between *araB* and *araO* and are *cis* dominant and *trans* recessive to the *araI*$^+$ allele. Some of the *araI*^{c} mutations affect the sensitivity of the operon to the *araC* activator without having any appreciable effect on the maximum level of induction or on the sensitivity to catabolite repression (1, 6); therefore they have been used to define a site in which *araC* activator functions. No mutations have been isolated which map in the controlling site region and make the operon insensitive to catabolite repression.

In this paper we report the isolation of mutants, termed *araX*^{c}, that have further increased constitutive expression of the *araBAD* operon in the absence of the regulatory gene product and decrease the sensitivity of the *araBAD* operon to catabolite repression. These mutations are *cis* acting and closely linked to the *araI*^{c} mutations.

MATERIALS AND METHODS

Media. L-broth, mineral L-arabinose (MA), mineral D-glucose (MG), mineral glycerol (MGlc), mineral L-arabinose-glycerol (MAGlc), and complex eosin methylene blue L-arabinose (EMBA) were de-

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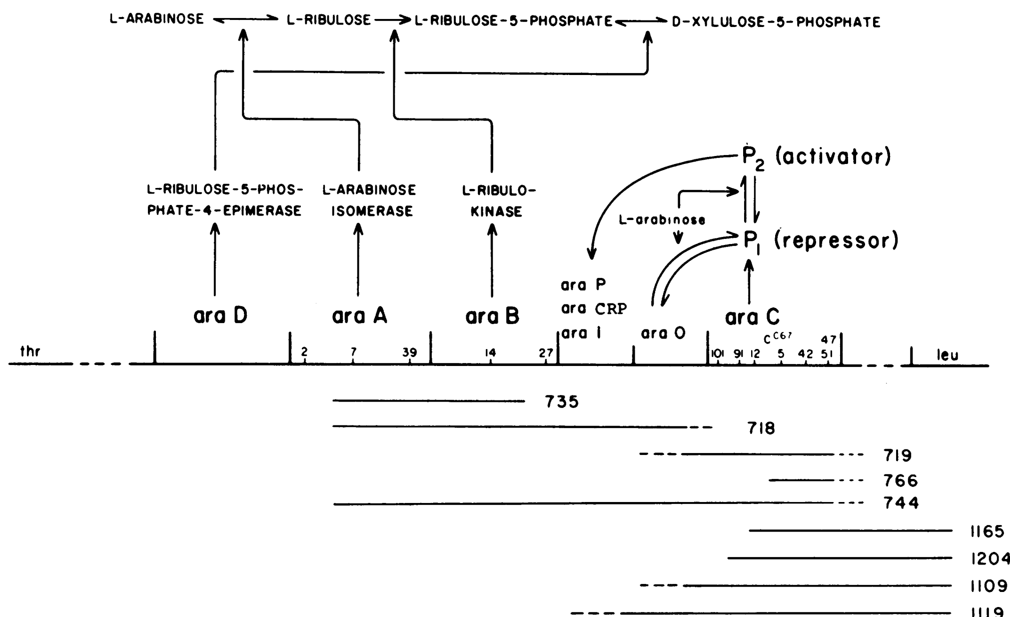

FIG. 1. *L-Arabinose operon and its regulatory gene. The L-arabinose operon is a cluster of three structural genes and their controlling sites, which lie between threonine and leucine on the E. coli linkage map. The three structural genes code for three enzymes that convert L-arabinose to D-xylulose-5-phosphate. An isomerase, coded for by araA, converts L-arabinose to L-ribulose. A kinase, coded for by araB, converts L-ribulose to L-ribulose-5-phosphate, and an epimerase, coded for by araD, converts L-ribulose-5-phosphate to D-xylulose-5-phosphate. The three structural genes are contiguous in the order araB, araA, and araD. The controlling elements, an initiator site (araI), an operator site (araO), the RNA polymerase initiation site (araP), and the site of action of the cAMP-CRP complex (araCRP) lie next to the araB gene. A regulatory gene (araC) lies near the operon to the right of the controlling sites.*

scribed previously (10). Mineral D-arabinose and mineral lactose were made in a manner similar to that for MA, except that D-arabinose or lactose was substituted for L-arabinose.

All liquid mineral media contained 0.4% of the carbohydrate or carbon source unless specifically stated otherwise. All liquid mineral media were supplemented with 0.05 mM $MnCl_2$, 0.05% yeast extract, and 0.004% of any required amino acid. cAMP, when used, was added to a final concentration of 2 mM unless stated otherwise.

Bacterial and phage strains. Bacteriophage P1*bt* was used for all transduction experiments. Propagation, storage, and transduction with the bacteriophage were described previously (10). The bacterial strains used, all *E. coli* B/r derivatives, are shown in Table 1.

The *araX*^{c} mutations, isolated in either strain *araI*^{c}*Δ766* or in strain *araI*^{c}*Δ1165*, were transduced into strain SB1636 (*araI*$^+$*Δ1165*) to insure that all of the mutant strains were isogenic for the rest of the chromosome.

The *araI*^{c}*C*$^+$ and *araI*^{c}*X*^{c}*C*$^+$ strains (SB2332 through SB2336) were constructed by transducing the *araI*^{c} *Δ1165* and the *araI*^{c}*X*^{c}*Δ1165* mutants to Leu$^+$ with P1*bt* grown on a strain containing *araΔ718* (SB1018). Deletion *718* removes *araI* and at least part of *araO*, assuring that the *araI*^{c} and *araI*^{c}*X*^{c} mutations would be placed *cis* to an *araC*$^+$ gene. Selection was on MG agar plates for Leu$^+$ transductants. The colonies on MG plates were then replica-plated onto MA agar plates. Colonies were picked from and purified on MA plates. Phage P1*bt* was grown on the supposed *araI*^{c}*C*$^+$ strains and then used to transduce strain SB1636 (*araI*$^+$*Δ1165*) to Ara$^+$. Selection was on mineral L-arabinose-L-leucine agar plates (MAL). The *araI*^{c}*Δ1165* transductants were distinguished from *araI*^{c}*C*$^+$ transductants by their slow growth on MA plates (colonies after 5 to 6 days at 37°C) and constitutive production of L-arabinose isomerase. The *araI*^{c}*X*^{c}*C*$^+$ transductants grow faster than the *araI*^{c}*X*^{c}*Δ1165* transductants, so it was possible with each *araI*^{c}*X*^{c} strain to easily find 200 *araI*^{c}*X*^{c}*Δ1165* transductants by replica picking onto MA and MAL plates. For each strain that was tested, we were able to find 4 to 6 transductants, from among the 200, that had the slower growth rate on MA expected for *araI*^{c}*Δ1165* or *araX*^{c}*Δ1165* strains, thus confirming that the *araI*^{c}*X*^{c}*C*$^+$ strains had been constructed.

