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Arabinose-Leucine Deletion Mutants of *Escherichia coli* B/r

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The control of *ara* gene expression was studied in mutants of *Escherichia coli* B/r containing deletions which fused the L-arabinose gene complex with the leucine operon (the normal gene order being *araDABIOC . . . leuDCBAO*). Complementation experiments with stable merodiploids showed that expression of *ara* genes *cis* to *araC-leu* deletions was controlled by the *trans*-acting product of the *araC* gene. Expression of *ara* genes *cis* to *araB-leu* deletions was under leucine control. These studies confirm the existence of a region between genes *araC* and *araB* essential for normal activator controlled expression of the *ara* structural genes. One deletion was characterized as an *araO-leu* deletion. Its effect on *ara* gene expression was unique in that *ara* genes were susceptible to potential regulation by both L-arabinose and leucine. These experiments suggest that two different species of messenger ribonucleic acid (mRNA) may be produced for the *ara-leu* region as a result of this deletion. One, under L-arabinose-activator control, is initiated in the L-arabinose region; the other, under leucine control, is initiated in the leucine region. The latter indicates that *araI* can be transcribed. Whether *araI* is transcribed in the former instance (mRNA made under activator control) remains to be established.

The L-arabinose gene complex in *Escherichia coli* B/r consists of structural genes, *araD*, *araA*, *araB*, controlling sites, initiator (*araI*) and operator (*araO*), and a regulatory gene, *araC*, linked in that order between markers *thr* and *leu* (Fig. 1), as well as an unlinked permease gene, *araE*, with presumably its controlling sites (8, 10–12, 14, 21, 31, 32; E. Englesberg et al., Proc. Nat. Acad. Sci. U.S.A., *in press*; and E. Englesberg et al., J. Mol. Biol., *in press*). [The term controlling sites is used here as defined by Epstein and Beckwith (13). The control of *araE* gene expression by the *araC* gene product will be covered in another paper.] Evidence has been presented that gene *araC* exerts a positive control over the L-arabinose structural genes B, A, and D (12, 31, 32; E. Englesberg et al., Proc. Nat. Acad. Sci. U.S.A., *in press*). The essential aspect of this positive control system is that the expression of the structural genes is normally "turned off" and that the presence of a specific cytoplasmic regulatory gene product is necessary for structural gene expression. This type of control is readily distinguished from a negative control system, i.e., the lactose operon, in which structural genes are normally "turned on" in the absence of a regulatory gene product (18). Inducible and repressible systems may be under positive or negative control, depending upon the state of the regulator.

The model for positive control of the L-arabinose operon, as originally proposed (12) and further developed (31, 32; E. Englesberg et al., Proc. Nat. Acad. Sci. U.S.A., *in press*; E. Englesberg et al., J. Mol. Biol., *in press*), is represented in Fig. 2. This model states that gene *araC* is a regulatory gene that produces a protein molecule, P1, a repressor which exists in equilibrium with P2, the activator, P2 attached to the initiator (P2-I), and P1 attached to the operator (P1-O), with the equilibrium being mainly in the direction of P1-O. Under those conditions, P2 is unable to evoke a significant expression of the structural genes *araBAD*. L-Arabinose, the inducer in this system, removes P1 from the operator site. P2, acting at the initiator (*araI*), stimulates expression of the structural genes. (It has not been specified whether activator acts at the level of transcription or translation.) It must be emphasized that the "absence" of P1 is a necessary, but not sufficient, condition for the expression of the structural genes. This implies that the structural genes are normally turned off at the initiator controlling site. P2 must be produced from P1 and act at *araI* to bring about the expression of this gene com-

