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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)

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Nineteen independent L-arabinose-utilizing "revertants" were isolated from *Escherichia coli B/r* containing a deletion (*Δ*719) that encompasses all known mutations in the regulator gene *araC*. The revertants contain the original deletion plus a secondary initiator constitutive mutation (I^c). All produce low constitutive levels of enzymes in the L-arabinose pathway. The I^c mutant sites are all closely linked to the deletion. Nine of the I^c sites, mapped with greater resolution, are located to the left of deletion 719, in the region containing the proposed initiator controlling site (*araI*) for this operon. The I^c alleles in all revertants tested are *cis*-dominant to the wild-type allele, I^+, and have no *trans* effect. All the strains are hyperinducible to varying degrees in the presence of a functioning C^+ allele in the *trans* position.

In merodiploids, the C^+ (even in the absence of inducer) and not the C^- alleles are able to stimulate the expression of the *araA* gene *cis* but not *trans* to the I^+ *Δ*719 and to most I^c*Δ*719 mutations. These results, together with other evidence, support a modified positive control model in which P1, the initial product of the *araC* gene, is a true repressor existing in equilibrium with P2, the activator, and with P1 and P2 attached to their respective controlling sites, the operator, *araO*, and the initiator, *araI*, located as follows: *araB*, *araI*, *araO*, *araC*. Evidence indicates that the repressor–operator site function is epistatic over the activator–initiator site function. The *cis* effect of deletion 719 in the presence of a *trans* acting C^+ allele is explained on the basis that this deletion desensitizes this operon to the repressor, P1, by excising the operator, and thus allows this operon to be activated by P2 whose presence would otherwise have remained cryptic.

Among 19 constitutive revertants of deletion 719, none could be identified as mutants in a regulatory gene of the negative control type. Therefore, no evidence could be found to support a model of negative control internal induction in this system.

1. Introduction

Evidence, previously presented (Englesberg, Irr, Power & Lee, 1965; Sheppard & Englesberg, 1966,1967), clearly demonstrates that the gene *araC* in the L-arabinose system is distinct from a regulator gene of a negative control system; *e.g.* the *i* gene

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of the β-galactosidase system. Whereas a product of the *araC* gene, the activator, is required for the expression of the structural genes in the L-arabinose system, the only known functional product of the *i* gene, the repressor, prevents the otherwise free expression of the related structural genes (Jacob & Monod, 1961). Thus deletions (Sheppard & Englesberg, 1966,1967) and nonsense mutations (Irr & Englesberg, 1967) of the *araC* gene lead to a pleiotropic negative phenotype, C^-, (non-inducible for the enzymes specifically involved in L-arabinose metabolism), while deletions or nonsense mutations of the *i* gene in the β-galactosidase system (Willson, Perrin, Cohn, Jacob & Monod, 1964; Bourgeois, Cohn & Orgel, 1965; Muller-Hill, 1966) lead to a pleiotropic constitutive phenotype. While the $araC^-$ alleles are recessive (*cis* and *trans*) to the alleles of the *araC* gene for inducibility (C^+) and constitutivity (C^c), lactose non-utilizing pleiotropic negative mutants of the *i* gene (i^s), on the other hand, are *cis* and *trans* dominant to the analogous alleles of that gene (Jacob & Monod, 1961; Willson *et al.*, 1964).

The above characteristics of the *araC* gene are consistent with a model of positive control (Englesberg *et al.*, 1965; Sheppard & Englesberg, 1966,1967). According to this model, the gene *araC* (C^+ allele, wild type) produces a product (Pl), an allosteric protein, which is in equilibrium with P2, the activator. In the absence of L-arabinose, the equilibrium is in the direction of P1. L-Arabinose shifts the equilibrium to P2. P2, by reacting with a controlling site, the initiator (*araI*), located in the region between genes *araB* and *araC*, stimulates the expression of the structural genes *araB*, *araA* and *araD*. The initiator is thus the site of action of activator and the site of initiation of gene expression. (It has not been specified as to whether activator functions at the transcriptional or translational level.) According to the model, ara^- mutants in the *C* gene, C^-, fail to produce a biologically active C product. C^c mutants produce substantial amounts of an activator (P3, P4 . . . P*n*) in the absence of L-arabinose, as a result of a primary alteration in the amino acid sequence of the C product. Thus C^- mutants, producing no biologically active C product, are *cis* and *trans* recessive to C^c and C^+. Through interaction with the *araI* site, activator produced by the C^c allele in the absence of L-arabinose and activator produced by the C^+ allele in the presence of L-arabinose "turns on" the L-arabinose structural genes *cis* to *araI*.

The existence of P1 and a complication to the model of pure positive control was indicated by the finding that, in the absence of inducer, C^+ is dominant to C^c. Apparently P1, produced by the *C* gene in the absence of the inducer, antagonizes the action of the activator produced by the C^c allele. There have been three hypotheses proposed to explain the dominance of C^+ to C^c. (1) P1 is a repressor and competes with P2 for attachment at the initiator site (Sheppard & Englesberg, 1966). (2) P1 is a repressor which attaches at a separate site, the operator, and circumvents the action of P2 (Sheppard & Englesberg, 1966). (3) Interaction between the possible subunits of P1 and P3 results in the production of an inactive molecule (Sheppard & Englesberg, 1967). In another paper (Englesberg, Squires & Meronk, 1969), evidence is presented showing that P1 acts as a true repressor with a separate site of attachment, the operator (*araO*), located between *araI* and the *araC* gene (see Fig. 1). This has recently been confirmed by Kessler & Englesberg (1969).

Several lines of evidence indicate that the controlling sites for this system, including *araI*, are located in the region between genes *araB* and *araC* (see Fig. 2). (1) Gene *araC* is not part of the *B,A,D* operon (Englesberg *et al.*, 1965; Sheppard & Englesberg,

1967). (2) Polarity is in the direction *B,A,D* (unpublished data of Katz & Englesberg, 1968 and Hogg & Englesberg, 1969). (3) Deletions that excise the region between *araB* and *araC* lead to an absolute pleiotropic negative, *cis* dominant, phenotype (Sheppard & Englesberg, 1967). (4) Deletions that end within the *B* gene and the leucine operon and thus excise the region between *araB* and *araC* remove the remaining structural genes in the L-arabinose *B,A,D* operon from the control by L-arabinose and gene *araC* (as demonstrated in heterogenotes) and place them under the control of the leucine regulator gene; whereas deletions that end within the *C* gene and the leucine operon do not affect the L-arabinose-gene *araC* control of the L-arabinose *B,A,D* operon (Kessler & Englesberg, 1969).

A model that has been most frequently proposed as an alternative to positive control is one based upon negative control modified by internal induction. According to this model, the arabinose operon is proposed to be actually under the negative control of a yet undiscovered repressor-forming regulatory gene (gene *R*) comparable to the *i* gene in the β-galactosidase system. Gene *C*, according to this model, is the structural gene for an enzyme that converts L-arabinose into the real inducer. This inducer reacts with the repressor produced by gene *R* and inactivates it. (Although there are a number of related negative control models different from one another by the function assigned to gene *araC*, they are all predicated upon the existence of a typical repressor-forming regulatory gene *R*.) Based upon this model, C^c mutants would provide an altered enzyme converting some internal metabolite into the inducer. Some mutants of this proposed regulatory gene that we might have been expected to find are R^- constitutives. It is argued that we have not searched hard enough for such mutants.