Construction of the *araI*^{c}*X*^{c}*Δ719 cya crp* strains (SB2346 through SB2352) began by transducing strain SB5614 (*araΔ1165 cya-4 crp*) to Leu$^+$ with phage P1*bt* grown on the *araI*^{c}*X*^{c}*Δ719* strains. The Leu$^+$ transductants were picked from the 0.2% MG plates to 2% MA plates. Light growth appeared on these plates after 4 days of incubation at 37°C. The *araI*^{c}*X*^{c} markers could be recovered from these strains as described above. The *araI*^{c}*X*^{c}*Δ719 cya crp* strains form colonies on 0.2% MG after 24 h, show

TABLE 1. *Bacterial strains*[a]

Strain	Genotype	Origin or reference
UP1000	F^- wild type	10
UP1030	F^- araB27	3
UP1276	F^- araC^{c}67	5
SB1018	Hfr 33 ara D139Δ718 his	16
SB1095	F^- araΔ719	16
SB1509	F^-araΔ1109 D-ara-5 leu str^r	13
SB1636	F^-araΔ1165 D-ara-5 leu str^r	13
SB1676	F^-araΔ766	9
SB1678	F^-araA2Δ1109 D-ara-5 leu str^r	11
SB2176	F^- araI^{c}103Δ766	9
SB2179	F^-araI^{c}110Δ766	9
SB2180	F^-araI^{c}115Δ766	9
SB2183	F^-araI^{c}127Δ766	9
SB2185	F^-araI^{c}102Δ1165 D-ara-5 leu str^r	9
SB2186	F^-araI^{c}103Δ1165 D-ara-5 leu str^r	9
SB2187	F^-araI^{c}104Δ1165 D-ara-5 leu str^r	9
SB2188	F^-araI^{c}107Δ1165 D-ara-5 leu str^r	9
SB2189	F^-araI^{c}110Δ1165 D-ara-5 leu str^r	9
SB2190	F^-araI^{c}115Δ1165 D-ara-5 leu str^r	9
SB2193	F^-araI^{c}127Δ1165 D-ara-5 leu str^r	9
SB2194	F^-araI^{c}131Δ1165 D-ara-5 leu str^r	9
SB2290	F^-araI^{c}110X^{c}52Δ1165 D-ara-5 leu str^r	EMS mutagenesis of SB2189
SB2291	F^-araI^{c}127X^{c}53Δ1165 D-ara-5 leu str^r	EMS mutagenesis of SB2193
SB2292	F^-araI^{c}127X^{c}54Δ1165 D-ara-5 leu str^r	EMS mutagenesis of SB2193
SB2300	F^-araI^{c}103X^{c}42Δ766	EMS mutagenesis of SB2176
SB2301	F^-araI^{c}110X^{c}44Δ766	EMS mutagenesis of SB2179
SB2302	F^-araI^{c}115X^{c}45Δ766	DES mutagenesis of SB2180
SB2305	F^-araI^{c}127X^{c}47Δ766	NTG mutagenesis of SB2183
SB2307	F^-araI^{c}103X^{c}42Δ1165 D-ara-5 leu str^r	P1bt (SB2300) × SB1636
SB2308	F^-araI^{c}110X^{c}44Δ1165 D-ara-5 leu str^r	P1bt (SB2301) × SB1636
SB2309	F^-araI^{c}115X^{c}45Δ1165 D-ara-5 leu str^r	P1bt (SB2302) × SB1636
SB2310	F^-araI^{c}127X^{c}47Δ1165 D-ara-5 leu str^r	P1bt (SB2305) × SB1636
SB2314	F^-araI^{c}110X^{c}52Δ1165 D-ara-5 leu str^r	P1bt (SB2290) × SB1636
SB2315	F^-araI^{c}127X^{c}53Δ1165 D-ara-5 leu str^r	P1bt (SB2291) × SB1636
SB2316	F^-araI^{c}127X^{c}54Δ1165 D-ara-5 leu str^r	P1bt (SB2292) × SB1636
SB2317	F^-araA2I110X^{c}44Δ719 D-ara-5 str^r	This paper; see Materials and Methods
SB2321	F'araB24/F^-araA2I^{c}110X^{c}44Δ719	This paper; see Materials and Methods
SB2322	F'araB24/F^-araA2I^{c}115X^{c}45Δ719	This paper; see Materials and Methods
SB2324	F'araB24/F^-araA2I^{c}127X^{c}54Δ719	This paper; see Materials and Methods
SB2325	F'araB24C^{c}67F^-araA2I^{c}110X^{c}44Δ710	This paper; see Materials and Methods
SB2326	F^-araI^{c}110 D-ara-5 str^r	P1bt (SB1018) × SB2189
SB2327	F^-araI^{c}115	P1bt (SB1018) × SB2190
SB2328	F^-araI^{c}127 D-ara-5 str^r	P1bt (SB1018) × SB2193
SB2329	F^-araI^{c}110X^{c}44 D-ara-5 str^r	P1bt (SB1018) × SB2308
SB2330	F^-araI^{c}115X^{c}45 D-ara-5 str^r	P1bt (SB1018) × SB2309
SB2331	F^-araI^{c}127X^{c}47 D-ara-5 str^r	P1bt (SB1018) × SB2310
SB2332	F^-araI^{c}127X^{c}54 D-ara-5 str^r	P1bt (SB1018) × SB2316
SB2333	F^-D-ara-5 str^r D-ara-5 str^r	P1bt (SB1018) × SB1636
SB2334	F^-araI^{c}110Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2308
SB2335	F^-araX^{c}44Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2308
SB2336	F^-araI^{c}110X^{c}44Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2308
SB2337	F^-araI^{c}115Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2309
SB2338	F^-araX^{c}45Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2309
SB2339	F^-araI^{c}115X^{c}45Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2309
SB2340	F^-araI^{c}127Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2310
SB2341	F^-araX^{c}47Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2310
SB2342	F^-araI^{c}127X^{c}47Δ719 D-ara-5 str^r	P1bt (SB1095) × SB2310

