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The *araI*^{c} Mutation in *Escherichia coli* B/r

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The *araI*^{c} allele is a *cis*-acting mutation which has been used to define the *araBAD* promoter in *Escherichia coli* B/r. Nineteen *araI*^{c} mutants were originally isolated by Englesberg and co-workers as Ara$^+$ "revertants" of an *araC* deletion mutant (Englesberg et al., J. Mol. Biol. **43**:281-298, 1969). The mutants constitutively expressed *araBAD* gene products in the absence of functional *araC* activator protein. Eight of the *araI*^{c} mutations have been cloned by in vivo recombination onto pBR322-*ara* hybrid plasmids. Restriction and DNA sequence analysis of these *araI*^{c} mutations showed that they result from a single base-pair change located at −35 in the *araBAD* promoter.

The initiator region, *araI*, of the *araBAD* operon in *Escherichia coli* B/r has been genetically defined by the isolation and characterization of 19 Ara$^+$ "revertants" of a strain containing the *araC* deletion 719 (3, 4, 14). The revertants contained the original deletion and a closely linked, but separable, secondary mutation which mapped between *araB* and *araO*. These mutants, designated *araI*^{c}, constitutively expressed the *araBAD* operon at 5 to 10% of the induced wild-type level. The *araI*^{c} allele in all cases was *cis* dominant to the wild-type *araI*$^+$ allele and exerted no *trans* effect. In the absence of inducer, an *araC*$^+$ allele in the *trans* position of merodiploid strains was able to stimulate expression of the *araA* gene *cis* to *araI*$^+$ Δ(*araCO*)*719* and *cis* to most of the *araI*^{c} Δ(*araCO*)*719* mutations. In a wild-type uninduced strain, *araC* activator protein (P2) was cryptic since the *araC* gene product was predominantly in the repressor form (P1). Deletion *719* excised all or part of the operator site and therefore, in the presence of a *trans* acting *araC*$^+$ allele, prevented P1 from binding. The small amount of P2 in equilibrium with P1 was able to partially stimulate *araBAD* expression *cis* to deletion *719*.

Englesberg et al. (3) found that the *araI*^{c} mutants had significantly different isomerase levels when the *araC*$^+$ allele was in the *trans* position. Their results suggested that some of the *araI*^{c} mutations had altered the sensitivity of the operon to the *araC* gene product and that the 19 *araI*^{c} mutants represented two groups of mutations within the initiator region. In one group, *araBAD* expression increased by varying degrees in the presence of an *araC*$^+$ allele, whereas in the other group, the expression was unaffected by an *araC*$^+$ allele. Depending on how the *araI*^{c} mutation had modified the *araI* site, *araC* activator, P2, may or may not further stimulate the operon above the level of the *araI*$^+$ Δ(*araCO*)*719* strain. These in vivo experiments suggested a direct interaction between *araC* activator protein and the DNA at the initiator site region as defined by the *araI*^{c} mutations.

In this study, we have examined the *araI*^{c} mutation at the molecular level to elucidate the nature of the activator-initiator site interaction. We describe the molecular cloning and DNA sequencing of *araI*^{c} mutants representing the various degrees of sensitivity to the *araC* gene product. We have also used an *araC* gene cloned onto a plasmid to reevaluate the *trans* stimulatory effect of the *araC* activator protein on the expression of *araA cis* to *araI*^{c} Δ(*araCO*)*719*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used or constructed in this study are described in Table 1. Plasmids containing the *araI*^{c} or *araI*$^+$ alleles were introduced into strain SB1652 or LA5 by transformation. The plasmid pTB2, containing Δ(*araPBA*)*718*, was introduced by transformation into strains containing the *araI*^{c} or *araI*$^+$ alleles.

Media and chemicals. The media employed have been previously described (9), with the following addition: the medium used to grow cells for the L-arabinose isomerase enzyme assay consisted of mineral salts (20) and 1% (wt/vol) casein hydrolysate. L-Arabinose (0.4%, wt/vol) and tetracycline-hydrochloride (10 μg/ml) were added as needed. Casein hydrolysate, L-arabinose, and tetracycline were purchased from Sigma Chemical Co., St. Louis, Mo. [δ-^{32}P]ATP was prepared as described by Johnson and Walseth (11).