In this paper, we describe the isolation and characterization of 19 Ara^+ revertants of an Ara^- mutant strain, SB1094, containing a deletion (*Δ*719) that covers all known point mutations in the *araC* gene and produces, as a result, a pleiotropic negative phenotype. The 19 revertants contain the original deletion and a closely-linked secondary mutation mapping within the *araI* region of the L-arabinose operon and producing a *cis*-dominant constitutive phenotype characteristic of initiator constitutive mutants (I^c). The fact that none of the revertants maps in a hitherto undescribed repressor gene and that none has the characteristics of R^- constitutives suggest that no such regulatory gene exists. Therefore, we find no support for the negative control internal induction model. On the other hand, this type of reversion pattern (I^c's but no R^-'s) is what we would expect on the basis of the positive control model.

2. Materials and Methods

The media, strains (see Table 1), and general procedures for matings, the preparation and verifications of the genotype of merodiploids have been previously described (Sheppard & Englesberg, 1967).

The following abbreviations are used in media designation: M, mineral base; Ara, L-arabinose; Thr, L-threonine; Leu, L-leucine; Met, L-methionine; Glu, glucose; CH, casein hydrolysate; Str, streptomycin.

Transducing lysates of phage P1bt were prepared by the method of Gross & Englesberg (1959) as modified by Boyer, Englesberg & Weinberg (1962).

Transduction experiments were carried out as previously described (Gross & Englesberg, 1959).

Deletion 719 was originally isolated in Hfr 33 *araD139 his*$^-$ as a result of a spontaneous mutation producing resistance to the L-arabinose inhibition (strain SB1122) (Sheppard &

TABLE 1

List of strains

Strain	Mating type	Genotype					Origin, source or reference
		L-Arabinose	*thr1*	*leuB1*	*his*	*str*	
UP1001	F⁻	*ara*⁺	+	+	+	s	Gross & Englesberg (1959)
UP1004	F⁻	*ara*⁺	−	−	+	s	Gross & Englesberg (1959)
UP1005	F⁻	*ara*⁺	−	−	+	r	From UP1004 spontaneous mutations
UP1002	F⁻	*ara*⁺	+	−	+	s	From UP1004 by transduction
UP1089	F⁻	*A2*	−	−	+	s	Gross & Englesberg (1959)
UP1080	F⁻	*A2*	+	−	+	s	From UP1089 by transduction
UP1027	F⁻	*B24*	+	+	+	s	Gross & Englesberg (1959)
UP1010	F⁻	*C3*	+	+	+	s	Gross & Englesberg (1959)
UP1092	F⁻	*C5*	−	−	+	s	Gross & Englesberg (1959)
UP1082	F⁻	*C5*	+	−	+	s	From UP1092 by transduction
SB1122	Hfr33	*D139Δ719*	+	+	−	s	Sheppard & Englesberg (1967)
SB1094	F⁻	*Δ719*	−	+	+	r	SB1122 × UP1005, this paper
SB2000 to SB2018	F⁻	*I°1 Δ719* to *I°19 Δ719*	−	+	+	r	From SB1094, this paper
SB3139	F′	F′ *C19/C19*	+/+	+/+	+	s	Sheppard & Englesberg (1967)
SB3116	F′	F′ *C12/C12*	+/+	+/+	+	s	Sheppard & Englesberg (1967)
SB3141	F′	F′ *C101/C101*	+/+	+/+	+	s	Sheppard & Englesberg (1967)
SB3107	F′	F′ *B24/B24*	+/+	+/+	+	s	Sheppard & Englesberg (1967)
SB3101	F′	F′ *A2/A2*	+/+	+/+	+	s	Sheppard & Englesberg (1967)
SB1509	F⁻	*Δ1109*	+	+	+	r	Kessler & Englesberg (1969)
SB3550	F′	F′ *A2/Δ719*	+/−	+/+	+	r	SB3101 × SB1094, this paper
SB3538	F′	F′ *A2/C3*	+/+	+/+	+	s	Cross SB3101 × UP1010, this paper
SB3147	F′	F′ *A2 C3/A2 C3*	+/+	+/+	+	s	Negative segregants of SB3538
SB3551 to SB3569	F′	F′ *A2/I°1 Δ719* to F′ *A2/I°19 Δ719*	+/−	+/+	+	r	SB3101 × SB2000 to SB2018, this paper
SB3571 to SB3589	F′	F′ *A2 C3/I°1 Δ719* to F′ *A2 C3/I°19 Δ719*	+/−	+/+	+	r	SB3147 × SB2000 to SB2018, this paper
SB2149	F⁻	*A2 I°1 Δ719*	+	+	+	s	Phage P1bt (SB2000) × UP1080, this paper

TABLE 1 [*continued*]

List of strains

Strain	Mating type	Genotype					Origin, source or reference
		L-Arabinose	*thr1*	*leuB1*	*his*	*str*	
SB2151	F⁻	*A2 I^c13 Δ719*	+	+	+	s	Phage P1bt (SB2012) × UP1080, this paper
SB2152	F⁻	*A2 I^c14 Δ719*	+	+	+	s	Phage P1bt (SB2013) × UP1080,
SB2153	F⁻	*A2 I^c17 Δ719*	+	+	+	s	Phage P1bt (SB2016) × UP1080, this paper
SB2154	F⁻	*A2 I^c19 Δ719*	+	+	+	s	Phage P1bt (SB2018) × UP1080, this paper
SB5312	F⁻	*A2 I⁺ Δ719*	+	+	+	s	Phage P1bt (SB1094) × UP1080, this paper
SB3617	F′	F′ *B24/A2 I^c1 Δ719*	+/+	+/+	+	s	SB3107 × SB2149, this paper
SB3619	F′	F′ *B24/A2 I^c13 Δ719*	+/+	+/+	+	s	SB3107 × SB2151, this paper
SB3620	F′	F′ *B24/A2 I^c14 Δ719*	+/+	+/+	+	s	SB3107 × SB2152, this paper
SB3621	F′	F′ *B24/A2 I^c17 Δ719*	+/+	+/+	+	s	SB3107 × SB2153, this paper
SB3622	F′	F′ *B24/A2 I^c19 Δ719*	+/+	+/+	+	s	SB3107 × SB2154, this paper
SB3636	F′	F′ *B24/A2 I⁺ Δ719*	+/+	+/+	+	s	SB3107 × SB5312, this paper

Abbreviations used: ara, L-arabinose; *A*, structural gene for L-arabinose isomerase; *B*, structural gene for L-ribulokinase; *C*, regulator gene in the L-arabinose system; *D*, structural gene for L-ribulose 5-phosphate 4-epimerase; *Δ*, deletion; his, histidine; leu, leucine; thr, threonine; str, streptomycin; r, resistant; s, sensitive; +, ability to synthesize or utilize; −, inability to synthesize or utilize.