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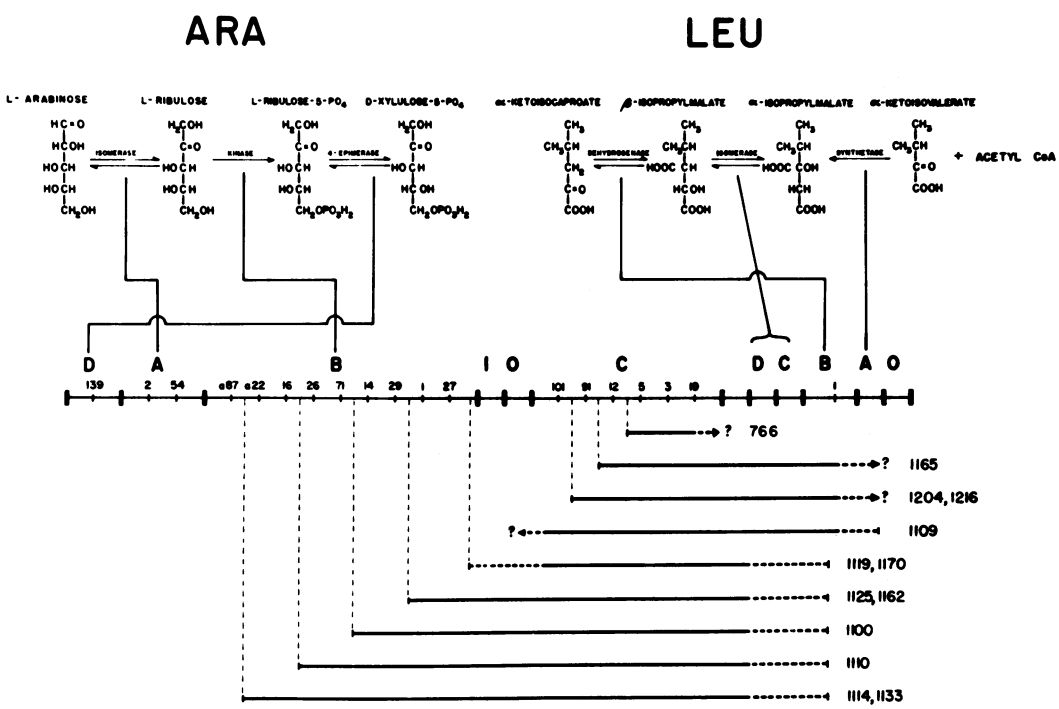

FIG. 1. *Genetic map of the* L-*arabinose and leucine regions in E. coli B/r. The genes coding for the indicated enzymes necessary for the catabolism of* L-*arabinose and the synthesis of α-ketoisocaproate are specified by a capital letter (9). In addition, C is the regulatory gene for the* L-*arabinose operon; O is the operator, the site for attachment of repressor; and I is the initiator, the site at which activator functions. Mutant sites are defined by a number. The solid horizontal lines indicate the region encompassed by the deletions, as determined by crosses with F' ara⁻ homogenotes and Hfr33 carrying the leuB1 mutation. The dashed lines extending the deletions are based on complementation and derepression studies.*

plex. The *ara* promoter, a genetic element defined as the site of initiation of messenger ribonucleic acid (mRNA) synthesis (17, 19), lies between *araC* and *araB*. Whether *araI* and the promoter are separate structural elements remains to be determined. The evidence supporting this model is presented elsewhere at length (12, 14, 15, 21, 31, 32; E. Englesberg et al., Proc. Nat. Acad. Sci. U.S.A., *in press*; E. Englesberg et al., J. Mol. Biol., *in press*).

In recent studies, it has been shown that it is possible to fuse the lactose operon to another operon by various interoperon deletions (2, 20). These deletions placed the lactose structural genes under the control of the effector and regulatory gene of the other operon, as predicted by the operon model. We decided to initiate a similar study with the intent of establishing the effect that interoperon deletions might have on the expression of the structural genes in the L-arabinose system. Such a study might yield further information regarding the nature of the controlling elements of the L-arabinose operon.

Deletions joining the L-arabinose region with

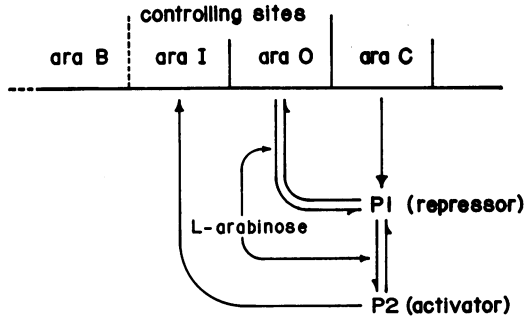

FIG. 2. *Model for positive control of the* L-*arabinose operon.*

the leucine operon (*ara-leu* deletions) seem to provide a suitable test system. The *ara* gene order with respect to the leucine region in *E. coli* B/r is *araDABIOC. . . leu* (11, 12, 14, 32; E. Englesberg et al., Proc. Nat. Acad. Sci. U. S. A., *in press*; E. Englesberg et al., J. Mol. Biol., *in press*). In *Salmonella typhimurium*, the *leu* gene order with respect to the L-arabinose region is *ara . . . leu-DCBAO* (23). Assuming that the *leu* gene order

in *E. coli* B/r is the same as that in *S. typhimurium*, then the spatial order of the controlling elements relative to the structural genes would be the same in both the arabinose and leucine regions; thus, functional *ara-leu* deletions might be found.