TABLE 1—*Continued*

Strain	Genotype	Origin or reference
SB2343	F^-*araX*^{c}*54Δ719 D-ara-5 str*^{r}	P1*bt* (SB1095) × SB2316
SB2344	F^-*araI*^{c}*127X*^{c}*54Δ719 D-ara-5 str*^{r}	P1*bt* (SB1095) × SB2316
SB2346	F^-*araI*^{c}*110X*^{c}*44Δ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB2336) × SB5614
SB2347	F^-*araI*^{c}*115X*^{c}*45Δ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB2339) × SB5614
SB2348	F^-*araI*^{c}*127X*^{c}*47Δ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB2342) × SB5614
SB2349	F^-*araI*^{c}*127X*^{c}*54Δ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB2344) × SB5614
SB2350	F^-araI^{c}110Δ719 D-ara-5 cya-4 crp str^r	P1*bt* (SB2334) × SB5614
SB2351	F^-*araI*^{c}*115Δ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB2337) × SB5614
SB2352	F^-*araI*^{c}*127Δ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB2340) × SB5614
SB2353	F^-*araΔ719 D-ara-5 cya-4 crp str*^{r}	P1*bt* (SB1095) × SB2345
SB2355	F^-*araΔ719 D-ara-5 str*^{r}	P1*bt* (SB1095) × SB1636
SB3101	F'*araA2*/F^-*araA2*	16
SB3107	F'*araB24*/F^-*araB24*	16
SB3164	F'*araB24C*^{c}*67*/F^-*araB24C*^{c}*67*	16
SB5614	F^-*araΔ1165 leu D-ara-5 cya-4 crp str*^{r}	11

[a] All strains are derivatives of *E. coli* B/r.

growth after 4 days, but do not form colonies after 6 days on 2% MA, and show no growth at all after 8 days on 0.2% MA. The growth response on MA plates could be due to the fact that catabolite repression of permease is more severe than catabolite repression of isomerase (11).

Construction of the F^-*araA2I*^{c}*X*^{c}*Δ719* strains is described below. Strain SB1678 (*araA2Δ1109 leu*) was transduced to Leu$^+$ by P1*bt* previously grown on the *araI*^{c}*X*^{c}*Δ719* strains (SB2339, SB2334, and SB2344). The Leu$^+$ transductants on 0.2% MG were picked to 0.2% MG and 0.2% MA plates. Ten to 15% of the transductants did not grow on 0.2% MA. These possible *araA2I*^{c}*X*^{c}*Δ719* transductants were verified as follows. The presence of the *araA2* marker was confirmed by the lack of recombinants in a mating with F'*araA2* (SB3101). P1*bt* phage grown on the transductants was used to transduce strain SB1636 (*araI*$^+$*Δ1165*) to Leu$^+$ on MA. Some Leu$^-$ transductants grew at the rate previously found for the *araI*^{c}*X*^{c}*719* strains, indicating that the *ara-I*^{c}*X*^{c} markers were present.

The merodiploids (SB2321 through SB2325) were made by mating strain SB3107 (F'*araB24*) or SB3164 (F'*araB24C*^{c}*67*) with the appropriate *araA2I*^{c}*Δ719* strains and were then identified by their Ara$^+$ phenotype on EMBA plates. EMBA-negative segregants from the diploids contain the *araA2* marker and produce L-ribulokinase constitutively.

Preparation of cell extracts and enzyme assays. Samples were collected and sonically disrupted, and enzyme assays were carried out by methods described previously (2, 5, 9). Protein determinations were performed by the method of Lowry et al. (14). Cultures of diploid strains were routinely checked for EMBA-negative segregants. For the data presented, the frequency of EMBA-negative segregants ranged from 5 to 15%.

Chemicals and mutagens. Ethyl methane sulfonate (EMS) and *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (NTG) were bought from Sigma Chemical Co.; diethyl sulfate (DES) was purchased from Eastman Organic Chemicals; and cAMP and L-arabinose were obtained from Calbiochem. L-[^{14}C]ribulose was prepared by the method of Englesberg (3) from L-[^{14}C]arabinose obtained from New England Nuclear Corp.

Regression analysis and standard errors. Regression analysis and determination of standard errors were carried out with the aid of a computer program from Biomedical Computer Programs, University of California, Los Angeles.

RESULTS

Selection of mutants of *araI*^{c}*ΔC* strains that have an increased growth rate on 0.2% MA plates. Method I. Strains missing the regulatory protein coded for by the *araC* gene will not grow on MA. However, when strains with deletions of the *araC* gene acquire *araI*^{c} mutations (5), they grow very slowly on MA and produce a negative reaction on EMBA. The slow growth on MA is due to the *araI*^{c} mutation, which makes the *ara* operon partially constitutive. The *araI*^{c}*Δ766* mutants (SB2175 through SB2184) were grown in L-broth overnight. Fresh L-broth cultures were prepared by transferring 1 ml of an overnight culture to 5 ml of L-broth and allowing growth for 2 h at 37°C. Cultures of each of the *araI*^{c}*Δ766* mutants were then mutagenized with one of the following: EMS, 6.2 mg/ml; DES, 7.7 mg/ml; or NTG, 100 μg/ml. The cultures were incubated at 37°C in the presence of the mutagen for 2 h, and then 0.5-ml volumes were transferred to 5 ml of 0.2% MA and allowed to grow for 24 h. The cultures were transferred twice more in MA to enrich for rapidly growing mutants, and then a set of EMBA plates was streaked with bacteria from the last enrichment tube. Only one colony was picked from each set of plates to insure independent mutants. The mutagenic and enrichment procedure produced mutants that were EMBA positive after 40 h of growth. EMBA-positive colonies represented less than 0.1% of the colonies on the EMBA plates. The

0.1% estimate is based on 200 distinguishable colonies per plate and the finding that only one in five plates had an EMBA-positive colony.

Method II. D-Arabinose is not metabolized by the enzymes coded for by the L-arabinose operon, nor is it an inducer of the L-arabinose operon. There is an inducible D-arabinose operon involved in the metabolism of D-arabinose (4). However, the fact that L-ribulokinase (from the L-arabinose operon) phosphorylates D-ribulose (first intermediate from D-arabinose) was made use of to select for increased constitutive expression of the *ara* operon in the $araI^{c}\Delta 1165$ *D-ara-5* strains. The $araI^{c}\Delta 1165$ *D-ara-5* strains contain a mutation in the D-arabinose kinase (*D-ara-5*). D-Arabinose induces the D-arabinose operon, but $ara\Delta 1165$ *D-ara-5* strains cannot grow on D-arabinose unless the L-arabinose operon provides sufficient constitutive L-ribulokinase or the *D-ara-5* mutation reverts. Thus, growth in D-arabinose can be used to enrich for strains with increased constitutive expression of the *ara* operon (4).

The $araI^{c}\Delta 1165$ *D-ara-5* strains were mutagenized in L-broth in a manner similar to that used to obtain the mutants of the $araI^{c}\Delta 766$ strains. However, instead of the cultures being transferred in 0.2% MAL, 0.2% mineral D-arabinose-L-leucine was used. After the cultures were transferred three times in the latter medium, each independently mutagenized culture was streaked onto a set of plates. Only one colony was picked from each set of plates to insure independent mutants. Colonies that were EMBA positive within 40 h were picked, and their growth characteristics were checked on MAL. The colonies that grew faster on MAL than the parent $araI^{c}\Delta 1165$ strains were isolated in pure culture. In this mutagenic and enrichment procedure, the EMBA-positive colonies also represented about 0.1% of the colonies on the EMBA plates. Phage P1*bt* was grown on each strain isolated by the two methods described above and used to transduce the mutant phenotypes into SB1636 ($araI^{+}\Delta 1165$) as a step in mapping the mutations and also as an assurance that the rest of the chromosome was isogenic.