Englesberg, 1967). This deletion has been shown to encompass the region of the *C* gene defined by the outermost C^- mutants available at that time; *C12* (left end) and *C19* (right end) (Sheppard & Englesberg, 1967). Another C^- mutant site (*C101*) has subsequently been mapped to the left of *C12*. Deletion 719 fails to recombine with *C101*. Thus deletion 719 encompasses, at least, the entire *C* gene as defined by point mutations *C101* and *C19* (see Fig. 2). To isolate deletion 719 free of the *D139* marker, strain SB1122 was crossed to strain F^- *thr1 leuB1* str^r1 and selection was carried out on M–Glu–Thr–Str–agar plates. Among the $Leu^+His^+Str^r$ recombinants, a *thr1 araD*$^+$ *Δ*719 str^r recombinant (strain SB1094) was isolated and characterized by tests on appropriate media and by progeny tests with F′*ara*$^-$/*ara*$^-$ homogenotes. To ensure the isolation of independent revertants of SB1094, 20 tubes containing L-broth were inoculated with approximately 200 cells of this strain. The cultures were incubated at 37°C with shaking overnight. 3 ml. of each independent culture was then incubated with 0·3 ml. of diethyl sulfate at 37°C for 30 min. 0·1 ml. of the treated culture was then diluted into 5·0 ml. of fresh L-broth, grown overnight, and samples were plated on M–Ara–Thr–Met–agar plates and incubated at 37°C. (Methionine was added as a precaution, in certain of these experiments, since it was found that some strains carrying the *thr1* marker develop a requirement for methionine after prolonged storage as slant cultures. Subsequent experiments, however, indicated that such a precaution was unnecessary with the cultures used in these experiments.) No revertants were detected until approximately 6 days of incubation. 19 revertants, one from each independent culture, were isolated in pure culture by restreaking twice on homologous media and the cultures were subsequently stored on nutrient agar slants and also lyophilized. No revertants were detected on plates inoculated with untreated cultures (10^{10} total bacteria).

For the preparation of cell-free extracts, all cultures were grown at 37°C with shaking in a medium containing mineral salts (Sheppard & Englesberg, 1967), 1% casein hydrolysate (Difco) and 0·4% L-arabinose when required. In early experiments, as will be indicated, cells were harvested in the exponential phase of growth. Subsequently, stationary phase cultures were employed. The latter invariably yielded cell-free extracts with higher and more reproducible L-arabinose isomerase activity than obtainable with exponential phase cultures. Extracts were prepared as previously described with slight modifications (Cribbs & Englesberg, 1964).

L-Ribulokinase assays were performed as previously described (Englesberg *et al.*, 1965).

L-Arabinose isomerase activity was performed as previously described (Englesberg *et al.*, 1965) except that the enzyme assay was determined at 30°C instead of 37°C, since the reaction was found to be linear for longer periods of time at the lower temperature.

Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951), using crystalline bovine serum albumin (California Corp. for Biochemical Research) as a standard.

3. Results

General properties of the revertants

Nineteen independent diethyl sulfate-induced, arabinose-utilizing revertants of strain SB1094 carrying deletion 719 were isolated. All grew poorly on M–Thr–Ara or M–Thr–Met–Ara media, producing colonies approximately 1-mm in diameter in 48 hours at 37°C as compared to colonies 3-mm in diameter for the wild type. All had low constitutive levels of L-arabinose isomerase and L-ribulokinase, ranging from 1·5 to 8·7 and 0·4 to 1·8%, respectively, of the wild-type induced levels with exponentially grown cultures (Table 2). (Enzyme levels were higher in stationary phase cultures, Table 6.) There appears to be a general lack of co-ordination between isomerase and kinase levels as compared to that of the wild type. (For an explanation, see Discussion.) None of the revertants showed any significant increase in isomerase or kinase levels when subjected to conditions of induction with L-arabinose as the inducer. This low constitutive expression of the L-arabinose gene cluster though

TABLE 2

Enzymic characterization of I^c*Δ719 revertants*

Strain	Arabinose genotype	L-Arabinose isomerase		L-Ribulokinase	
		Non-induced	Induced	Non-induced	Induced
SB2000	*I*^{c}1 *Δ*719	1·8	1·6	0·17	0·14
SB2001	*I*^{c}2 *Δ*719	1·3	1·7	0·11	0·15
SB2002	*I*^{c}3 *Δ*719	1·1	2·1	0·06	0·14
SB2003	*I*^{c}4 *Δ*719	1·0	1·0	0·11	—
SB2004	*I*^{c}5 *Δ*719	2·1	3·5	0·24	0·23
SB2005	*I*^{c}6 *Δ*719	1·1	1·2	0·08	0·09
SB2006	*I*^{c}7 *Δ*719	2·9	4·0	0·18	0·24
SB2007	*I*^{c}8 *Δ*719	0·7	0·9	0·10	—
SB2008	*I*^{c}9 *Δ*719	1·7	2·4	0·12	0·18
SB2009	*I*^{c}10 *Δ*719	2·3	2·5	0·12	0·20
SB2010	*I*^{c}11 *Δ*719	1·0	1·1	0·07	0·08
SB2011	*I*^{c}12 *Δ*719	2·3	2·0	0·11	0·09
SB2012	*I*^{c}13 *Δ*719	0·5	0·6	—	—
SB2013	*I*^{c}14 *Δ*719	1·3	1·6	—	—
SB2014	*I*^{c}15 *Δ*719	0·8	0·4	0·10	—
SB2015	*I*^{c}16 *Δ*719	0·9	1·2	0·06	0·06
SB2016	*I*^{c}17 *Δ*719	1·6	1·1	0·07	0·09
SB2017	*I*^{c}18 *Δ*719	1·4	1·6	0·11	0·10
SB2018	*I*^{c}19 *Δ*719	0·8	0·9	0·14	—
SB1094	*I*$^+$ *Δ*719	0·10	0·17	0·008	<0·01
UP1001	*I*$^+$*C*$^+$ (wild type)	0·08	33·4	0·03	13·4

Cell-free extracts were prepared from cells in the exponential phase of growth. Enzyme activity is expressed in μmoles of product formed/hr/mg protein.

refractory to induction is apparently sufficient to permit the slow growth observed with these mutants.

To avoid the confusion of a duplicate nomenclature, we shall refer to the revertant mutant sites now as *I*^{c} (initiator constitutive) and will justify this later. For convenience, the combined phenotype of *I*^{c}*Δ*719, characterized by slow growth with L-arabinose as the carbon source, will be indicated by the symbol Ara$^{+\,s1}$, in contrast to the wild-type L-arabinose phenotype, designated as Ara$^+$ and the L-arabinose non-utilizing phenotype as Ara$^-$.

(i) *Presence of the deletion*

When each of the revertants (strains SB2000 to SB2118) were crossed with F′*araC19*/*araC19* and F′*araC101*/*araC101* homogenotes, each carrying an *ara*$^-$ mutant site that mapped at either end of the *C* gene, no wild-type Ara$^+$ recombinants were detected. However, when F′*araA*$^-$/*araA*$^-$ and F′*araB*$^-$/*araB*$^-$ homogenotes were used as donors, wild-type Ara$^+$ recombinants were obtained in each case. Thus it is evident that these revertant strains still contained a deletion encompassing known point mutations in the *C* gene characteristic of the deletion in the parent strain SB1094.

