THE L-ARABINOSE OPERON IN ESCHERICHIA COLI B/r: A GENETIC DEMONSTRATION OF TWO FUNCTIONAL STATES OF THE PRODUCT OF A REGULATOR GENE*

By Ellis Englesberg, Craig Squires, and Frank Meronk, Jr.

DEPARTMENT OF BIOLOGICAL SCIENCES, UNIVERSITY OF CALIFORNIA (SANTA BARBARA)

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Abstract.—The product of the regulator gene *araC* in the L-arabinose gene complex exists in two functional states: P1, the repressor, and P2, the activator, presumably in equilibrium with each other, and with P1 and P2 attached to their respective controlling sites, *araO*, the operator, and *araI*, the initiator. The controlling sites are linked in the following order with respect to genes *araB* and *araC: BIOC*. Two *C* gene deletions (Δ719 and Δ766) serve to define the newly described *araO* site, and to place it adjacent to the left end of the *C* gene. We have suggested that deletion 719 deletes, in addition to the *C* gene, part or all of the *araO* site. Deletion 766 leaves the *araO* site intact. Complementation analysis employing stable merodiploids indicates that the repressor-operator site function is epistatic over the activator-initiator site function. It is necessary both for activator (P2) to be present and for repressor (P1) to be absent at their respective controlling sites (*araI* and *araO*) for full expression of the L-arabinose operon.

The L-arabinose gene–enzyme complex in *Escherichia coli* B/r has been shown to consist of structural genes *araD*, *araA*, *araB*, controlling site *araI* (the initiator), and regulatory gene *araC*, linked in that order between the markers *thr* and *leu* and the unlinked gene, *araE*, concerned with the active transport of L-arabinose, with its corresponding controlling sites.[1–3] Several lines of evidence have been presented indicating that gene *araC* controls in a positive fashion the expression of the structural genes in this system. A detailed analysis of this evidence is presented in another publication.[4] Briefly, it has been shown that deletions[1–3] and nonsense mutations[5] of the *C* gene lead to the production of a pleiotropic-negative phenotype (C^-) which is recessive (*cis* and *trans*) to both the wild-type (C^+) and constitutive (C^c) alleles of that gene. The findings that unlinked suppressors restore activity of C^- mutants and that some revertants of these C^- mutants form a thermolabile *C* gene product indicate that the activator is at least partially a protein molecule.[5] No support has been found for the existence of another L-arabinose regulatory gene producing a repressor specific for the L-arabinose system.[4, 5] Several lines of evidence indicate that the controlling sites are located in the region between genes *B* and *C:* (1) polarity is in the direction *BAD*;[6, 7] (2) gene *araC* is not part of the *BAD* operon, since nonsense and deletion mutations in the *araC* gene[1–3, 5] and deletion mutations that end within the *araC* gene and the *leu* operon do not affect the L-arabinose-gene *araC* control of the L-arabinose operon, while deletion mutations that end within the *araB* gene and the *leu* operon remove the remaining structural genes of the operon from the control of L-arabinose and gene *araC*

(as demonstrated in merodiploids) and place them under the control of the *leu* regulator gene;[8] and (3)initiator constitutive mutants (I^c) map within this region and produce low-constitutive, *cis*-dominant phenotypes (I^c is dominant to I^+ with no *trans* effect) in a genetic background lacking an active *C* gene.[4]

The I^c mutants were isolated as revertants of the Ara$^-$ deletion mutant (Δ)719, a deletion encompassing all known *ara*$^-$ mutant sites in the regulator gene, *araC*.[4] The revertants contain the original deletion plus the secondary I^c mutation. In the complementation analysis with merodiploids of the type F′A2I$^+$C$^+$/A$^+$I^{c}Δ719 and F′A2I$^+$C$^+$/A$^+$I$^+$Δ719, it was observed that the constitutive L-arabinose isomerase levels (see legend, Fig. 2) of most of the former and the basal level of the latter were significantly higher than those found for the corresponding F$^-$ haploid strains. When a C^- allele was substituted for the C^+ in the exogenote (F′A2C3), the L-arabinose isomerase levels were reduced to those characteristic of the respective F$^-$ haploid strains. Thus, it appears that the product of the C^+ allele, in the absence of inducer, does affect an increase in expression of the *araA* gene, the structural gene for the L-arabinose isomerase, *cis* to Δ719.