Mapping the mutations. P1*bt*, grown on a strain carrying $ara\Delta 719$ (SB1095) or $ara\Delta 766$ (SB1676), was used to transduce $araI^{c}\Delta 1165$ and $araI^{c}X^{c}\Delta 1165$ to Leu^{+}. The Leu^{+} transductants, selected on MG plates, were replica-plated to MA. The ratio of colonies on MA to MG was used to determine the percent recombination of the mutations with the ends of the deletions (MA × 100/MG = percent recombination). The transductions are diagrammed in Fig. 2. The mapping data (Table 2) show that $ara\Delta 719$ gave 0.3 to 0.6% and $ara\Delta 766$ gave 2.0

TABLE 2. *Mapping of the $araI^{c}$ and $araI^{c}X^{c}$ mutations against the deletions 719 and 766*[a]

Recipient		No. of transductants								
		P1*bt* ($ara\Delta 719$)			P1*bt* ($ara\Delta 766$)			P1*bt* (*araB27*)		
Strain	Genotype	2% MA	2% MG	%	2% MA	2% MG	%	2% MA	2% MG	%
SB2307	$F^{-}araI^{c}103X^{c}44\Delta 1165$	14	4,452	0.32						
SB2308	$F^{-}araI^{c}110X^{c}44\Delta 1165$	38	8,428	0.45	90	4,020	2.2			
SB2309	$F^{-}araI^{c}115X^{c}45\Delta 1165$	25	7,874	0.32	89	2,960	3.0			
SB2310	$F^{-}araI^{c}127X^{c}45\Delta 1165$	22	6,444	0.34	84	3,670	2.3			
SB2314	$F^{-}araI^{c}110X^{c}52\Delta 1165$	38	6,138	0.62						
SB2315	$F^{-}araI^{c}127X^{c}53\Delta 1165$	27	6,254	0.43						
SB2316	$F^{-}araI^{c}127X^{c}54\Delta 1165$	24	5,566	0.43	106	4,460	2.4			
SB2185	$F^{-}araI^{c}102\Delta 1165$	49	11,370	0.43						
SB2186	$F^{-}araI^{c}103\Delta 1165$	14	4,650	0.30						
SB2187	$F^{-}araI^{c}104\Delta 1165$	39	9,180	0.43						
SB2188	$F^{-}araI^{c}107\Delta 1165$	99	10,480	0.95						
SB2189	$F^{-}araI^{c}110\Delta 1165$	33	7,352	0.45	162	5,230	3.1			
SB2190	$F^{-}araI^{c}115\Delta 1165$	37	6,370	0.58	139	5,340	2.6			
SB2193	$F^{-}araI^{c}127\Delta 1165$	25	4,428	0.57	100	3,670	2.7			
SB2194	$F^{-}araI^{c}131\Delta 1165$	22	7,278	0.30						
SB1636	$F^{-}araI^{+}\Delta 1165$	0	1,600	<0.06	0	3,030	<0.03			
SB1636	$F^{-}araI^{+}\Delta 1165$							44	1,194	3.7
SB1509	$F^{-}araI^{+}\Delta 1109$							26	3,227	0.8

[a] The $araI^{c}\Delta 1165$ and the $araI^{c}X^{c}\Delta 1165$ mutants were transduced to *leu*$^{+}$ by bacteriophage P1*bt* grown on strains carrying either $ara\Delta 719$ (SB1095) or $ara\Delta 766$ (SB1676). The *leu*$^{+}$ transductants were selected on MG and counted. The number of *ara*$^{+}$ *leu*$^{+}$ transductants was then determined by replica-plating the colonies on MG onto MA. Ability to synthesize the amino acid leucine, *leu*$^{+}$; ability to utilize the sugar L-arabinose, *ara*$^{+}$.

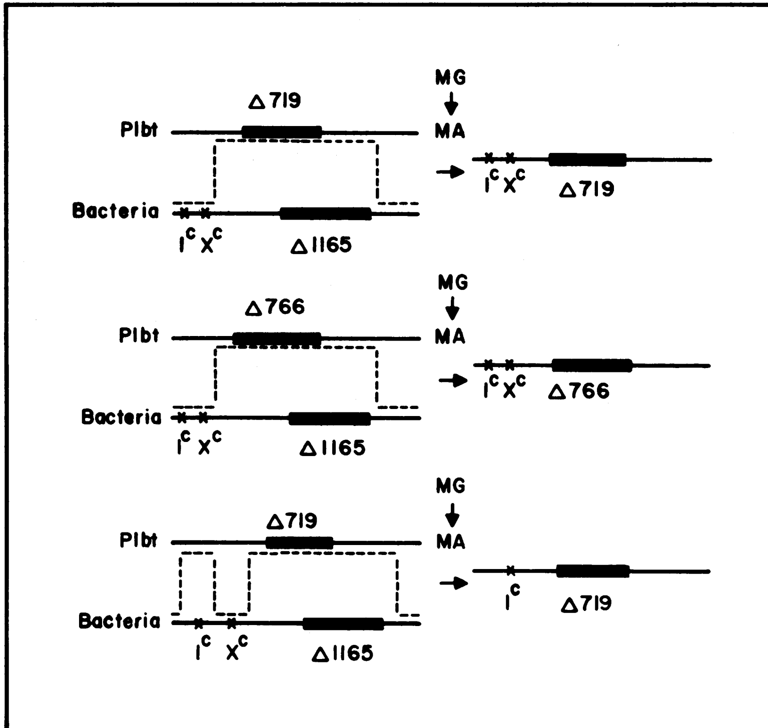

FIG. 2. *Diagram of transductions. The transductions used for mapping the araX^c mutations are shown. The media used in the transductions are indicated: MA, mineral 0.2% L-arabinose; MG, mineral 0.2% D-glucose.*

to 3.0% Ara$^+$ transductants with the *ara-I^{c}X^{c}Δ1165* strains. Similarly, these two deletions gave 0.3 to 0.6% and 2.6 to 3.1% Ara$^+$ transductants, respectively, with the *araI^c-Δ1165* strains. Strain *araΔ1109* gave 0.8% arabinose-positive transductants with *araB27*, and *araΔ1165* gave 3.7% arabinose-positive transductants with *araB27*. Also, 90 to 97% of the arabinose-positive transductants of *ara-I^{c}X^{c}Δ1165* have growth characteristics similar to the *araI^{c}X^{c}Δ1165* strains. These observations indicate that the *araI^c* and *araX^c* mutations are very near each other and map in the region between *araB* and *araO*.