Enzymes. Restriction endonucleases and polynu-

TABLE 1. *List of bacterial strains and plasmids*

Strain[a]	Genotype/phenotype[b]	Origin or reference
Bacterial strain		
SB2001	F$^-$ *araI*^{c}*2* Δ(*araCO*)*719 thr* Str^r	(3)
SB2002	F$^-$ *araI*^{c}*3* Δ(*araCO*)*719 thr* Str^r	(3)
SB2004	F$^-$ *araI*^{c}*5* Δ(*araCO*)*719 thr* Str^r	(3)
SB2006	F$^-$ *araI*^{c}*7* Δ(*araCO*)*719 thr* Str^r	(3)
SB2007	F$^-$ *araI*^{c}*8* Δ(*araCO*)*719 thr* Str^r	(3)
SB2009	F$^-$ *araI*^{c}*10*Δ(*araCO*)*719 thr* Str^r	(3)
SB2010	F$^-$ *araI*^{c}*11* Δ(*araCO*)*719 thr* Str^r	(3)
SB2094	F$^-$Δ(*araCO*)*719 thr* Str^r	(3)
SB1652	F$^-$Δ(*araCOPB leuBCD*)*1119 leu-6 dau-5* Str^r	(13)
LA5	F$^+$/F$^-$Δ(*araCOPBA*)*744 lac gal pro thi hsdS*	(7)
UP1000	F$^-$Str^r	(8)
Plasmid		
pAH15	pBR322 *bla*::Δ(*araCOPB*)*747*	(9)
pTB1	pBR322 *bla*::*araC*$^+$*B*$^+$	(7)
pTB2	pBR322 *bla*::Δ(*araPBA*)*718*	(7)
pTB3	pBR322 *bla*::Δ(*araCO*)*719*	(7)
pTB5	pBR322 *bla*::Δ(*araC*)*766*	(7)
pCH1	pBR322 *bla*::*araI*^{c}*3* Δ(*araCO*)*719* Δ(*araCOPB*)*747*	This paper
pCH2	pBR322 *bla*::*araI*^{c}*5* Δ(*araCO*)*719*	This paper
pCH3	pBR322 *bla*::*araI*^{c}*10* Δ(*araCO*)*719* Δ(*araCOPB*)*747*	This paper
pCH4	pBR322 *bla*::araI^{c}4 Δ(*araCO*)*719* Δ(*araCOPB*)*747*	This paper
pCH5	pBR322 *bla*::*araI*^{c}*11* Δ(*araCO*)*719*	This paper
pCH6	pBR322 *bla*::araI^{c}2 Δ(*araCO*)*719* Δ(*araCOPB*)*747*	This paper
pCH7	pBR322 *bla*::*araI*^{c}*7* Δ(*araCO*)*719*	This paper
pCH8	pBR322 *bla*::*araI*^{c}*1* Δ(*araCO*)*719* Δ(*araCOPB*)*747*	This paper
pCH9	pBR322 *bla*::*araI*^{c}*3* Δ(*araCO*)*719*	This paper
pCH10	pBR322 *bla*::*araI*^{c}*10* Δ(*araCO*)*719*	This paper
pCH11	pBR322 *bla*::*araI*^{c}*2* Δ(*araCO*)*719*	(9)
pAH11	pBR322 *bla*::*araI*^{c}*8* Δ(*araC*)*766*	This paper

[a] All strains are derivatives of *E. coli* B/r with the exception of LA5, which is from *E. coli* K-12 strain RR1 and contains the *ara* region from *E. coli* B/r.

[b] Abbreviations used: *ara*, L-arabinose; *A*, structural gene for L-arabinose isomerase; *B*, structural gene for L-ribulokinase; *C*, regulatory gene for L-arabinose system; *O*, operator site of the controlling region; *P*, promoter site of the controlling region; Δ, deletion; ::, insertion.

cleotide kinase were isolated or purchased as described previously (1). Bacterial alkaline phosphatase was purchased from Worthington Biochemical Co., Freehold, N.J.

Construction and screening of recombinant plasmids. Plasmids carrying the *araI*^{c} mutation were constructed by in vivo recombination as described by Horwitz et al. (9). Restriction analysis of recombinant plasmids was performed by digesting 10 μl of phenol-extracted crude cleared lysate DNA with 1 U of *Bam*HI or *Hha*I in a total volume of 20 μl for 3 h at 37°C. *Bam*HI buffer consisted of 50 mM Tris-hydrochloride (pH 7.8), 5 mM $MgCl_2$, 25 mM NaCl, and 10 mM $(NH_4)_2SO_4$. *Hha*I buffer consisted of 50 mM Tris-hydrochloride (pH 7.4), 5 mM $MgCl_2$, and 0.5 mM dithiothreitol.

Transformation. Transformation was performed as described by Cohen et al. (2). Purified plasmid DNA (1 μg in 1 to 4 ml) or phenol-extracted crude cleared lysate DNA (5 μl) was mixed with 0.4 ml of competent cells.

Preparation of DNA. Plasmid DNA was isolated for screening from clones suspected of containing the *araI*^{c} mutation by the procedure described by Horwitz et al. (10). Large quantities of purified plasmid DNA were prepared as described by Norgard et al. (18).

DNA sequence analysis. Purified plasmid DNA was restricted with *Bam*HI and 5′ end labeled with [δ-^{32}P]ATP as described by Greenfield et al. (7). After restriction with *Hin*fI or *Hae*III, the appropriate single end-labeled fragments were isolated by preparative gel electrophoresis and subjected to the base-specific chemical cleavage reactions of Maxam and Gilbert (16).

Enzyme assay. Cell extracts for assay of L-arabinose isomerase activity were prepared from cells harvested at stationary phase of growth (16-h cultures), as described by Englesberg et al. (3). L-Arabinose isomerase was measured as previously described (3). Protein was estimated by the method of Lowry et al. (15), using crystalline bovine serum albumin (Miles Laboratories, Inc., Kankakee, Ill.) as a standard.