(ii) *Mapping of the revertant mutant sites*

Cross I. P1bt transducing phage, prepared with each of the revertant strains SB2000 to SB2018 and the parental deletion strain SB1094, were used as donors in

TABLE 3

Cross I I^c*Δ719* (*donor*) × leuB1 (*recipient*)

Donor Strain	Donor Arabinose genotype	Selected Leu$^+$ analyzed	Unselected Ara^{+s1} among Leu$^+$†	Leu$^+$Ara^{+s1} Leu$^+$ (%)	Unselected Ara$^-$ among Leu$^+$‡	Leu$^+$Ara$^-$ Leu$^+$ (%)
SB2000	*I*^{c}1 *Δ719*	150	79	52	1	0·67
SB2001	*I*^{c}2 *Δ719*	333	171	52	3	0·90
SB2002	*I*^{c}3 *Δ719*	444	248	56	1	0·23
SB2003	*I*^{c}4 *Δ719*	270	144	53	1	0·37
SB2004	*I*^{c}5 *Δ719*	590	319	54	4	0·68
SB2005	*I*^{c}6 *Δ719*	299	154	52	1	0·34
SB2006	*I*^{c}7 *Δ719*	298	168	56	1	0·34
SB2007	*I*^{c}8 *Δ719*	323	175	54	2	0·62
SB2008	*I*^{c}9 *Δ719*	303	172	57	2	0·66
SB2009	*I*^{c}10 *Δ719*	168	95	57	1	0·60
SB2010	*I*^{c}11 *Δ719*	298	161	54	2	0·67
SB2011	*I*^{c}12 *Δ719*	407	227	56	1	0·24
SB2012	*I*^{c}13 *Δ719*	300	169	56	1	0·33
SB2013	*I*^{c}14 *Δ719*	555	311	56	5	9·90
SB2014	*I*^{c}15 *Δ719*	70	37	45	1	0·14
SB2015	*I*^{c}16 *Δ719*	300	189	63	1	0·33
SB2016	*I*^{c}17 *Δ719*	740	416	56	5	0·68
SB2017	*I*^{c}18 *Δ719*	586	311	53	5	0·85
SB2018	*I*^{c}19 *Δ719*	554	313	56	1	0·18
SB1094	*Δ719*	120	0	—	76	63

Phage P1bt was grown for two cycles on each of the revertants of strain SB1094, *Δ719*. The complete genotype of the revertants, strains SB2000 to SB2018, is *thr*$^-$*I*^{c}1–19*Δ719* *str*^{r}. The phage were used in transduction experiments with UP1002 *leuB1* as the recipient. Selection was for Leu$^+$ on M–Glu–agar. Leu$^+$ transductants were then picked to M–Glu–agar (20/plate). The plates were incubated overnight at 37°C and replica plated on to M–Ara–agar and as a control on to M–Glu–agar. In some crosses threonine and threonine and methionine were included in the medium. This had little effect on the results. (*thrI* cotransduces only about 2% of the time with *leuB1* (Gross & Englesberg, 1959).) As controls, phage were grown on the original deletion mutant, strain SB1094, and crossed to *leuB1*. Leu$^+$ transductants were selected and screened in the same manner as described above. In addition *leuB1* was plated on M–Glu–agar without phage to detect the presence of spontaneous Leu$^+$ revertants. No such revertants were observed.

† The phenotype of *I*^{c}*Δ719* mutants is Ara^{+s1} (slow growth on agar medium with L-arabinose as carbon source; in 48 hr at 37°C, colony size is approximately 1 mm in diameter as compared to 3mm for the wild type). In replica plating to M–Ara from a patch of cells on M–Glu it is a simple matter to distinguish between Ara$^+$ (wild type), Ara^{+s1} (slow grower) and Ara$^-$ (no growth). In 48 hr Ara$^+$ produces a heavy patch; Ara^{+s1}, a light but definite patch, and Ara$^-$, no patch (no growth) at all. The unselected Ara^{+s1} clones scored therefore represent the *I*^{c}*CΔ719* genotypes in this experiment. Several of these transductants from each cross were analyzed by progeny tests by crossing them to F′ *ara*$^-$ homogenotes. In each case the presence of *Δ719* was confirmed.

‡ The phenotype of *Δ719* is Ara$^-$ (no growth on agar medium with L-arabinose as carbon source). All Ara$^-$ transductants were analyzed by progeny tests against F′ Ara$^-$ homogenotes. In every case they were shown to contain the original deletion *Δ719*.

crosses with *leuB1* (strain UP1002) as the recipient (Table 3). We selected for Leu$^+$ and analyzed these recombinants to determine the L-arabinose phenotype. Among the Leu$^+$ recombinants, three different L-arabinose phenotypes were obtained; Ara$^+$, Ara^{+s1} and Ara$^-$. Several Ara^{+s1} colonies from each cross and all L-arabinose negative clones were purified and shown by progeny tests, as previously described, to contain the deletion *Δ719*. The frequency of co-transduction of *I*^{c}*Δ719* with the leucine marker, *i.e.* Ara^{+s1}Leu$^+$, is approximately 50% (the differences observed

are probably not significant) and is similar to that obtained in the control cross, *Δ719* × *leuB1*, for co-transduction of the parental deletion 719 with the leucine marker (Ara⁻Leu⁺). The low frequency of Leu⁺Ara⁻ recombinants in the experimental crosses is probably due to a rare crossover event resulting in the transfer of the original deletion 719 to the Ara⁺Leu⁻ recipient cell. (Although I^cC^+ recombinants probably occur in this population, they are probably not distinguishable from wild-type recombinants on arabinose agar plates. This conclusion is based upon the findings (see below) that merodiploids of the type $F'A2C^+/A^+I^cΔ719$ have inducible levels of L-arabinose isomerase close to fully induced wild-type cells. In addition, a strain in which I^c marker has been isolated in an otherwise wild-type L-arabinose genetic background ($A^+B^+I^c13C^+$) is indistinguishable from wild-type colonies on mineral L-arabinose agar plates.) It is clear from this evidence that the I^c mutations in strain SB1094, carrying the deletion 719 and producing the Ara^{+s1} phenotype, are separable mutational events from the deletion mutation itself. Furthermore, since the frequency of Ara⁻ among the Leu⁺ transductants is very low, the I^c mutations are closely linked to the deletion 719 or to the *leuB1* locus.