Separating and characterizing the *araI^c* and *araX^c* mutations. Transductants from the mapping experiments with *araΔ719* were picked from MA and restreaked onto MA to check their growth characteristics. Three to 10% of the transductants formed colonies similar in size to those that contain *araI^c* mutations, and less than 1% formed intermediate size colonies that might be expected for those which contain *araX^c* mutations if one assumes that the effects of the *araI^c* and *araX^c* mutations are additive. L-Arabinose isomerase activity of the suspected *araI^{c}Δ719*, araX^c*Δ719*, and *araI^{c}X^{c}Δ719* transductants is, respectively, 4 to 5, 10 to 15, and 17 to 22 μmol of L-ribulose formed per h per mg of protein (Table 3). For each mutant considered, one intermediate type transductant, *araX^{c}Δ719*, could be found. Thus, the *araI^c* mutation can be separated from the *araX^c* mutation. The rate of synthesis of L-arabinose isomerase in the *araI^{c}X^c* strains is close to the sum of the rates in the *araI^c* and *araX^c* strains.

Maximum rate of expression of the *araBAD* operon is similar in *araI^{c}X^c* strains. The *ara-I^{c}X^{c}C$^+$* strains, their *araI^{c}C$^+$* parents, and the *araI$^+$C$^+$* wild-type strain show no significant differences in the maximum rate of expression of the *araBAD* operon (Table 4). L-Arabinose isomerase and L-ribulokinase are coordinately produced in these strains. The *araI$^+$C$^+$*, *araI^{c}110C$^+$*, and *araI^{c}110X^{c}44C$^+$* strains gave initial rates of L-arabinose isomerase synthesis of 166, 171, and 185 μmol of L-ribulose formed per h per mg of protein, respectively (Fig. 3). We do not feel that these values are significantly different. The medium employed in these experiments was chosen so as to minimize the effect of catabolite repression and thus allow for maximum rates of expression.

Evidence for altered sensitivity to catabo-

TABLE 3. *Growth rate and L-arabinose isomerase activity in strains containing the araX^c allele*[a]

Strain	Genotype	Colony diameter (mm) on MA		L-Arabinose isomerase activity
		45 h	65 h	
SB2334	F$^-$araI^{c}110X$^+$Δ719	0.1	0.5	4.2 ± 0.9
SB2335	F$^-$araI$^+$X^{c}44Δ719	0.3	1.0	15.2
SB2336	F$^-$araI^{c}110X^{c}44Δ719	0.7	1.7	21.8 ± 2.4
SB2337	F$^-$araI^{c}115X$^+$Δ719	0.1	0.5	4.1
SB2338	F$^-$araI$^+$X^{c}45Δ719	0.2	1.0	12.5 ± 2.1
SB2339	F$^-$araI^{c}115X^{c}45Δ719	0.9	1.7	20.7 ± 2.1
SB2340	F$^-$araI^{c}127X$^+$Δ719	0.1	0.5	4.8
SB2343	F$^-$araI$^+$X^{c}54Δ719	0.2	1.0	12.7 ± 1.0
SB2344	F$^-$araI^{c}127X^{c}54Δ719	0.9	1.9	20.1 ± 2.1

[a] The F$^-$araI^{c}X^{c}Δ1165 leu strains were transduced to leu$^+$ by bacteriophage P1bt grown on strains carrying araΔ719. The leu$^+$ transductants were selected on MG. The ara$^+$ leu$^+$ transductants were identified by replica-plating the colonies onto MG and MA. The growth rate of the ara$^+$ leu$^+$ transductants was estimated by streaking on MA plates and measuring the increase in colony diameter with time. Exponentially growing cells in MGlc were employed for isomerase determinations. Isomerase activity represents the average of four or more assays of different cell extracts and is measured as micromoles of L-ribulose formed per hour per milligram of protein. The average error for duplicate experiments is indicated in some cases.

TABLE 4. *Induced levels of L-arabinose isomerase and L-ribulokinase in the araI^{c}C$^+$ and araI^{c}X^{c}C$^+$ strains*[a]

Strain	Genotype	L-Arabinose isomerase activity	L-Ribulokinase activity
SB2329	F$^-$araI^{c}110X^{c}44C$^+$	86.5 ± 0.5	26.1
SB2326	F$^-$araI^{c}110X$^+$C$^+$	88.3	22.5
SB2330	F$^-$araI^{c}115X^{c}45C$^+$	88.5 ± 4.0	27.6
SB2327	F$^-$araI^{c}115X$^+$C$^+$	91.0	22.0
SB2331	F$^-$araI^{c}127X^{c}47C$^+$	83.6 ± 4.0	22.0
SB2328	F$^-$araI^{c}127X$^+$C$^+$	87.5	22.0
SB2332	F$^-$araI^{c}127X^{c}54C$^+$	84.5	25.7
SB2333	F$^-$araI$^+$X$^+$C$^+$	93.3 ± 5.0	22.2
SB1636	F$^-$araI$^+$X$^+$Δ1165	0.2	0.1

[a] L-Arabinose isomerase and L-ribulokinase levels were measured in cells growing exponentially in MAGlc plus 2 mM cAMP. Two different experiments are combined in this table, and the average enzyme activities are indicated. Isomerase and kinase are measured as the micromoles of product formed per hour per milligram of protein.

lite repression. L-Arabinose isomerase specific activities were determined for the araI^{c}Δ719 parents and for the araI^{c}X^{c}Δ719 mutants growing in MGlc and MG. The values in Table 5 indicate that the araI^{c}Δ719 strains were catabolite repressed approximately 55 to 65%, whereas the araI^{c}X^{c}Δ719 strains were only catabolite repressed 25 to 35%. The two araX^{c}Δ719 mutants were catabolite repressed 20 to 40%. Results similar to those found for the araI^{c}Δ719 and araI^{c}X^{c}Δ719 strains were found for the araI^{c}Δ1165 and araI^{c}X^{c}Δ1165 strains (data not shown).