In the model for positive control,[1] it was proposed that the initial product of the regulator gene, *araC*, is an allosteric protein (P1) that is converted by inducer into P2, the activator, which reacts at the initiator site and "turns on" gene expression. The existence of P1 was first indicated by the demonstrated dominance of C^+ to C^c.[1-3] It was suggested that P1 might be a true repressor and that P1 competes with P2 for the same functional site, *araI*, or has a separate site of attachment and thus prevents the expression of the operon in the presence of the constitutive activator.[1-3]

In this paper, we compare the results of complementation experiments performed with strains containing Δ719 as compared with those performed with Δ766, a deletion whose left end terminates within the *C* gene. We present evidence that (1) the *C* gene product, P1, produced in the absence of inducer, does exist as a true repressor with a specific site of attachment, the operator (*araO*), and is presumed to be in equilibrium with P2, the activator, and P1 and P2 attached to their respective controlling sites *araO* and *araI*; (2) the order of controlling sites in reference to genes *araB* and *araC* is *araB*, *araI*, *araO*, *araC*; and (3) that the repressor-operator site function is epistatic over the activator-initiator site function. It is necessary both for P2 to be present and for P1 to be absent at their respective controlling sites for full expression of the L-arabinose operon. The stimulation of the expression of gene *araA cis* to Δ719 in merodiploids containing a *trans* C^+ allele is explained on the basis that Δ719 extends into the region between *araC* and *araB* and excises all or a portion of *araO*. Thus, this strand is sensitized to the small amounts of P2 produced by the C^+ allele in the *trans* position that would otherwise have remained cryptic.

Materials and Methods.—With the exceptions given below, the materials and methods employed in the experiments described in this paper are essentially the same as previously described.[4] The strains used are described in Table 1.

The isolation and characterization of deletion 719 has already been presented.[4] Deletion 766 was originally isolated in strain SB5088 F$^-$ *thr*$^-$ *D*139 *str*^{r} as a result of

a spontaneous mutation making the strain resistant to the L-arabinose inhibition.[1, 3, 9] To isolate the deletion free of the *D*139 marker, phage P1bt grown on the wild type was crossed to strain SB1064 F⁻ *thr*⁻ *D*139Δ766 *str*ʳ and selection was for Thr⁺ on mineral-glucose agar plates. Thr⁺ transductants were scored by matings with F′ Ara⁻ homogenotes for the *D*139 marker and for the deletion. Transductants of the genotype *thr*⁺ *araD*⁺Δ766 *str*ʳ were isolated in pure culture and a nonlysogenic strain (SB1114) was employed in this study. The extent of deletion 766 was determined by matings with pertinent F′ Ara⁻ homogenotes. Strain SB1114 failed to yield any Ara⁺ recombinants with F′ *araC*19/*araC*19; F′*araC*3/*araC*3; F′*araC*5/*araC*5; but did yield Ara⁺ recombinants with F′*araC*12/*araC*12; F′*araC*101/*C*101; and F′*araB*27/*araB*27. This places the left end of 766 within the *C* gene between mutant sites *araC*5 and *araC*12.