Cross II. In a second series of crosses the same donor phages prepared on each of

TABLE 4

Cross II $I^cΔ719$ (*donor*) × C5 leuB1 (*recipient*)

Donor		Total Ara^{+s1} analyzed	$Ara^{+s1}Leu^+$	$\frac{Ara^{+s1}Leu^+}{Ara^{+s1}} \times 100$
Strain	Arabinose genotype			
SB2000	I^c1 Δ719	280	172	61
SB2001	I^c2 Δ719	280	165	59
SB2002	I^c3 Δ719	350	233	67
SB2003	I^c4 Δ719	279	193	69
SB2004	I^c5 Δ719	140	86	61
SB2005	I^c6 Δ719	140	97	69
SB2006	I^c7 Δ719	349	236	68
SB2007	I^c8 Δ719	350	233	67
SB2008	I^c9 Δ719	140	102	73
SB2009	I^c10 Δ719	350	185	53
SB2010	I^c11 Δ719	350	254	73
SB2011	I^c12 Δ719	131	83	63
SB2012	I^c13 Δ719	350	246	70
SB2013	I^c14 Δ719	140	68	49
SB2014	I^c15 Δ719	350	239	68
SB2015	I^c16 Δ719	140	73	52
SB2016	I^c17 Δ719	140	78	56
SB2017	I^c18 Δ719	140	85	61
SB2018	I^c19 Δ719	140	75	54

Phage P1bt as used in Cross I was employed in transduction experiments with *araC5 leuB1* as the recipient. 0·1 ml. of a 1/10 dilution of each of the transducing mixtures and the culture of *araC5 leuB1* without phage were plated in duplicate on M–Thr–Ara–agar plates to select for arabinose utilizing colonies. After 6 days incubation at 37°C Ara^{+s1} transductants were then picked onto homologous media (20/plate) and subsequently replica plated on to M–Glu–Thr–agar and M–Glu–Leu–Thr–agar as a plating control. The number (1 to 2/plate) of Ara^+ (large colonies) appearing in 48 hr were approximately the same in all cases including that of the control cross (donor *Δ719 leuB1* × *araC5 leuB1* recipient) and on plates seeded with the *C5 leuB1* recipient strain alone and probably are revertants of *araC5*. A few small colonies, on the average 5/ml., appeared in 6 days on these control plates.

the revertant strains were crossed to *araC5 leuB1* as the recipient (Table 4). In this case selection was for arabinose-utilizing recombinants on M–Ara–Thr–Leu agar plates. The number of Ara$^+$ (wild type, large colony types) found (on the average 1/plate) were the same as on the control plates containing the cross, deletion 719 × *araC5 leuB1*, and on plates containing just the recipient and are therefore probably the result of spontaneous reversion of the *araC5* mutation. The remainder, slow growing revertant-type recombinants (Ara$^{+\,sl}$), were observed after six days of incubation in each of these crosses, at an average frequency of approximately 2×10^4/ml. plated as compared to 5/ml. with the control described above. (*I*^{c} *araC5* recombinants probably yield an Ara$^{+\,sl}$ phenotype.) These recombinants were picked to homologous medium and replica-plated onto M–Glu–Thr–Agar to determine their leucine phenotype. The percentage of the Ara$^{+\,sl}$ that were Leu$^+$ in each of the crosses varied from 49 to 73. (Because of the relatively small numbers assayed, these differences are probably not significant.) These frequencies of co-transduction of *I*^{c}*Δ*719 with *leuB1* are in close agreement with the previous observations of Gross & Englesberg (1959).

The results of the crosses, with *araC5 leuB1* as recipient, demonstrate that the revertant mutant sites must be closely linked to the deletion itself and thus to the *C* gene since there is a high frequency of segregation of the Leu character among the *I*^{c}*Δ*719 transductants.

Cross III. In a third series of crosses the same donor phages from ten of the revertants were crossed with the Ara$^-$ strain SB1509 containing the large deletion 1109 (Table 5). This deletion encompasses a genetic region extending from *araC101*, the mutant site closest to the *B* gene, to and including genes in the leucine operon (Kessler & Englesberg, 1969), and is phenotypically Ara$^-$Leu$^-$. Arabinose-utilizing recombinants were selected on M–Ara–Leu–agar medium. Only the slow growing type of arabinose-utilizing clones (Ara$^{+\,sl}$) were observed in these crosses. These

TABLE 5

Cross III I^c*Δ719 (donor)* × *Δ1109*

Strain	Donor Arabinose genotype	Selected Ara$^{+\,sl}$ analyzed	Unselected Leu$^-$ among Ara$^{+\,sl}$	$\frac{\text{Ara}^{+\,sl}\text{Leu}^-}{\text{Ara}^{+\,sl}}$ (%)	Spontaneous reversion frequency†
SB2000	*I*^{c}1 *Δ*719	1000	1	0·1	<0·02
SB2001	*I*^{c}2 *Δ*719	995	0	<0·10	<0·02
SB2002	*I*^{c}3 *Δ*719	477	2	0·42	<0·1
SB2005	*I*^{c}6 *Δ*719	914	3	0·33	<0·02
SB2008	*I*^{c}9 *Δ*719	1000	4	0·40	<0·02
SB2009	*I*^{c}10 *Δ*719	1000	6	0·60	<0·02
SB2012	*I*^{c}13 *Δ*719	1000	4	0·4	0·02
SB2013	*I*^{c}14 *Δ*719	1000	4	0·4	<0·02
SB2016	*I*^{c}17 *Δ*719	1000	5	0·5	0·08
SB2018	*I*^{c}19 *Δ*719	1000	3	0·3	0·06

Phage P1bt as used in Cross I was employed in transduction experiment with strain SB1509 containing deletion 1109, as described in Table 2. Only Ara$^{+\,sl}$ transductants were found in these crosses.

† The spontaneous reversion $= \dfrac{\text{Ara}^{+\,sl} \text{ revertants of } \Delta 1109 \text{ (control)}}{\text{Ara}^{+\,sl} \text{ (transductants)}} \times 100$

recombinants were picked to homologous medium and replica-plated to score for Leu$^-$. In nine out of the ten crosses, Leu$^-$ types were found at a very low frequency ranging from 0·1 to 0·6%. In the cross with $I^c2\Delta719$, no Leu$^-$ recombinants were isolated. The Ara$^{+\,sl}$Leu$^-$ recombinants were isolated in pure culture and verified by progeny testing with F'*araC*$^-$/*C*$^-$ homogenotes to contain a deletion that encompasses the *C* gene and were shown to be not revertible to Leu$^+$. This evidence indicates, therefore, that these recombinants contain the original deletion of the recipient used in these crosses.

Cross II has established that the revertant mutant sites were closely linked to the *araC* gene. The fact that we were able to cross, in nine out of ten cases, the revertant mutant sites into strain SB1509 containing deletion 1109, indicates that these revertant mutant sites must lie to the left of deletion 1109 and therefore to the left of the *araC* gene; that is, the side of the *C* gene closest to gene *araB*. In the cross with $I^c2\Delta719$, we presume that I^c2 lies less than 0·1% recombination units from the deletion 1109. The fact that all 19 revertants produce a similar phenotype, that the mutant sites are closely linked to *araC* and that nine have been shown to map to the left of the *C* gene (as defined by deletion 1109), forms the basis for placing all 19 mutant sites within the initiator region of the L-arabinose complex located between genes *araB* and *araC*.

(iii) *Complementation analysis of the revertants*

Cis-*dominance test.* We initially constructed merodiploids of the type F'$A2I^+C^+$/$A^+I^c\Delta719$ for each of the $I^c\Delta719$ revertants plus, as a control, merodiploid F'$A2I^+C^+$/$A^+I^+\Delta719$, and analyzed these merodiploids and their related F$^-$ haploid strains for L-arabinose isomerase activity. If the constitutive levels of isomerase produced by the revertants $I^c\Delta719$ were the result of mutation in the initiator region (*araI*), one would expect that the isomerase levels of the merodiploids would be similar to those found with the respective haploid $I^c\Delta719$ strains; *i.e.* I^c should be *cis*-dominant to I^+.