If the wild-type araC$^+$ is introduced *cis* to the araI^{c}X^c mutations, it is found that the araI^{c}X^c mutations still alter the *ara* operon's sensitivity to catabolite repression. Initial rates of L-arabinose isomerase synthesis in MAGlc and in mineral arabinose-glucose (MAG) indicate that ara^{c}110X^{c}44C$^+$ is less sensitive to catabolite repression than araI^{c}110C$^+$ and araI$^+$C$^+$ (Fig. 4). The wild type, araI$^+$C$^+$, was severely catabolite repressed and gave an initial rate of isomerase synthesis in glycerol and glucose of 133 and 20 U/mg of protein, respectively (catabolite repression [CR] = 85%). The araI^{c}110C$^+$ gave values for the initial rate of isomerase synthesis in glycerol and glucose of 129 and 56 U/mg of protein, respectively (CR = 57%), whereas araI^{c}X^{c}44C$^+$ gave values for the initial rate of isomerase synthesis in glycerol and glucose of 160 and 94 U/mg of protein, respectively (CR =

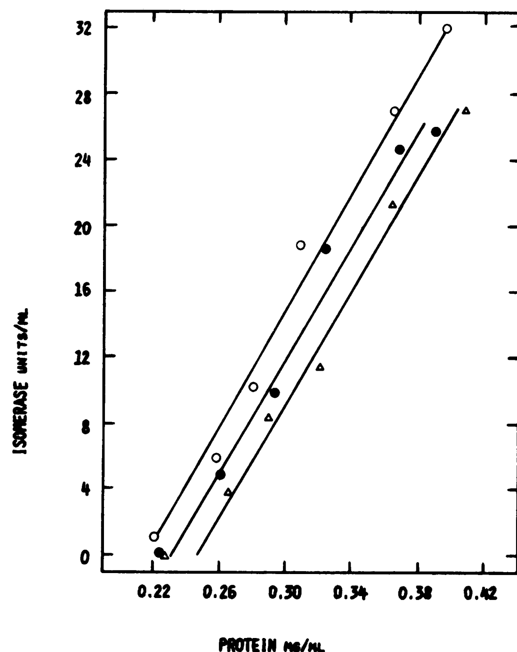

FIG. 3. *Initial rate of isomerase synthesis. The strains araI$^+$C$^+$, araI^{c}110C$^+$, and araI^{c}110X^{c}C$^+$ were grown in MGlc containing 2 mM cAMP. The first samples of the cultures were taken, and then the cultures were induced with 0.4% L-arabinose. The curves were fitted to the points by the method of least squares, and the standard errors were determined by using a computer program. Symbols: △, F$^-$araI$^+$C$^+$ (166 ± 10 U/mg of protein); ●, F$^-$araI^{c}110C$^+$ (171 ± 21 U/mg of protein); ○, F$^-$araI^{c}110X^{c}44C$^+$ (185 ± 10 U/mg of protein).*

41%). The severity of catabolite repression in the different induced strains (*araI*$^+$*C*$^+$, *araI*^{c}*C*$^+$, and *araI*^{c}*X*^{c}*C*$^+$) showed the same pattern of differences as in the noninduced strains (*araI*$^+$*C*^{c}, *araI*^{c}*Δ719*, and *araI*^{c}*X*^{c}*Δ719*).

A more definitive determination of the resistance of the various mutants to catabolite repression can be achieved by looking at expression of the *araBAD* operon in a *crp*$^-$ *cya*$^-$ background. The *crp*$^-$ *cya*$^-$ strains do not make detectable cAMP receptor protein (*crp*$^-$) or a functional adenylcyclase (*cya-4*). These mutations alone or together do not allow growth of *araI*$^+$*C*$^+$ (wild-type) strains on mineral 0.2% L-arabinose plates. Table 6 shows that the *araI*^{c}*X*^{c}*Δ719 crp*$^-$ *cya*$^-$ mutants grown in L-broth have L-arabinose isomerase differential rates of 10 to 12 U/mg of protein compared with the *araI*^{c}*X*^{c}*Δ719* strains, which have 8 to 14 U/mg of protein. There is no significant difference between the isomerase values of the *araI*^{c}*X*^{c}*Δ719 crp*$^-$ *cya*$^-$ mutants and the isomerase values of the *araI*^{c}*X*^{c}*Δ719* strains. The isomerase values of the *araI*^{c}*Δ719 crp*$^-$ *cya*$^-$ mutants and the *araI*^{c}*Δ719* strains also are very smilar but approximately 10-fold lower than the *araI*^{c}*X*^{c}*Δ719*-containing strains.

TABLE 5. *Comparison of cultures grown in MG and in MGlc to determine the severity of catabolite repression of L-arabinose isomerase*[a]

Strain	Genotype	Isomerase activity		
		MGlc	MG	%CR
SB2334	F$^-$*araI*^{c}*110X*$^+$*Δ719*	4.1 ± 0.2	1.7 ± 0.1	58
SB2337	F$^-$*araI*^{c}*115X*$^+$*Δ719*	4.2 ± 0.3	1.5 ± 0.2	65
SB2340	F$^-$*araI*^{c}*127X*$^+$*Δ719*	4.8 ± 0.6	1.8 ± 0.1	63
SB2336	F$^-$*araI*^{c}*110X*^{c}*44Δ719*	20.6 ± 2.6	15.2 ± 1.2	26
SB2339	F$^-$*araI*^{c}*115X*^{c}*45Δ719*	20.6 ± 2.0	14.5 ± 0.8	30
SB2342	F$^-$*araI*^{c}*127X*^{c}*47Δ719*	16.8 ± 0.4	11.5 ± 1.1	31
SB2344	F$^-$*araI*^{c}*127X*^{c}*54Δ719*	18.1 ± 1.0	12.3 ± 0.9	32
SB2338	F$^-$*araI*$^+$*X*^{c}*45Δ719*	13.7 ± 1.5	10.9 ± 0.2	20
SB2343	F$^-$*araI*$^+$*X*^{c}*54Δ719*	12.6 ± 1.0	7.6 ± 0.6	40
UP1276	F$^-$*araI*$^+$*X*$^+$*C*^{c}*67*	38.3 ± 1.4	8.6 ± 0.8	77

[a] The strains were grown in mineral medium and harvested in the exponential phase of growth. L-Arabinose isomerase activities, the average of five or more values, are reported as micromoles of L-ribulose formed per hour per milligram of protein. The standard deviation from the average is indicated. %CR, Percent catabolite repression, defined as the ratio of the isomerase values in MG and MGlc.