TABLE 1. *List of strains.*

Strain	Genotype: Mating type	Genotype: L-arabinose	Genotype: thr1	Genotype: leuB1	Genotype: str	Origin, source or reference
SB5088	F⁻	*D*139	−	+	*r*	1, 3, 9
SB1064	F⁻	*D*139Δ766	−	+	*r*	Spontaneous mutation
SB1114	F⁻	Δ766	+	+	*r*	From SB1064 by transduction (this paper)
SB1094	F⁻	Δ719	−	+	*r*	4
SB2012	F⁻	*I*ᶜ13Δ719	−	+	*r*	4
SB2018	F⁻	*I*ᶜ19Δ719	−	+	*r*	4
SB5316	F⁻	*I*ᶜ13Δ766	+	+	*r*	By transduction (this paper)
SB5317	F⁻	*I*ᶜ19Δ766	+	+	*r*	By transduction (this paper)
SB1509	F⁻	Δ1109	+	−	*r*	8
SB3101	F′	F′*A*2/*A*2	+/+	+/+	*s*	3
SB3107	F′	F′*B*24/*B*24	+/+	+/+	*s*	3
SB3114	F′	F′*C*3/*C*3	+/+	+/+	*s*	3
SB3115	F′	F′*C*5/*C*5	+/+	+/+	*s*	3
SB3116	F′	F′*C*12/*C*12	+/+	+/+	*s*	3
SB3129	F′	F′*B*27/*B*27	+/+	+/+	*s*	3
SB3139	F′	F′*C*19/*C*19	+/+	+/+	*s*	3
SB3141	F′	F′*C*101/*C*101	+/+	+/+	*s*	3
SB3147	F′	F′*A*2C3/*A*2C3	+/+	+/+	*s*	4
UP1010	F⁻	*C*3	+	+	*s*	10
UP1009	F⁻	*A*2	+	+	*s*	10
UP1029	F⁻	*B*26	+	+	*s*	10
SB5161	F⁻	*C*101	+	+	*s*	4

Symbols and abbreviations: *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; leu, leucine; thr, threonine; str, streptomycin; *r*, resistant; *s*, sensitive; plus, ability to synthesize or utilize; minus, inability to synthesize or utilize.

*I*ᶜ13 and *I*ᶜ19 mutant sites[4] were crossed into F⁻Δ766 (SB1114) in two steps. First, phage P1bt grown on strains SB2012 (*I*ᶜ13) and SB2018 (*I*ᶜ19) was used to infect F⁻Δ1109 (SB1509) (a deletion of *araC* extending into the leucine operon[8]). We selected Ara⁺ (slow-growing) transductants and scored for those that were Leu⁻, indicating transfer of the *I*ᶜ mutation to Δ1109.[4] Second, phage P1bt was grown on the two *I*ᶜΔ1109 derivatives and used to infect F⁻Δ766 (SB1114). We selected for transfer of the *I*ᶜ's on mineral-arabinose agar without leucine. The only progeny that could grow received the *I*ᶜ-mutation from the phage while retaining the *leu*⁺ (and thus the linked Δ766) of the recipient strain. The two *I*ᶜΔ766 strains isolated SB5316 (*I*ᶜ13) and SB5317 (*I*ᶜ19) grew slowly on mineral-arabinose agar and showed the recombination pattern of Δ766 when cross-streaked against F′*C*⁻ homogenotes.

Construction of merodiploids: Matings were carried out as previously described.[3] In crosses between strain SB3101 and strains SB1094, SB2012, SB2018, SB1114, SB5316, and SB5317, the mating mixture was streaked out on eosin methylene blue (EMB)

L-arabinose agar plates. In a normal mating, an appreciable fraction of the population is Ara$^+$. Ara$^+$ clones are isolated and their genotype determined by noting the segregation of negative clones and picking several positive colonies (usually ten) from each positive clone to L-broth and demonstrating the segregation of Ara$^-$ clones (note: $I^c\Delta 719$ and $I^c\Delta 766$ appear Ara$^-$ on EMB plates) containing either *araA*2 and $\Delta 719$ or $\Delta 766$. The latter was determined by cross-streaking against the following homogenotes on mineral L-arabinose threonine agar plates: (strain SB3101) F'*A*2, (strain SB3107) F'*B*24, (strain SB3141) F'*C*101, and (strain SB3139)F'*C*19. The I^c character was scored by the typical slow growth on mineral-arabinose agar plates. This character is always associated with the deletion.