Our experiments demonstrated that in a few cases the isomerase levels of the merodiploids were the same but, in a majority of cases, the levels were higher than those of the F$^-$ haploid strains (Table 6). The control merodiploid, F'$A2I^+C^+$/$A^+I^+\Delta719$, however, also showed a significant increase in isomerase activity over that of the F$^-A^+I^+\Delta719$ haploid. Because of this increased isomerase activity in the control merodiploid, we considered the possibility that such increases occurring in the merodiploids might obscure a *trans*-dominant effect of I^+ on I^c. Therefore, before we can fully assess the significance of these complementation analyses, it is necessary for us to understand the cause of these increases in isomerase activity and attempt to eliminate this effect, so as to uncover a possible cryptic *trans* effect of I^+.

We do know that this unexpected increase in non-induced isomerase levels is not the result of recombination and segregation in the merodiploid cultures. First of all, if by a recombination event, the episome in the control merodiploid now carried $A^+I^+C^+$, even if there were several copies of the episome per nucleus, this could not explain the increase in basal level of isomerase from 0·1 to 3·4 units, since the wild-type strain has a basal level of isomerase of only 0·1 unit. Similarly, in the experimental set of merodiploids, to explain the large increases in constitutive isomerase levels, one might suppose that a recombination event might have occurred producing

TABLE 6

L-Arabinose isomerase activity of merodiploids of the type F′ A2I^+C$^+$/A$^+$I^c*Δ719 and* F′ A2I^+C3/A$^+$I^c*Δ719*

Endogenote	haploid	L-Arabinose isomerase Non-induced diploid exogenote F′ *A2I$^+$C$^+$*	F′ *A2I$^+$C3*	Induced diploid exogenote F′ *A2I$^+$C$^+$*
I^{c}1 Δ719	5·2	6·8(2)		56
I^{c}2 Δ719	3·5	3·9		44
I^{c}3 Δ719	3·6	12	4·5	54
I^{c}4 Δ719	4·0	7·4(2)	3·9	44
I^{c}5 Δ719	5·8	11	4·1(3)	47
I^{c}6 Δ719	3·6	4·6	4·1(2)	52
I^{c}7 Δ719	4·8	9·8(2)		51
I^{c}8 Δ719	2·8	16	3·6	52
I^{c}9 Δ719	2·9	4·3	3·9	39
I^{c}10 Δ719	2·0	7·4		53
I^{c}11 Δ719	5·5	17		55
I^{c}12 Δ719	2·5	2·9	2·6	62
I^{c}13 Δ719	3·6	5·6	4·9	50
I^{c}14 Δ719	3·5	7·5		57
I^{c}15 Δ719	3·2	6·6		69
I^{c}16 Δ719	3·2	4·0(3)	4·2	57
I^{c}17 Δ719	3·5	5·2	4·0	80
I^{c}18 Δ719	2·1	4·7		42
I^{c}19 Δ719	2·4	4·6	3·4	63
A$^+$I$^+$ Δ719	0·1	3·4(4)	0·1	49
WT	0·08(28·8)†			
A2 I$^+$C$^+$	0·1			

Cell-free extracts were prepared from cells in the stationary phase of growth. Enzyme activity is expressed in μmoles of product formed/hr/μg protein. The haploid F$^-$ strains were initially analyzed as two groups with *I^{c}10Δ719* present in each group. There was no significant difference in the isomerase levels of *I^{c}10Δ719* in each run. In analyzing the non-induced merodiploids, usually the corresponding F$^-$ haploid strains were run in conjunction with the corresponding merodiploid and the isomerase levels were normalized to the isomerase levels of the F$^-$ haploid strain as initially determined in the group run of F$^-$ strains. In most cases the correction was a minor one. The analysis of the induced merodiploid was performed in 4 batches (*I$^+$Δ719*, *I^{c}1Δ719* to *I^{c}4Δ719*; *I^{c}5Δ719* to *I^{c}9Δ719*; *I^{c}10Δ719* to *I^{c}14Δ719*; *I^{c}15Δ719* to *I^{c}19Δ719*) together with the corresponding haploid strains. The isomerase values listed for the former are uncorrected. The figure in parenthesis indicates the number of independent analyses performed.

† Induced L-arabinose isomerase activity.

an episome of the genotype *A$^+$I^{c}C$^+$*. This, however, is a very unlikely event since, as we have shown, *I^c* is very closely linked to deletion 719. Besides, as shown by Englesberg *et al.* (1969), the *C$^+$* allele in this case (no deletion 719 in a *cis* position) would have an epistatic effect on the function of the *I^c* allele. In any case, by an analysis of the merodiploid cultures used for the preparation of enzyme extracts for L-arabinose isomerase activity, we have been able to rule out an explanation based upon genetic recombination. Each of the ten Ara$^+$ clones, isolated on eosin–methylene blue–Ara, from each culture, was shown to segregate Ara$^-$ progeny which were *A2C$^+$*.

To explore this phenomenon further, a second series of merodiploids was constructed and analyzed in which a C^- allele was substituted for the C^+ allele in the exogenote (F′$A2I^+C3/A^+I^c\Delta719$ as well as a control merodiploid, F′$A2I^+C3/A^+I^+\Delta719$) (Table 6). The L-arabinose isomerase levels of these merodiploids were determined and compared with those of the corresponding F$^-I^c\Delta719$ strains and F$^-I^+\Delta719$. In all cases the isomerase levels of the merodiploids containing the $A^-I^+C^-$ alleles in the exogenote were similar to the isomerase levels of the respective F$^-$ haploid strains. Thus the increases in isomerase activity found with some merodiploids of the type F′$A2I^+C^+/A^+I^c\Delta719$ and with merodiploid F′$A2I^+C^+/A^+I^+\Delta719$ are due to the product of the C^+ allele produced in the absence of the inducer. (See Discussion for explanation of this effect.) By eliminating this effect of the C^+ allele we have established, unambiguously, in the 11 cases tested, that I^+ has no effect on the constitutive expression of *araA cis* to I^c. Thus I^c is dominant to I^+.

It will be noted that the merodiploid F′$A2I^+C^+/A^+I^+\Delta719$ is hyperinducible in the presence of L-arabinose; the isomerase activity is nearly twice that of the fully-induced wild type. Merodiploids of the type $A2I^+C^+/A^+I^c\Delta719$ are in all cases also hyperinducible. The induced levels found are higher than those of the fully induced wild type and in some cases higher than those found for the control merodiploid $A2I^+C^+/A^+I^+\Delta719$. An explanation of differences in basal and induced levels of isomerase, found for the various I^c in merodiploids of the type F′$A2I^+C^+/A^+I^c\Delta719$, will be presented in the Discussion.