Repressor reduces the constitutive expression due to the *araX*^{c} allele. Englesberg et al. showed that the *araC* repressor reduces the constitutive expression of *araI*^{c} mutations (7). To determine the effect of the *araC* repressor on the *araX*^{c} mutations, L-arabinose isomerase ac-

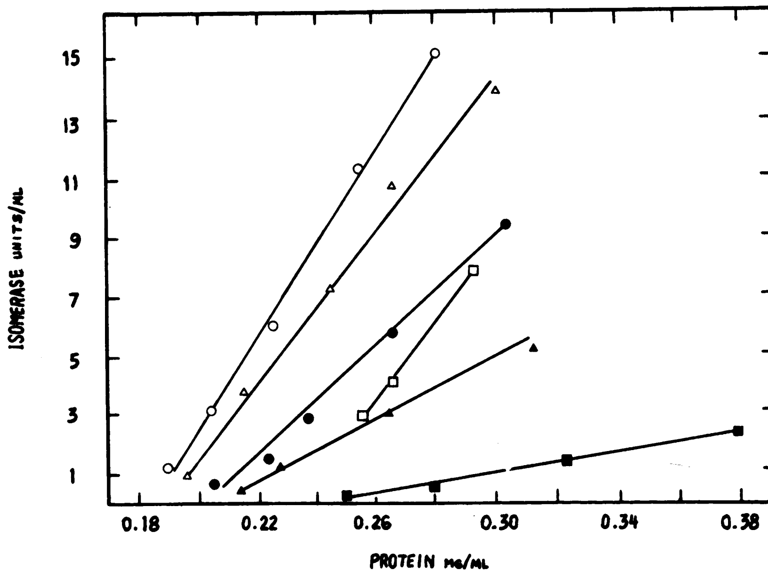

FIG. 4. *Initial rate of isomerase synthesis. A wild-type strain (SB2333), F*$^-$*araI*^{c}*110X*^{c}*44C*$^+$ *(SB2329), and F*$^-$*araI*^{c}*110C*$^+$ *(SB2326) were grown in MGlc and MG. Samples of the exponentially growing cultures were taken, and then 0.4% L-arabinose was added to the cultures. Samples of the exponentially growing induced cultures were taken. Isomerase was measured as moles of L-ribulose formed per hour per milligram of protein. Units, micromoles of L-ribulose formed per hour. The curves were fitted to the points by the method of least squares, and the standard errors were determined by using a computer program. Symbols:* ○, *F*$^-$*araI*^{c}*110X*^{c}*44C*$^+$ *in MAGlc;* ●, *F*$^-$*araI*^{c}*110X*^{c}*44C*$^+$ *in MAG;* △, *F*$^-$*araI*^{c}*110C*$^+$ *in MAGlc;* ▲, *F*$^-$*araI*^{c}*110C*$^+$ *in MAG;* □, *F*$^-$*araI*$^+$*C*$^+$ *in MAGlc;* ■, *F*$^-$*araI*$^+$*C*$^+$ *in MAG.*

TABLE 6. *Comparison of araI^c and araI^cX^c mutations in strains missing CRP, adenylcyclase, and the araC protein*[a]

Strain	Genotype	L-Arabinose isomerase differential rate of synthesis
SB2334	$F^-araI^c110X^+\Delta719$	1.6
SB2350	$F^-araI^c110X^+\Delta719$ *cya-4 crp*	1.2
SB2336	$F^-araI^c110X^c44\Delta719$	14.2
SB2346	$F^-araI^c110X^c44\Delta719$ *cya-4 crp*	10.1
SB2337	$F^-araI^c115X^+\Delta719$	1.3
SB2351	$F^-araI^c115X^+\Delta719$ *cya-4 crp*	1.1
SB2339	$F^-araI^c115X^c45\Delta719$	11.2
SB2347	$F^-araI^c115X^c45\Delta719$ *cya-4 crp*	11.5
SB2340	$F^-araI^c127X^+\Delta719$	0.9
SB2352	$F^-araI^c127X^+\Delta719$ *cya-4 crp*	0.5
SB2342	$F^-araI^c127X^c47\Delta719$	10.0
SB2348	$F^-araI^c127X^c47\Delta719$ *cya-4 crp*	11.9
SB2344	$F^-araI^c127X^c54\Delta719$	8.2
SB2349	$F^-araI^c127X^c54\Delta719$ *cya-4 crp*	10.4
SB2353	$F^-araI^+X^+\Delta719$ *cya-4 crp*	0.1
SB2355	$F^-araI^+X^+\Delta719$	0.1

[a] Differential rates of L-arabinose isomerase synthesis were determined from five measurements. Isomerase is measured as micromoles of L-ribulose formed per hour per milligram of protein. Defective or missing gene that normally codes for the adenylcyclase, *cya-4*. Defective or missing gene that normally codes for CRP, *crp*. Cells were grown in L-broth and harvested in the exponential phase of growth.

tivity was measured in $araC^+$ and $araC\Delta1165$ strains containing the $araI^cX^c$ or the $araI^c$ mutations alone. In the absence of L-arabinose, the $araC^+$ allele reduces L-arabinose isomerase activities three- to fourfold in both the $araI^c$ and the $araI^cX^c$ strains (Table 7).

***araX^c* mutations are *cis* acting.** In this set of experiments, we asked whether the constitutive expression and the decreased sensitivity to catabolite repression caused by the $araX^c$ mutation act in *cis* or in *trans*. The merodiploid $F'araB24/F^-araA2I^c110X^c44\Delta719$ (SB2321) was constructed, and the differential rates of synthesis of L-arabinose isomerase and L-ribulokinase were determined. If the $araI^cX^c$ mutations are *trans* acting as well as *cis* acting, then L-arabinose isomerase should be synthesized as efficiently in strain SB2321 as in $F^-araI^c110X^c44C^+$ (SB2329). If, however, the $araI^cX^c$ mutations do not stimulate in *trans*, then no L-arabinose isomerase should be produced. The data (Table 8) show that the $araI^cX^c$ mutations stimulate the syntheis of L-ribulokinase synthesis, the product of the *araB* gene which is in *cis*, but do not stimulate the synthesis of L-arabinose isomerase, the product of the *araA* gene which is in *trans*. Thus, both the $araI^c$ and $araX^c$ mutations exert an effect in *cis*. However, this experiment does not totally rule out a *trans* effect because it is possible that repression by the $araC^+$ allele in the absence of L-arabinose (Table 7) could have masked a *trans* effect by the $araI^cX^c$ mutations. To eliminate this possibility, strain SB2325 ($F'araC^c67B24/araA2I^c110X^c44\Delta719$) was constructed. The $araC^c$ protein does not interact at *araO* to cause repression and allows us to measure catabolite repression in *cis* and *trans* in the absence of the inducer L-arabinose without the possible complication caused by repressor interacting with the operator on the episome. It was found that L-arabinose isomerase, the product of the *trans* gene, was catabolite repressed 62%, whereas L-ribulokinase, the product of the *cis* gene, was catabolite repressed only 22%. This is compared with the $F^-araI^+X^+C^c67$ (UP1276) strain in which L-arabinose isomerase and L-ribulokinase are catabolite repressed 74 and 60%, respectively, and strain SB3164 ($F'araB24I^+X^+C^c67/F^-araB24I^+X^+C67$) in which L-arabinose isomerase is 59% repressed. The lack of catabolite repression *cis* to the $araI^cX^c$ markers must be caused by these markers since it does not occur in $araI^+X^+$ strains. Thus, the $araI^cX^c$ mutations have only a *cis* effect.