RESULTS

Cloning of *araI*^{c} mutations. We have previously described a method utilizing in vivo recombination for cloning *ara* deletion and point mutations onto plasmids (9). As part of this study we used plasmid pTB1, which contains all of *araC* and *araB*, to construct plasmid pAH15. The plasmid pAH15 contains *ara* deletion *747*, which covers a small part of *araB*, all of the *ara* controlling site region, and all of *araC* (Fig. 1a). The plasmid pAH15 was subsequently used to clone, by in vivo recombination, several *ara* controlling site mutations, including the initiator constitutive mutation, *araI*^{c}*2*.

In this study, we have used the plasmid pAH15 to clone, by in vivo recombination, 7 of the 19 independently isolated *araI*^{c} mutations representing the different classes of response to

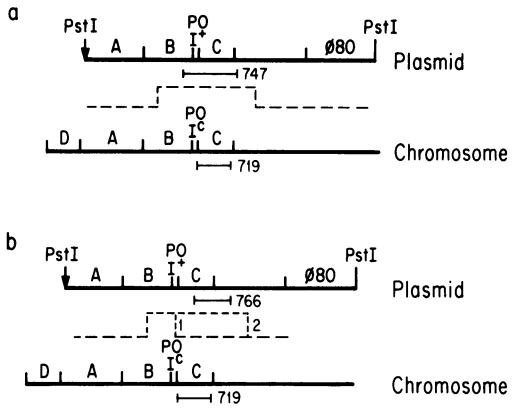

FIG. 1. *The recombination events that placed the araI^c mutation onto plasmids. (a) The recombination event between pAH15 and an araI^c mutation; (b) possible recombination events between pTB5 and an araI^c mutation. Recombination event no. 2 generated plasmid pAH11.*

araC activator (see above). The *araI*^{c} strains were transformed with plasmid pAH15, and transformants were selected on tryptone-yeast extract agar supplemented with tetracycline. Crude cleared lysates were prepared from cultures of purified transformants as described above. A recombination event which places the *araI*^{c} mutation onto the plasmid pAH15 is shown in Fig. 1a. This crossover event places both the *araI*^{c} mutation and deletion *719* onto the plasmid pAH15, since deletion *747* overlaps the region excised by deletion *719*. The presence of a rare recombinant plasmid carrying the *araI*^{c} mutation was detected by transforming strain SB1652 with the crude lysate DNA. This strain is *araB*, and constitutively synthesizes the *araA* and *araD* gene products (13); it is not complemented by pAH15 (10). A recombinant plasmid containing *araB*$^+$*I*^{c} Δ(*CO*)*719* is, however, able to complement strain SB1652 since the *araB*$^+$ gene product is produced constitutively by the plasmid (10). The transformed cells were plated on MacConkey-arabinose-tetracycline medium and incubated at 37°C. At 24 h, Ara$^+$ clones were obtained at a frequency of 0.06 to 0.1%. Each clone suspected of carrying the *araI*^{c} mutation was purified by successive restreaking on MacConkey-arabinose-tetracycline medium. One additional *araI*^{c} mutation (*araI*^{c}*8*) was cloned by in vivo recombination, using the plasmid pTB5 as described by Horwitz et al. (10). The possible recombination events that yield an *araI*^{c} plasmid, using plasmid pTB5, are shown in Fig. 1b.

Strain SB1652 was again transformed with crude plasmid DNAs isolated from the Ara$^+$ clones to confirm the presence of the *araI*^{c} mutation. With four of the eight *araI*^{c} plasmids, 100% of the Tc^r transformants were Ara$^+$. The other four plasmids generated both Ara$^+$ and Ara$^-$ Tc^r colonies when transformed into strain SB1652. The Ara$^-$ clones occurred at a frequency of 1 to 5%. To determine whether these Ara$^-$ colonies resulted from contamination of the crude lysates with pAH15 or from some other factor, single Ara$^+$ colonies were isolated from each clone and purified by two successive restreaks on MacConkey-arabinose-tetracycline medium. For each clone, an overnight tryptone-yeast extract-tetracycline broth culture was prepared from a single, isolated Ara$^+$ colony and diluted to yield approximately 900 colonies when plated on MacConkey-arabinose-tetracycline medium. The result was a mixture of Ara$^+$ and Ara$^-$ colonies in which 1 to 5% of the colonies were Ara$^-$. This suggested that the Ara$^-$ colonies were generated by unstable *araI*^{c} plasmids which were constructed during the in vivo cloning and were not the result of contamination of the crude lysate DNA with pAH15. The nature of these plasmids is examined below.