In crosses between F'*A2C*3 homogenote (SB3147) and SB1094, SB2012, SB2018, SB1114, SB5316, and SB5317, contraselection of the F' donor occurred on mineral-glucose-streptomycin plates. Individual clones were isolated and tested for transfer of the episome by cross-streaking against appropriate F$^-$ haploid strains F$^-$*A*2, F$^-$*B*26, F$^-$*C*3, and F$^-$101. Merodiploids of the type F'*A2C*3/*A*$^+$*I*$^+\Delta 766$ and F'*A2C*3/*A*$^+$*I*$^+\Delta 719$ do not grow on mineral arabinose and when cross-streaked against F$^-$*A*2, yield a small number of Ara$^+$ recombinants as a result of episomal mobilization of the *A*$^+$ allele on the chromosome. When cross-streaked against F$^-$*B*26, a heavy complementation reaction is evident, whereas a completely negative response is elicited with F$^-$*C*3. When cross-streaked against F$^-$*C*101, a small number of recombinants is apparent as a result of recombination between *araC*3 and *araC*101.

Merodiploids of the type F'*A2C*3/*A*$^+$*I*$^c\Delta 766$ and F'*A2C*3/*A*$^+$*I*$^c\Delta 719$ were tested as described above and behaved in a similar manner except that slow growth on mineral arabinose due to the I^c allele was apparent in each case, verifying the presence of the I^c marker.

In the preparation of cell-free extracts in the case of the F'*A2C*$^+/\Delta 766$ (I^c or I^+) or F'*A2C*$^+/\Delta 719$ (I^c or I^+) merodiploids, a sample of the cells employed in the preparation of the extracts was streaked on EMB L-arabinose and scored for Ara$^-$ segregants. In addition, usually ten Ara$^+$ clones were analyzed as described above for the identity of Ara$^-$ segregants. With merodiploid cultures of the type *A*$^-$*C*$^-/\Delta 766$ and *A*$^-$*C*$^-/\Delta 719$, besides cross-streaking the cultures against the F$^-$ strains listed above, ten clones isolated on nutrient agar were tested in a similar manner. The enzymatic data reported here are the result of experiments in which segregation was 20% or less and in which the genotype of the exogenote and endogenote was verified as present in at least eight out of ten of the tested clones.

Results.—A comparison of the trans effect of C$^+$ and C$^-$ with mutants containing deletions 719 and 766: As shown in Table 2, and as previously demonstrated,[4] as a result of the expression of the *araA* gene *cis* to deletion 719, there is a large constitutive increase (35×) in L-arabinose isomerase activity in a merodiploid containing *A*$^-$*C*$^+$ in the exogenote, over the isomerase activity of the original haploid F$^-$ deletion strain or a merodiploid containing *A*$^-$*C*$^-$ in the exogenote. These experiments demonstrate that it is the product of the *C*$^+$ allele (in the absence of the inducer) that causes the increased expression of gene *araA cis*

TABLE 2. *A comparison of the trans effect of C$^+$ and C$^-$ on mutants containing deletions 719 and 766.*

	Endogenote	
	A$^+$*I*$^+\Delta 719$	*A*$^+$*I*$^+\Delta 766$
Exogenote	L-Arabinose Isomerase*	
None (haploid)	0.10	0.09
F' *A2C*$^+$	3.53	0.20
F' *A2C*3	0.12	0.13

* L-Arabinose isomerase activity is expressed as μmoles of ribulose formed per hour per milligram of protein.

to deletion 719. When similar experiments were conducted with a strain containing deletion 766, the left end of which terminates within the *C* gene, there was a twofold increase in isomerase activity in the merodiploid containing the C^+ allele over that of the F^- haploid deletion strain, or a merodiploid containing a C^- allele in the exogenote. Thus the deletions modify the effect of the *C* gene product.

A comparison of the trans effect of C^+ and C^- on the constitutive expression of I^c alleles in strains containing deletion 719 and deletion 766: There is a striking difference in the effect that the C^+ allele has on the constitutive expression of I^c alleles in strains containing deletions 766 and 719. In merodiploids $F'A2I^+C^+/A^+I^c13\Delta719$ and $F'A2I^+C^+/A^+I^c19\Delta719$, there is an increase in constitutive isomerase activity over that found in the F^- haploid strains. This increase in isomerase activity disappears if a C^- allele is substituted for the C^+ allele in the exogenote (Tables 3 and 4). These results are similar to those previously reported.[4] This is a further demonstration of the *cis* dominance of I^c to I^+ and the role of the product of the C^+ allele in stimulating the expression of the structural genes *cis* to deletion 719, as observed in the merodiploids described above.