DISCUSSION

In this paper we have described the isolation and characterization of mutants of the $araI^c\Delta C$ strains that have higher constitutive levels of *araBAD* expression than the parent strains. The phenotype is the result of a secondary mu-

TABLE 7. *Effect of the araC repressor on the araX^c allele*[a]

Strain	Genotype	L-Arabinose isomerase activity
SB2308	$F^-araI^c110X^c44\Delta1165$	17.0
SB2329	$F^-araI^c110X^c44C^+$	3.8
SB2189	$F^-araI^c110\Delta1165$	4.3
SB2326	$F^-araI^c110C^+$	1.1
SB2310	$F^-araI^c127X^c47\Delta1165$	18.8
SB2331	$F^-araI^c127X^c47C^+$	5.1
SB2193	$F^-araI^c127\Delta1165$	4.0
SB2328	$F^-araI^c127C^+$	1.2
UP1000	$F^-araI^+C^+$	0.3
SB1636	$F^-araI^+\Delta1165$	0.1

[a] The strains were grown in mineral 0.4% glycerol-leucine media and harvested in the exponential phase of growth. L-Arabinose isomerase activity (moles of L-ribulose formed per hour per milligram of protein) was assayed as described in the text.

TABLE 8. *Cis-trans test*[a]

Strain	Genotype	L-Arabinose isomerase activity			L-Ribulokinase activity		
		MGlc	MG	%CR	MGlc	MG	%CR
SB2321	F'*araB24I*$^+$*X*$^+$*C*$^+$/F$^-$*araA2I*^{c}*110X*^{c}*44Δ719*	0.3			2.7		
SB2322	F'*araB24I*$^+$*X*$^+$*C*$^+$/F$^-$*araA2I*^{c}*115X*^{c}*45Δ719*	0.4			2.6		
SB2324	F'*araB24I*$^+$*X*$^+$*C*$^+$/F$^-$*araA2I*^{c}*127X*^{c}*54Δ719*	0.5			2.9		
SB2329	F$^-$*araI*^{c}*110X*^{c}*44C*$^+$	4.7			1.6		
SB2317	F$^-$*araA2I*^{c}*110X*^{c}*44Δ719*	<0.1			3.8		
SB2336	F$^-$*araI*^{c}*110X*^{c}*44Δ719*	28.5			3.8		
UP1000	F$^-$*araI*$^+$*X*$^+$*C*$^+$	0.2			<0.1		
UP1276	F$^-$*araI*$^+$*X*$^+$*C*^{c}*67*	61	16	74	3.0	1.2	60
SB2325	F'*araB24I*$^+$*X*$^+$*C*^{c}*67*/F$^-$*araA2I*^{c}*110X*^{c}*44Δ719*	73	28	62	1.9	1.5	21
SB3164	F'*araB24I*$^+$*X*$^+$*C*^{c}*67*/F$^-$*araB24I*$^+$*X*$^+$*C*^{c}*67*	66	27	59	<0.1	<0.1	

[a] The differential rates of synthesis of L-arabinose isomerase and L-ribulokinase were determined in exponentially growing cultures. L-Arabinose isomerase and L-ribulokinase activities are expressed as micromoles of product formed per hour per milligram of protein. %CR is defined in the footnote to Table 5.

tation (*araX*^{c}) in the controlling site region that is closely linked to, but can be segregated from, the original *araI*^{c} mutant site. This increased constitutivity of the *araΔCI*^{c}*X*^{c} is probably responsible for the more rapid growth rate on 0.2% MA plates and the EMBA-positive reaction.

Besides affecting the constitutive expression of the *araBAD* operon in the absence of *araC*, the *araX*^{c} and *araI*^{c} alleles resemble one another in several other respects. Both affect the expression of the *araBAD* operon only in the *cis* position. They also do not alter the maximum rate of expression of the *araBAD* operon and therefore do not reside in the proposed *araP* region. In the presence of the *araO* site, the expression of the *araBAD* operon in *araI*^{c} and in *araX*^{c} mutants is reduced, but not completely repressed, by the *araC* repressor. The fact that repression is not complete may indicate that the site for repressor binding or action may be changed by the *araI*^{c} mutations. Independent analysis of the effect of the *araC* repressor on the constitutive expression of the *araX*^{c} mutants has not been performed. In any case, the remaining expression of the *araX*^{c} allele in the presence of *araC* repression is similar to that of the *araI*^{c} allele. Some of the *araI*^{c} alleles in the absence of *araO* were sensitive to small amounts of activator produced by *araC* in the absence of L-arabinose (6). In one of the *araX*^{c} strains in which this property was examined, the results were somewhat ambiguous. Thus, we can make no comment at this time about the sensitivity to activator.

What does distinguish the *araX*^{c} from the *araI*^{c} is the strong effect of the former in reducing the sensitivity of the operon to catabolite repression. Bass et al. (1) found that the *araI*^{c}*Δ719* (6) mutants tested in casein hydrolysate and casein hydrolysate glucose medium showed no pronounced changes in sensitivity to catabolite repression. In comparing the *araI*^{c}*Δ766* mutants (9) in mineral glucose and mineral glycerol medium, we found that the *araI*^{c} mutants tested in this work showed from 58 to 63% catabolite repression, whereas the *araX*^{c} *araI*^{c} types were catabolite repressed from 26 to 32% and the *araX*^{c} types alone were catabolite repressed from 20 to 40%. In a *crp*$^-$ *cya*$^-$ background, the double mutants (*araI*^{c}*X*^{c}*Δ719*) show a differential rate of L-arabinose isomerase synthesis 10-fold greater than the *araI*^{c}*Δ719 crp*$^-$ *cya*$^-$ strains. Although *araΔ719I*^{c}*crp*$^-$ *cya*$^-$ strains show an increase in the expression of the *araBAD* operon when compared with *araΔ719 crp cya* strains, the activity was very low and difficult to evaluate. In any case, it is possible to conclude that the *araX*^{c} mutation, in affecting the constitutive expression of the *araBAD* operon in the absence of the *araC* gene product and in reducing significantly the dependence of the expression of this operon on cAMP and CRP, is an alteration in a region in which *araI* and the proposed *araCRP* may overlap. This suggests that possibly *araI* and *araCRP* may not be distinct regions at all. However, a second possibility is that the *araX*^{c} mutation has created a new site that does not require CRP and *araC* protein for RNA polymerase to initiate transcription. In this case, a different message may be made in *araC* deletion strains: different in that the 5′ end will be distinguishable from the 5′ end of the wild-type *araBAD* messenger RNA. We are presently testing these possibilities.

ACKNOWLEDGMENTS

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