Physical evidence for recombination. The crossover event that occurred to place the *araI*^{c} mutations onto the plasmid pAH15 also placed deletion *719* on the plasmid (Fig. 1a). The presence of deletion *719* on the *araI*^{c} plasmids was detected as follows. The plasmid pAH15 carried deletion *747* (Fig. 1a), which eliminated the *Bam*HI site in the *araBAD* promoter (10). Since *araC* deletion *719* does not eliminate this *Bam*HI site (12), a reciprocal crossover event that places *araI*^{c} Δ(*CO*)*719* onto the plasmid pAH15 should restore the *Bam*HI site in the promoter. A representative *araI*^{c} plasmid DNA and pAH15 DNA digested with *Bam*HI are shown in Fig. 2 (lanes 3 and 5). The presence of Δ(*araCO*)*719* on the *araI*^{c} plasmids was confirmed by comparing the *Bam*HI restriction patterns of pTB1 which contained an intact *araC* gene (Fig. 2, lane 2), and pCH7, which contained *araI*^{c}*7* Δ(*araCO*)*719* (Fig. 2, lane 3). The 4.5-kilobase *araC* fragment present in pTB1 (Fig. 2, arrow 2) was replaced by a 3.53-kilobase fragment (Fig. 2, arrow 4) generated by deletion *719*. The difference in the size of the two fragments was consistent with the 972 base pairs which are removed by Δ(*araCO*)*719* (17).

The plasmid pAH11, which contained the *araI*^{c}*8* mutation, was constructed as described above by in vivo recombination, using the plasmid pTB5 (Fig. 1b). Restriction analysis demonstrated that plasmid pAH11 contained deletion *766*, using the approach described previously (10) (data not shown). Thus, crossover event no. 2 (Fig. 1b) occurred to produce this plasmid.

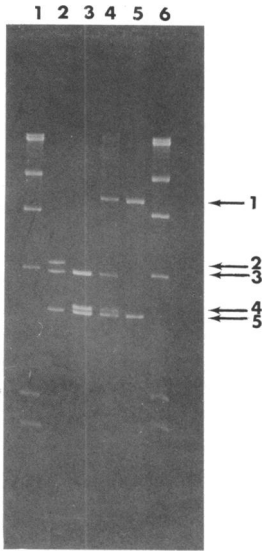

FIG. 2. *Agarose gel electrophoresis of ara plasmid DNAs digested with BamHI. DNA samples (1 μg) were digested with 1 U of enzyme in a total volume of 20 μl for 3 h at 37°C. Lanes 1 and 6, lambda DNA digested with HindIII; lane 2: pTB1; lane 3: pCH7; lane 4: pCH1; lane 5: pAH15. Arrow 1, the fused araCOPBA fragment resulting from deletion 747, which eliminated the BamHI site in the araBAD promoter. Arrow 2, the 4.5-kilobase araC fragment (10). Arrow 3, the araBA fragment. Arrow 4, the araC fragment containing deletion 719. Arrow 5, the 3.5-kilobase fragment containing primarily pBR322 DNA.*

The plasmids which gave a mixture of Ara$^+$ and Ara$^-$ colonies upon retransformation of SB1652 were also restricted with *Bam*HI. A representative digest (Fig. 2, lane 4) demonstrated that these plasmids contained both deletion *719* and deletion *747*. This was indicated by the presence of the high-molecular-weight fragment created by deletion *747* (Fig. 2, arrow 1) and the two lower-molecular-weight fragments created by deletion *719* (Fig. 2, arrows 3 and 4). The presence of more than one copy of pBR322 was indicated by the greater intensity of the pBR322 fragment (Fig. 2, arrow 5) relative to the two higher-molecular-weight bands immediately above this fragment. These results suggest a "double-plasmid" structure generated by a single crossover event between pAH15 and an *araI*^{c} Δ(*araCO*)*719* recombinant plasmid at some point during the in vivo cloning procedure.

Isolation of single Ara$^+$ plasmids from double plasmids. The occurrence of Ara$^-$ Tc^r segregants when SB1652 was transformed with the double plasmids might be due to a recombination event which regenerates plasmid pAH15. This was tested by preparing crude lysates from representative Ara$^-$ segregant colonies and digesting the plasmid DNA with *Bam*HI. The *Bam*HI restriction pattern was identical to that of PAH15 (Fig. 2, lane 5). Recombination of pAH15 out of the double plasmid to yield a single *araI*^{c} Δ(*araCO*)*719* plasmid, might also occur with equal frequency. Clones carrying the single *araI*^{c} plasmid might be detected on MacConkey-arabinose-tetracycline plates since the resultant plasmid, which is smaller, might complement SB1652 more strongly because it is present in more copies per cell. This was tested by transforming SB1652 with several double plasmids and plating so that approximately 900 colonies arose on each plate. Several colonies which produced a stronger Ara$^+$ reaction than the other Ara$^+$ colonies were isolated for each plasmid. These colonies were purified by restreaking on MacConkey-arabinose-tetracycline plates, and crude cleared lysates were prepared. *Bam*HI restriction analysis revealed that in three out of four cases, single plasmids were obtained. Only the single plasmids were used in the experiments described below to eliminate any problems associated with a heterogeneous plasmid population.