In contrast to the results with deletion 719, with similar merodiploids constructed with deletion 766 ($F'A2I^+C^+/A^+I^c13\Delta766$, $F'A2I^+C^+/A^+I^c19\Delta766$), there is a severe repression of the expression of the I^c alleles. This repression of constitutive synthesis of isomerase by the *araA* gene *cis* to the deletion is removed when a C^- allele is substituted for the C^+ allele in the exogenote. Thus, in merodiploids containing deletion 766, there is a severe epistatic effect of the C^+ allele on the expression of the isomerase gene *cis* to the deletion. As with deletion 719, with merodiploids containing the C^- allele in the exogenote, the *cis* dominance of I^c to I^+ is demonstrated.

Discussion.—The phenotypic expression of gene *araA*, the structural gene for L-arabinose isomerase, is affected differently by a C^+ allele in a *trans* position, depending upon whether the *araA* gene is linked to deletion 719 or 766. The

TABLE 3. *A comparison of the trans effect of C^+ on I^c in mutants containing deletions 719 and 766.*

	Endogenote	
	$A^+I^c13\Delta719$	$A^+I^c13\Delta766$
Exogenote	L-Arabinose Isomerase*	
None (haploid)	4.0	6.9
$F'A2I^+C^+$	6.2	0.93
$F'A2I^+C3$	4.9	4.4

* See Table 2 for L-arabinose isomerase activity of $F^-A^+I^+\Delta719$ and $F^-A^+I^+\Delta766$.

TABLE 4. *A comparison of the trans effect of C^+ on I^c in mutants containing deletions 719 and 766.*

	Endogenote	
	$A^+I^c19\Delta719$	$A^+I^c19\Delta766$
Exogenote	L-Arabinose Isomerase*	
None (haploid)	4.5	5.0
$F'A2I^+C^+$	9.1	0.75
$F'A2I^+C3$	3.4	3.3

* See Table 2 for L-arabinose isomerase activity of $F^-A^+I^+\Delta719$ and $F^-A^+I^+\Delta766$.

mapping of these two deletions indicates that the left end of deletion 766 terminates within the *C* gene between mutant sites *araC*5 and *araC*12. On the other hand, deletion 719 covers all known point mutant sites in gene *araC*. This leaves the termination of the left end of Δ719 undetermined, somewhere between *araB*27 and *araC*101. Mutants containing either deletion fail to recombine with *araC*19, the *ara*$^-$ mutant site farthest to the right in the *C* gene, and are both Leu$^+$. This places the right end of both deletions somewhere between *araC*19 and the leucine operon. It has been shown that it is possible to delete the entire region from *araC* to the leucine operon and, except for the leucine requirement, the phenotype characteristic of such deletion mutants with respect to the L-arabinose operon *BAD* is identical to that of *araC*$^-$-point mutants.[8] Therefore, since there is no evidence to indicate that a difference in the termination point of the right arm of these deletions, if there were any, would make any difference in our analysis of the effect of these deletions on the function of this *ara* operon, at this point we choose to ignore the possibility.

A modified model for positive control designed to explain the experimental data presented in this paper is shown in Figure 1. In essence, this model is similar to one proposed earlier in which the dominance of C^+ to C^c was explained

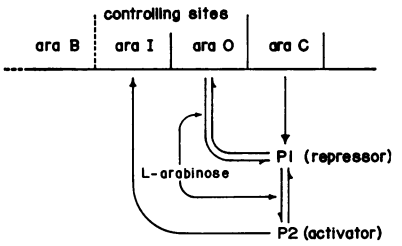

FIG. 1.—Positive control model.