Cis–trans *dominance test*. The *cis* dominance of I^c to I^+ is characteristic of mutations at a controlling site producing a constitutive phenotype. If this were the case, the I^c alleles should have no *trans* effect. To test for a *trans* effect, we constructed and analyzed merodiploids of the type F′$A^+B^-I^+C^+/A2B^+I^c\Delta719$ for five of the I^c mutations, and as a control, merodiploid, F′$A^+B^-I^+C^+/A^-B^+I^+\Delta719$. Non-induced and induced (in some cases) isomerase and kinase activity was determined for each of the merodiploids and the appropriate haploid strains. In all five cases, there was no demonstrable *trans* effect of the I^c alleles (Table 7). For instance, F$^-A^+B^+I^c\Delta719$ has a constitutive level of isomerase equal to 4·36. When *A2*, a mutation in the *araA* structural gene, was introduced into this strain, isomerase activity was reduced to less than 0·01 unit. This F$^-$ strain still carries the constitutive marker as evidenced by its kinase activity. F$^-A^+B24I^+C^+$ has a basal isomerase level of 0·35 unit. If the I^c allele has a *trans* effect, the *araA* gene in the episome should be activated and the merodiploid would be expected to have, at a minimum, approximately 4 units of isomerase activity. A basal level of 0.37 unit of isomerase was obtained with the control merodiploid (no I^c allele), as would be predicted. A value of 0·43 was obtained with the experimental merodiploid (F′$A^+B24I^+C^+/A^-2B^+I^c1\Delta719$), a value not significantly different from 0·37. The constitutive production of kinase in the merodiploid is evidence for the presence of a functional I^c allele. Essentially similar results were obtained for each of the I^c mutants tested. Besides the enzymic analysis, the genotype of each merodiploid culture employed in the preparation of cell-free extracts was verified. Crossover and segregation was negligible in the experiments recorded. Therefore with the five I^c alleles tested, I^c is found to be *trans* recessive to I^+.

It will be noted that kinase activity did not increase in the merodiploids containing the I^c allele in the endogenote, while there was a small but significant increase in kinase activity in the control merodiploid containing the I^+ allele in the endogenote. On the basis of the demonstrated co-ordinate expression of the structural genes

TABLE 7

L-Arabinose isomerase and L-ribulokinase
Activity of merodiploids of the type F′ A⁺B24I⁺C⁺/A2B⁺I^c*Δ719*

Strain	Arabinose genotype	Non-induced		Induced	
		Kinase	Isomerase	Kinase	Isomerase
SB3617	F′ *A⁺B24I⁺C⁺/A2 B⁺I^{c}1 Δ719*	0·52	0·43	11	88
SB2149	F⁻ *A2B⁺I^{c}1 Δ719*	0·55	0·01		
SB2000	F⁻ *A⁺B⁺I^{c}1 Δ719*	0·38	4·36		
SB3619	F⁻ *A⁺B24I⁺C⁺/A2B⁺I^{c}13 Δ719*	0·64	0·72	10	73
SB2151	F⁻ *A2B⁺I^{c}13 Δ719*	0·61	0·01		
SB2012	F⁻ *A⁺B⁺I^{c}13 Δ719*	0·35	3·52		
SB3620	F′ *A⁺B24I⁺C⁺/A2B⁺I^{c}14 Δ719*	0·58	0·36	12	94
SB2152	F⁻ *A2B⁺I^{c}14 Δ719*	0·61	0·01		
SB2013	F⁻ *A⁺B⁺I^{c}14 Δ719*	0·38	5·60		
SB3621	F′ *A⁺B24I⁺C⁺/A2B⁺I^{c}17 Δ719*	0·46	0·44	8·9	99
SB2153	F⁻ *A2B⁺I^{c}17 Δ719*	0·46	0·01		
SB2016	F⁻ *A⁺B⁺I^{c}17 Δ719*	0·61	4·63		
SB3622	F′ *A⁺B24I⁺C⁺/A2B⁺I^{c}19 Δ719*	0·38	0·35	7·6	79
SB2154	F⁻ *A2B⁺I^{c}19 Δ719*	0·49	0·02		
SB2018	F⁻ *A⁺B⁺I^{c}19 Δ719*	0·41	4·86		
SB3636	F′ *A⁺B24 I⁺C⁺/A2B⁺I⁺ Δ719*	0·17	0·37	9·7	78
SB5312	F⁻ *A2B⁺I⁺ Δ719*	0·01	0·01		
SB1094	F⁻ *A⁺B⁺I⁺ Δ719*	0·01	0·16		
UP1001	F⁻ *A⁺B⁺I⁺C⁺*	0·01	0·08	7·2	19
UP1027	F⁻ *A⁺B24C⁺*	0·01	0·35		

See Table 2 for explanations.

araB, *araA*, *araD* (Englesberg *et al.*, 1965), one would have expected similar results to those obtained with the isomerase. We do not fully understand this discrepancy. It is possible that the absence of a co-ordinate increase in kinase activity may be a reflection of increased instability of the kinase molecule at low concentrations, in the absence of substrate as a result of the I^c mutations. This possible instability of the kinase in I^c mutants is indicated by the finding that the ratio of kinase activity to isomerase activity in the F⁻ strains of the type $A^+B^+I^c\Delta719$ is about three to five times less than the comparable ratio in the induced wild type.

4. Discussion

(a) *The nature of* I^c *mutants*

From an Ara⁻ strain of *Escherichia coli* containing a deletion that excises most, if not all of gene *araC*, 19 independent revertants have been isolated. All the revertants were shown to be the result of a secondary mutation (I^c) closely linked to but separable from the deletion itself and all possess the characteristics required of mutations in the initiator region of the L-arabinose operon.

(1) They produce a *cis*-dominant constitutive phenotype, and all those tested showed no *trans* effect.

(2) All 19 revertant mutant sites map within the initiator region of the L-arabinose operon. Three-factor transduction crosses, with phage previously grown on each of the revertants and with *leuB1* and *araC5 leuB1* as recipients, have established that each of the 19 revertants map within the *ara leu* region of the chromosome and are closely linked to and separable from the *araC* segment of the chromosome. In nine out of ten cases, it was possible to cross, at low frequency, the I^c mutant sites into a strain containing deletion 1109, a deletion extending from *araC* to the leucine operon. This placed nine I^c mutant sites to the left of the deletion, and thus to the left of *araC*, in the region proposed to contain the initiator site for the L-arabinose operon. We presume that the exceptional site (I^c2) is also to the left of the *C* gene but so close to deletion 1109 that, in the number of transductants analyzed, we failed to pick up an $I^c\Delta1109$ recombinant. Because of the close linkage of all 19 mutant sites to deletion 719 and the similarity of their phenotypes, it seems likely that all 19 mutant sites reside within the same region.

(3) Several of the revertants that have the same level of L-arabinose isomerase activity in the haploid state (in the presence or absence of the inducer) have significantly different hyperinducible levels of this enzyme in merodiploids containing a C^+ allele in the *trans* position. Thus in these cases, the I^c mutation, besides permitting expression of the structural genes *cis* to it in the absence of a *C* gene product, has altered the sensitivity of the operon to the *C* gene product. Thus, by definition, these mutations must have occurred in the initiator region of the operon.

(b) *A single regulatory gene for the* ara-OIBAD *operon*

We have been unable to isolate any revertants of a *C* gene deletion which have the properties of mutations in a proposed repressor gene (*R*). In both the *lac* and *gal* systems, where negative control has been established by genetic evidence, mutations to constitutivity in the repressor gene occurs at a relatively high frequency (Willson *et al.*, 1964; Shapiro, 1967). It is expected that, at least, nonsense mutants and deletions would abolish the activity of a repressor gene. The failure to find any such mutants for the Ara system down to a *spontaneous* frequency of less than 1×10^{-10} is a strong indication that no such repressor gene exists. The possibility, however, is not completely ruled out by these studies, since it is conceivable that such a gene does exist but either is essential to bacterial growth or is present in duplicate copies.