Restriction and DNA sequence analysis of *araI*^{c} mutations. A second series of *araI*^{c} mutants was isolated by Gielow et al. (5) as Ara$^+$ revertants of *araC* deletion *766*, subsequent to 2-aminopurine mutagenesis. Six of these *araI*^{c} Δ*766* mutations were previously cloned by in vivo recombination onto the plasmid pBR322, and the location of these mutations was determined by DNA sequence analysis (10). All of the *araI*^{c} mutations occurred at position −35 in the *araBAD* promoter and resulted from an AT → GC transition which created a *Hha*I site (10). If any of the *araI*^{c} mutations cloned in the present study resulted from the same base-pair change at position −35, the new *Hha*I site would have been present. Restriction analysis with *Hha*I of plasmids carrying the various *araI*^{c} mutations *cis* to Δ(*araCO*)*719* as well as an *araI*$^+$ Δ(*araCO*)*719* plasmid (pTB3) revealed that all of the cloned *araI*^{c} mutations contained a new *Hha*I site within the controlling site region (data not shown). This experiment established that all of the *araI*^{c} mutations examined resulted from at least one base-pair change at position −35 of the *araBAD* promoter.

The DNA sequence of three *araI*^{c} promoters was determined to confirm the presence of the AT → GC transition at position −35 and to look for additional mutations. Three *araI*^{c} plasmids (pAH11, pCH7, and pCH11) which contained mutations representing different sensitivities to *araC* activator protein (3 and above) were chosen for DNA sequence analysis because they

were more likely to contain a second mutation in the *ara* controlling site region. The strategy employed for DNA sequence analysis was essentially that of Horwitz et al. (10). The plasmids pCH7 and pCH11 were digested with *Bam*HI and 5′ end labeled as described above. The DNAs were then restricted with *Hin*fI, generating the following relevant *Bam*HI/*Hin*fI fragments: a 110-base pair fragment extending from the *Bam*HI site in the *araBAD* promoter into *araB* and a 186-base pair fragment containing deletion *719*, which extends from the *Bam*HI site in the promoter through the end of *araC*. Plasmid pAH11 was sequenced in an identical manner, except that *Hae*III was the enzyme used to generate single end-labeled fragments. The fragments that were sequenced included a 86-base pair fragment containing part of the controlling site region and *araB* and a 350-base pair fragment containing part of the controlling site region and *araC*. Isolated end-labeled DNA fragments were sequenced by the chemical method of Maxam and Gilbert (16). All three of the $araI^c$ mutations sequenced had the single base-pair change at position −35 which resulted in the AT → GC transition predicted by the *Hha*I restriction analysis. No other mutations were found within the entire *ara* controlling site region. A representative sequencing gel showing the sequence of the 110-base pair *Bam*HI/*Hin*fI fragment of plasmid pCH7, which contained the $araI^c7$ mutation, is shown in Fig. 3 (left gel). A gel showing the wild-type DNA sequence of this region is also presented in Fig. 3 for comparison (right gel).

Physiological characterization: L-arabinose isomerase activity. The DNA sequence and *Hha*I restriction analyses of the cloned $araI^c$ mutants have demonstrated that the $araI^c$ mutations isolated by Englesberg et al. (3) probably all result from the same base-pair change in the *araBAD* promoter. It was therefore necessary to reexamine the in vivo assay used by Englesberg et al. (3) to measure the effect of *araC* activator protein on *araA* gene expression *cis* to the $araI^c$ Δ*719* mutations. L-Arabinose isomerase activity was measured in the previous study under induced and uninduced conditions (± L-arabinose), using merodiploid strains of the type $F'araA\text{-}C^+/F^-araA^+I^c$ Δ*719*. One possible explanation for the variation of isomerase levels between the different $araI^c$ mutations containing $F'araC^+$ in *trans* was the instability of the F-factor carrying the $araC^+$ allele. Since there was no selection for maintenance of the $F'araC^+$, this element could have been present in zero to four copies per cell. As a result, the low levels of isomerase in some of the strains may have been due to a population of cells which contained on the average a single $F'araC^+$ allele *trans* to the $araI^c$ mutation. To test this hypothesis, the effect on L-arabinose isomerase activity of $araC^+$ *trans* to the $araI^c$ Δ(*araCO*)*719* mutations was measured as described by Englesberg et al. (3) (see above), with the following modification: instead of using an $F'araC^+$, *the* $araC^+$ allele was placed in *trans* to the $araI^c$ mutations by transforming representative $araI^c$ strains ($araI^c2$, *-7*, *-8*, *-11*) and the appropriate $araI^+$ Δ(*CO*)*719* control with the Tc^r $araC^+$ plasmid, pTB2 (7). This plasmid is a derivative of pBR322 containing *ara* deletion *718*, which extends from *araA* through *araB*, leaving *araC* intact. The maintenance of pTB2, and therefore the presence of a functional $araC^+$ allele in the population of cells, was insured by inclusion of tetracycline in the medium used to grow cells for the measurement of L-arabinose isomerase activity. Three independent assays were performed. During each assay, the analysis of all induced and uninduced strains was performed concurrently to minimize experimental variation. In Table 2, the uninduced L-arabinose isomerase levels of the representative $araI^c$ Δ(*araCO*)*719* strains containing pTB2 and the appropriate haploid wild-type and $araI^+$ Δ(*araCO*)*719* controls are shown. Within any single assay, there was a small variation in the L-arabinose isomerase levels for the various pTB2-containing $araI^c$ strains. A comparison of L-arabinose isomerase in any given pTB2-containing strain over the three independent assays, however, showed an equal amount of variation in the enzyme levels. Evaluation of the arithmetic means of the enzyme levels for the three independent assays for each plasmid-containing strain demonstrated that the L-arabinose isomerase level of each strain was not significantly different. The variation between the arithmetic means for each plasmid-containing strain was dramatically less than the standard deviation resulting from the analysis of data from three independent assays for each plasmid-containing strain. The average level of L-arabinose isomerase in induced cultures was 164 ± 18 μmol of product formed per h per mg of protein and was not significantly different for all $araI^c$ mutants tested. We conclude from the data in Table 2 that the variations in the uninduced levels of L-arabinose isomerase in the presence of the $araC^+$ allele in *trans* is not a reflection of physiologically significant differences between the strains, but rather a result of normal variations occurring in the in vivo assay itself.