on the basis that P1, the product of the *C* gene produced in the absence of inducer, acts as a true repressor with a separate site of action, the operator. It differs in that we can now more precisely define and locate the operator site. To review the model briefly, it is proposed that *araC* is a regulator gene which produces, in the absence of inducer, a repressor, P1, which is at least partially a protein molecule. P1 exists in equilibrium with P1 attached to an operator site (O) located between *araC* and *araI* (the initiator site), P2 (the activator), and P2 attached to *araI*. The inducer removes P1 from the operator[11, 12] and shifts the equilibrium to P2. P2 reacts at the initiator site and turns on the expression of the structural genes *cis* to the initiator. The basal level of expression of the L-arabinose operon *OIBAD* probably is determined by the relative effective concentrations of P1 and P2 and their respective affinities for *araO* and *araI*. In this positive control system, it is not sufficient to remove P1 from the operator in order to achieve the expression of the structural genes concerned. P2 must be produced and react at *I* to stimulate the expression of these genes.[1, 3] This model now provides a logical explanation for the dominance of C^+ to C^c and the different effects of C^+ on the expression of structural genes *cis* to I^cΔ719

and $I^c\Delta 766$ if we assume in the latter case that deletion 719 terminates outside of the *C* gene and encompasses *araO* but leaves intact *araI*. Experiments have shown that deletion 766 does terminate within the *C* gene and therefore must leave intact the postulated *araO* site (Fig. 2).

The repressive epistatic effect of the C^+ allele on the constitutive expression of the I^c alleles in strains containing deletion 766 and the absence of such a repressive effect on the I^c alleles *cis* to deletion 719 can now be understood on the basis that P1 produced by the C^+ allele (in the absence of inducer) reacts with the operator site *cis* to deletion 766 and prevents the complete expression of the I^c alleles. Such a repressive effect does not occur in strains containing deletion 719 because this deletion excises the operator site. The large increase in isomerase activity in the merodiploid of $F^-I^+\Delta 719$ containing a *trans* C^+ allele as compared to only a minor increase with a similar merodiploid of $F^-I^+\Delta 766$ is also consistent with the model. Strains containing $I^+\Delta 719$, lacking an operator

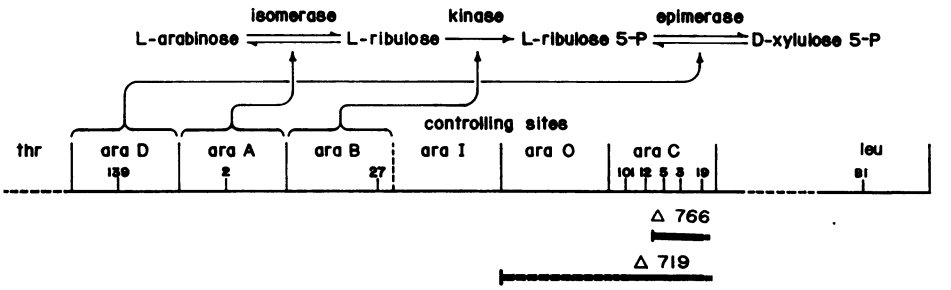

FIG. 2.—The L-arabinose gene-enzyme complex.

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 employed 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 portions of the lines extending the deletion are based upon complementation and enzymatic analysis.

region, are able to detect the small quantities of P2 (activator) that exist in equilibrium with P1. This small quantity of P2 is able to partially "turn on" the expression of the genes *cis* to this deletion. No large increase in isomerase activity would be expected with the strain containing $I^+\Delta 766$ with the operator and initiator sites both present.

The simplest explanation of the dominance of C^+ to $C^{c\,1,\,3}$ in the light of the evidence that we have presented in this paper is that the C^+ allele produces a repressor which attaches to the operator site on both the exogenote and the endogenote and prevents the constitutive activator from functioning. The presence of P1 at the operator site takes precedence over the action of the constitutive activator at the initiator site.

Our evidence therefore indicates that the *C* gene product has a dual function. In the presence of an operator site and in the absence of the inducer, the *C* gene product is mainly present in the configurational state of a functional repressor

but with detectable amounts of activator present that probably represent another configurational state of the molecule. In the presence of inducer and an initiator site, repressor function is no longer detectable and full activation of the operon occurs probably as a result of a shift in equilibrium of the *C* product to the activator configuration.

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