Other properties of the *ara* operon and the *araC* gene, as indicated in the discussion below, suggest strongly that the *C* gene product directly interacts with the *ara* operon-controlling elements. Thus it is unlikely that the *C* gene functions as proposed in the negative control internal induction model.

(c) *Modified positive control model*

The increase in L-arabinose isomerase activity in merodiploid $A^-I^+C^+/A^+I^+\Delta719$ and with most $I^c\Delta719$ mutants in merodiploids of the type $A^-I^+C^+/A^+I^c\Delta719$, in the absence of the inducer, over that of the corresponding F^- haploid strains has been examined in greater detail by Englesberg *et al.* (1969). We have shown in this article that this increase is the result of a *trans*-acting C^+ allele on the activity of the *araA* gene *cis* to the deletion, since the substitution of a C^- allele in the merodiploids described above does away with this increase in isomerase activity. Thus a product

formed by the C^+ allele (in the absence of inducer) is responsible for this phenomenon. The fact that the isomerase level in merodiploid $A^-I^+C^+/A^+I^+\Delta 719$ is approximately 34 times higher than the basal level of the wild type ($A^+I^+C^+$) and that there is no *trans* effect (compare isomerase and kinase levels of UP1027, SB5312 and SB3636 in Table 7 and corresponding data in Table 6) suggests that deletion 719 must have a significant role in this increased expression of the *cis araA* gene. This is in fact shown to be the case, since no such increase in basal or constitutive levels of isomerase is found when deletion 766 (a deletion whose left end terminates within the *C* gene between two C^- point mutants) is used in place of deletion 719 (Englesberg *et al.*, 1969). In fact, with merodiploids of the type $A^-I^+C^+/A^+I^c\Delta 766$, the C^+ allele (but not the C^- allele) on the episome severely depresses the constitutive expression of the *araA* gene *cis* to the I^c mutations.

To explain these results, Englesberg *et al.* (1969) have proposed a modification of the simplified model for positive control, as described in the Introduction. They propose that (1) P1, the initial product of the *araC* gene, is a repressor with a site of attachment, the operator, located between *araI* and *araC*; (2) P1 is in equilibrium with P2, the activator, and with P1 and P2 attached to their respective controlling sites *araO*, the operator, and *araI*, the initiator; (3) L-arabinose, the inducer, removes P1 from *araO* and shifts the equilibrium to P2. P2 acts at *araI* and thereby stimulates

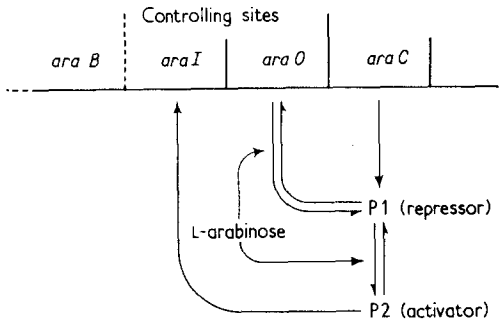

FIG. 1. Positive control model.

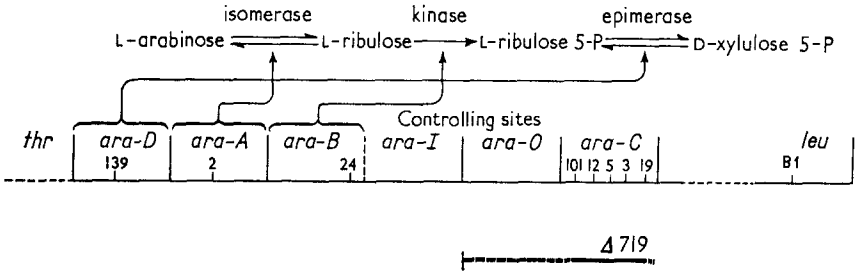

FIG. 2. The L-arabinose gene–enzyme complex.

Structural genes: *araB*, L-ribulokinase; *araA*, L-arabinose isomerase; *araD*, L-ribulose 5-phosphate 4-epimerase.

Controlling sites: *araI*, initiator site—this is the position of I^c mutations and the site for activator (P2) function. *araO*, operator site—this is the site for repressor (P1) function.

The numbers indicate the mutants used in this study.

Deletions—solid lines indicate the portion of the genome excised by the deletion as determined by genetic mapping with F′ Ara⁻ homogenotes. The dashed portion of the lines extending the deletion are based upon complementation, and enzymic analysis.

the expression of the operon. It is necessary both for P2 to be present at *araI* and for P1 to be removed from *araO* for full expression of the operon to occur (Fig. 1). With the exception of a more precise positioning of the operator, this model is essentially the one previously proposed as a possibility to explain the dominance of C^+ to C^c (Sheppard & Englesberg, 1967).

According to this model, the increase in isomerase levels in merodiploids of the type $A^-I^+C^+/A^+I^+\Delta 719$ over that of $F^-A^+I^+\Delta 719$ is explained on the basis that deletion 719 excises all or part of the operator site (Fig. 2). In the absence of a functional operator site, the amount of P2 existing in equilibrium with P1 is able to partially turn on the expression of the structural genes *cis* to the deleted operator. Depending upon how the I^c mutation has modified the initiator site, the amount of P2 present in the absence of inducer may or may not further activate the structural genes *cis* to $I^c\Delta 719$. However, in the presence of inducer, there is sufficient P2 to hyperinduce the operon in all the I^c mutants analyzed, although the I^c mutations appear to govern the extent of hyperinducibility.

(d) *Elimination of other explanations for the properties of the* C *gene*

The properties of regulatory mutants in the maltose (Schwartz, 1967) and rhamnose (Power, 1967) systems indicate that the structural genes for these pathways are also under positive control. Schwartz (1967) has proposed other hypotheses as alternatives to positive control to explain these properties. In terms of the *ara* operon these would be (1) that the supposed positive control gene *araC* actually produces an enzyme that converts L-arabinose into a true inducer (this we have eliminated: see Discussion above); (2) that the *araC* gene produces a protein subunit required for the activity of the other enzymes in the *ara* operon; and (3) that *araC* is a structural gene for a component of the L-arabinose permease system.

Both of these alternatives have been eliminated in the L-arabinose system. First of all it has been shown that it is possible to obtain high levels of L-arabinose isomerase activity, similar to that obtainable in the wild type, in mutants containing an *araB-leu* deletion; *i.e.* a deletion that cuts out *araI*, *araO*, and *araC* and fuses the L-arabinose isomerase structural gene (*araA*) to the leucine operon. Thus it is unlikely that gene *araC* provides a required polypeptide chain for the L-arabinose isomerase (Kessler & Englesberg, 1969). (There are also many other cogent arguments against this model.) Second, it has been recently shown that C^- mutants do have L-arabinose permease activity (Englesberg, unpublished results), although lower than that of a reference *araA* strain. Thus although there is evidence that the *C* gene controls permease activity (see also Englesberg *et al.*, 1965), certain C^- mutants that are recessive to C^+ and C^c alleles are still able to concentrate L-arabinose internally.

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