DISCUSSION

We have used in vivo recombination to construct hybrid plasmids containing the $araI^c$ con-

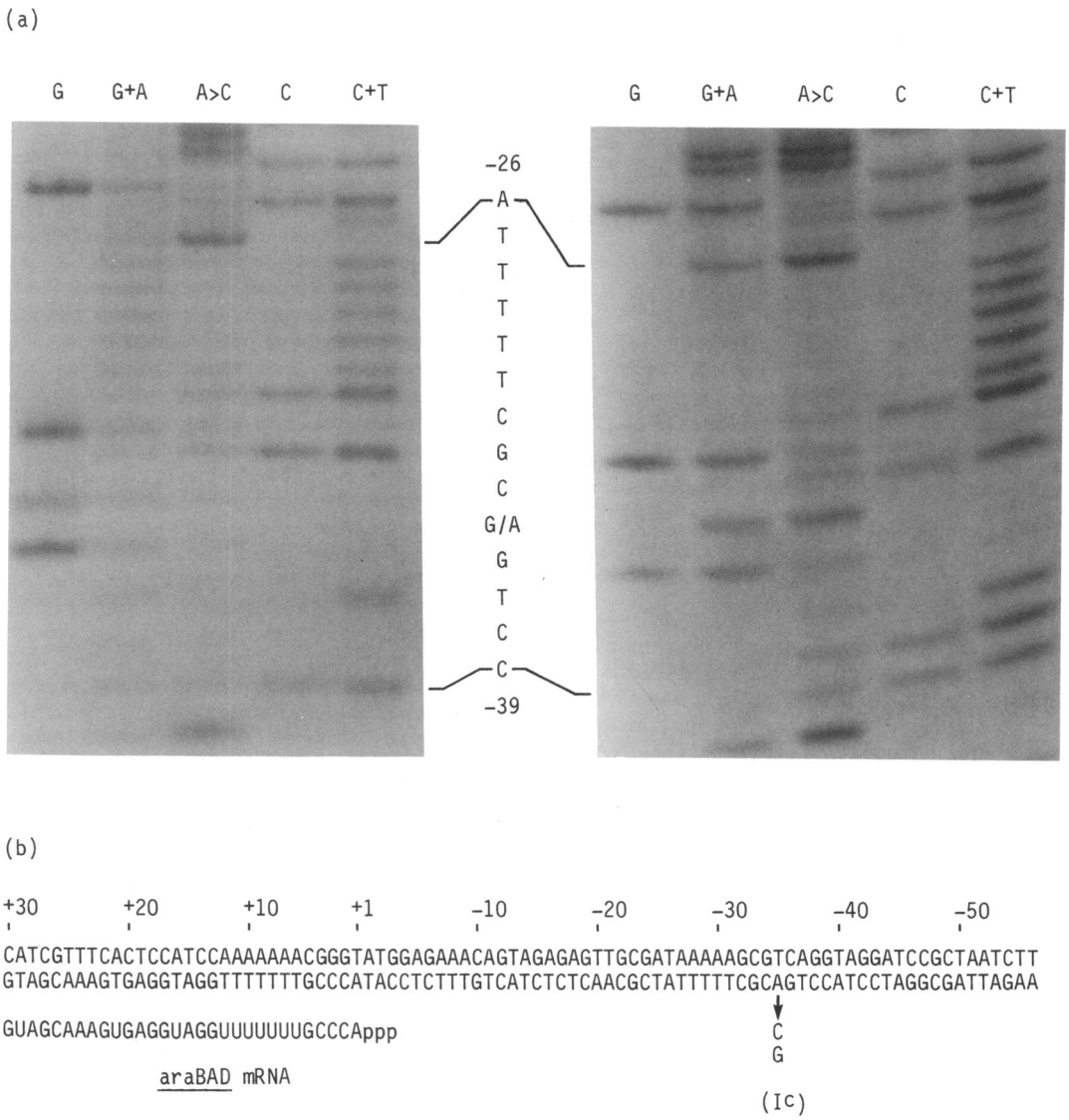

FIG. 3. *(a) Autoradiograms of 15% polyacrylamide gels showing the position of the araI*^{c} *mutation. (Right) The araI*^{c} *sequence from position −43 to −26. (Left) The wild-type sequence from position −43 to −26. (b) The DNA sequence of the araBAD promoter and adjacent regions showing the location of the araI*^{c} *mutation. The sequence was determined as described in the text.*

trolling site mutation. This procedure utilized recombination between the Δ(*araCOPB*)*747* plasmid, pAH15, and the chromosome of an *araI*^{c} Δ(*araCO*)*719* strain. The crossover event which placed the *araI*^{c} mutation and the *araC* deletion *719* onto the plasmid is shown in Fig. 1. Seven independently isolated *araI*^{c} mutations were cloned in this manner. *Bam*HI restriction analysis demonstrated that the initiator site region, and therefore the *araI*^{c} mutation, had been restored in the hybrid plasmids and that *araC* deletion *719* was present *cis* to the *araI*^{c} allele (Fig. 2). One additional *araI*^{c} mutation (*araI*^{c}*8*) was also cloned by in vivo recombination, using the plasmid pTB5 as previously described (10). *Hha*I restriction analysis of the seven *araI*^{c}-containing plasmids constructed in this study revealed that in all cases, the hybrid plasmid contained a new *Hha*I site not present in an *araI*$^+$ Δ(*araCO*)*719* plasmid. The new *Hha*I site was shown to be located within the controlling site region, suggesting that all seven *araI*^{c} mutations resulted from at least one base-pair change at the same location in the *ara* promoter

TABLE 2. *L-Arabinose isomerase activity: noninduced levels*[a]

Strain genotype (plasmid)	Activity			
	1	2	3	Average ± SE
F⁻*araI*ᶜ*2* Δ(*araCO*)*719* (pTB2)[b]	21.3	24.6	13.8	19.9 ± 6.5
F⁻*araI*ᶜ*7* Δ(*araCO*)*719* (pTB2)	22.1	17.5	16.0	18.5 ± 2.3
F⁻*araI*ᶜ*8* Δ(*araCO*)*719* (pTB2)	17.6	29.2	7.6	18.1 ± 7.3
F⁻*araI*ᶜ*11* Δ(*araCO*)*719* (pTB2)	24.8	27.0	13.5	21.7 ± 5.5
F⁻*araI*⁺ Δ(*araCO*)*719* (pTB2)	5.0	4.2		4.6 ± 0.4
F⁻*ara*⁺ (UP1000)	0.714	0	1.0	0.57 ± 0.38
F⁻*araI*⁺ Δ(*araCO*)*719*	1.08	0.099	0	0.39 ± 0.40

[a] Cell-free extracts were prepared from cells in the stationary phase of growth. Enzymatic activity is in micromoles of product formed per hour per milligram of protein. SE, Standard error.

[b] pTB2 is a plasmid containing the *araC* gene cloned in pBR322. It has the following genotype: Δ(*araABP*)*718*.

site region.

Since all of the *araI*ᶜ mutations examined by *Hha*I restriction analysis contained a base-pair change at −35, the possibility of a second base-pair change in the *ara* controlling site region was examined on a molecular level. DNA sequence analysis of three hybrid plasmids containing *araI*ᶜ Δ(*araCO*)*719* mutations from both groups (two from the group affected by *araC*⁺ in *trans*) revealed only the AT → GC transition at position −35 of the *araBAD* promoter, which had created the *Hha*I restriction site (Fig. 3). No other base-pair changes were found in the entire controlling site sequence.

The results of our restriction and DNA sequence analysis of the *araI*ᶜ mutations are inconsistent with the results of the in vivo assay utilized by Englesberg et al. (3) to measure levels of stimulation by an *araC*⁺ allele on expression of the *araA* gene *cis* to the *araI*ᶜ Δ(*araCO*)*719* mutations. We therefore reevaluated the methodology used in the in vivo assay, specifically, the type of merodiploids employed in the previous study. An F′ plasmid carrying an *araC*⁺ allele was placed in *trans* to the *araI*ᶜ mutations (3). Under the growth conditions employed in preparation of cell-free extracts, no selection was present to prevent segregation of the F-factor within the cell population. Variation in expression of the *araA* gene *cis* to the various *araI*ᶜ mutations, therefore, may have resulted not from physiological differences among the *araI*ᶜ mutants, but rather from the number of F′-*araC* plasmids per cell. We have tested this possibility by repeating the in vivo assay, utilizing stable merodiploids. This was accomplished by placing an *araC*⁺ Tcʳ plasmid, pTB2 (7, Table 2), in the *trans* position to four representative *araI*ᶜ mutants by transformation. The presence of the *araC*⁺ allele was maintained by growth of the merodiploids in a medium containing tetracycline. L-Arabinose isomerase assays of cell-free extracts were measured in the manner used by Englesberg et al. (3). We found that the induced and uninduced levels of L-arabinose isomerase were not significantly different (Table 2; see above) among the four *araI*ᶜ strains examined, reflecting similar levels of stimulation by the *araC*⁺ gene product. We feel that the use of a stable merodiploid to carry out these in vivo assays resulted in a more accurate measurement of the physiological state of the *araI*ᶜ strains.

Our examination of the *araI*ᶜ mutations on a molecular level indicates that they do not define the P2 binding site. Thus, the *araI*ᶜ mutation, located at position −35, occurs in the region suggested to be the initial recognition site for RNA polymerase (6, 10). Recent binding studies have shown that the *araC* protein in the activator form binds not in the −35 region, where the *araI*ᶜ mutation is located, but rather upstream from this region at position −40 to −78 (19). It was previously suggested that the *araI*ᶜ mutation may allow RNA polymerase interaction on the DNA in the absence of the activator protein (10). If this is the case, the presence of the *araC* activator produced by an *araC*⁺ allele *trans* to the *araI*ᶜ mutation may merely further facilitate, either directly or indirectly, this interaction.

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LITERATURE CITED

1. **Cass, L. G., A. H. Horwitz, C. G. Miyada, L. Greenfield, and G. Wilcox.** 1980. The *araC* regulatory gene mRNA contains a leader sequence. Mol. Gen. Genet. **180**:219–226.
2. **Cohen, S. N., A. C. Y. Chang, and L. Heu.** 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2119.
3. **Englesberg, E., D. Sheppard, C. Squires, and F. Meronk, Jr.** 1969. An analysis of "revertants" of a deletion mutant in the *C* gene of the L-arabinose gene complex in *Escherichia coli* B/r: isolation of initiator constitutive mutants (*I*ᶜ). J. Mol. Biol. **43**:281–298.
4. **Englesberg, E., and G. Wilcox.** 1974. Regulation: positive control. Annu. Rev. Genet. **8**:219–242.
5. **Gielow, L., M. Largen, and E. Englesberg.** 1971. Initiator constitutive mutants of the L-arabinose operon (*OIBAD*) of *Escherichia coli* B/r. Genetics **69**:289–302.

6. **Gilbert, W.** 1976. Starting and stopping sequences for the RNA polymerase, p. 193–205. *In* R. Losick and M. Chamberlin (ed.), RNA polymerase. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Greenfield, L., T. Boone, and G. Wilcox.** 1978. DNA sequence of the *araBAD* promoter in *Escherichia coli* B/r. Proc. Natl. Acad. Sci. U.S.A. **75**:4724–4728.
8. **Gross, J., and E. Englesberg.** 1959. Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1bt. Virology **9**:314–331.
9. **Horwitz, A. H., L. Heffernan, L. Cass, C. G. Miyada, and G. Wilcox.** 1980. Construction of pBR322-*ara* hybrid plasmids by in vivo recombination. Mol. Gen. Genet. **179**:615–625.
10. **Horwitz, A. H., C. Morandi, and G. Wilcox.** 1980. Deoxyribonucleic acid sequence of *araBAD* promoter mutants of *Escherichia coli*. J. Bacteriol. **142**:659–667.
11. **Johnson, R. A., and T. F. Walseth.** 1979. The enzymatic preparation of (α-^{32}P)ATP, (α-^{32}P)GTP, (^{32}P)cAMP, and (^{32}P)cGTP, and their use in the assay of adenylate and guanylate cyclase and cyclic nucleotide phosphodiesterases. Adv. Cyclic Nucleotide Res. **10**:135–167.
12. **Kaplan, D., L. Greenfield, T. Boone, and G. Wilcox.** 1978. Hybrid plasmids containing the *araCBAD* genes of *Escherichia coli*. Gene **3**:177–189.
13. **Kessler, D., and E. Englesberg.** 1969. Arabinose-leucine deletion mutants of *Escherichia coli* B/r. J. Bacteriol. **98**:1159–1169.
14. **Lee, N.** 1978. Molecular aspects of *ara* regulation, p. 389–409. *In* J. H. Miller and W. S. Reznikoff (ed.), The operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Lowry, O., N. Rosenbrough, A. Farr, and R. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages, p. 499–560. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 65. Academic Press, Inc., New York.
17. **Miyada, C. G., A. H. Horwitz, L. G. Cass, J. Timko, and G. Wilcox.** 1980. DNA sequence of the *araC* regulatory gene from *Escherichia coli* B/r. Nucleic Acids Res. **8**:5267–5274.
18. **Norgard, M., K. Emigholz, and J. Manihan.** 1979. Increased amplification of pBR322 plasmid deoxyribonucleic acid in *Escherichia coli* K-12 strains RR1 and χ1776 grown in the presence of high concentrations of nucleoside. J. Bacteriol. **138**:270–272.
19. **Ogden, S., D. Haggerty, C. M. Stoner, D. Kolodrubetz, and R. Schleif.** 1980. The *Escherichia coli* L-arabinose operon: binding sites of the regulatory proteins and a mechanism of positive and negative regulation. Proc. Natl. Acad. Sci. U.S.A. **77**:3346–3350.
20. **Sheppard, D., and E. Englesberg.** 1967. Further evidence for positive control of the L-arabinose system by gene *araC*. J. Mol. Biol. **25**:443–454.