This paper reports the isolation and the genetic and physiological characterization of some *ara-leu* deletion mutants of *E. coli* B/r. A preliminary report of this work has already been presented (D. P. Kessler and E. Englesberg, Bacteriol. Proc., p. 50, 1967).

MATERIALS AND METHODS

Media. L broth, mineral base, and complex eosin methylene blue L-arabinose agar (EMBara) have been described (32). The mineral base was supplemented with 0.4% glycerol (Gly), 0.2% glucose (G), 0.2% L-arabinose (A), 0.004% L-leucine (L), 0.008% DL-threonine (T), and 1.5% Difco agar as required. Streptomycin was employed at 300 μg/ml. In the chemostat experiments, a single component of the GlyTL medium was limited at either 0.0012% L-leucine, 0.0024% DL-threonine, or 0.085% glycerol.

Bacterial strains. Some of the strains used in this study are given in Table 1. The *leu*⁻ mutation (14) carried in strains SB5000, SB5078, SB3002, and UP1142 abolishes the activity of β-isopropylmalate dehydrogenase (Umbarger, *personal communication*) and is here designated as *leuB1*. A number of F′ Ara⁻ homogenotes (32), carrying different *araA*, *araB*, and *araC* mutations, were used in genetic mapping experiments (see Fig. 1 for some of the mutant sites employed). Mutants containing deletions were isolated in strain SB5003. The *araD139* mutation in several deletion mutants was removed by cotransduction with the *thr* marker by use of phage P1bt grown on the wild-

TABLE 1. *List of strains*[a]

Strain	Sex	Genotype	Source or reference	Use
SB5000	F⁻	*leuB1 D-ara-5*[b]	N. Lee	As recipient in transduction experiment to construct SB5002
SB5112	F⁻	*thr araD139*	Englesberg et al. (11)	Donor in transduction experiment to construct SB5002
SB5002	F⁻	*thr araD139 D-ara-5*	P1bt (SB5112) × SB5000	To construct SB5003
SB5003	F⁻	*thr araD139 D-ara-5 str*^{r}*2*	Spontaneous mutant of SB5002	Parental strain of *ara-leu* deletion mutants
SB5010	F⁻	*thr*	P1bt (UP1001) × UP1142	Recipient in transduction experiments (Table 3)
SB5078	F⁻	*araC101 leuB1*	E. Englesberg, *unpublished data*	Donor in transduction experiment (Table 3)
SB5021	F⁻	*thr araD139 D-ara-5 str*^{r}*2 leu-6*	Spontaneous mutant of SB5003	Recipient in transduction experiments to construct Δ*ara-leu leu6* doubles (Table 6)
SB3002	Hfr	*leuB1 his*	Originally from Boyer (3); same origin as HfrB1, 0..try..gal.. leu	Genetic mapping experiments
UP1001	F⁻	Wild type	Gross and Englesberg (14)	Donor in transduction experiments to construct SB5010 and the *thr*$^+$ *araD*$^+$ derivatives of the *ara-leu* deletion mutants
UP1142	F⁻	*thr araA54 leuB1*	Cribbs and Englesberg (8)	Recipient in transduction experiment to construct SB5010
SB1085	F⁻	Δ*766 str*^{r}	Englesberg, Squires, and Meronk[c]	Donor in transduction experiments (Table 8)
SB2078	F⁻	Δ*766 ara*^{c}*1*	Englesberg, Squires, and Meronk[c]	Donor in transduction experiments (Table 7)

[a] Deletion indicated by Δ.

[b] The *D-ara-5* marker (mutation in the D-ribulokinase structural gene) is not pertinent to this study and further reference to it in the text will be omitted.

[c] Proc. Nat. Acad. Sci. U.S.A., *in press*.

type strain UP1001. In all strain constructions involving deletion mutants, the presence of the deletion was verified by mating experiments with the appropriate Ara⁻ homogenotes. Stable merodiploids (Tables 4 and 5) were constructed as previously described (32). Streptomycin was added to the EMBara agar to counterselect against the Ara⁻ homogenote.

Isolation and screening of L-arabinose-resistant mutants. The growth of L-ribulose 5-phosphate 4-epimerase-deficient strains (*araD*⁻ mutants) is inhibited by L-arabinose (11). Ara⁻ mutants resistant to this inhibition contain, in addition to the *araD* mutation, a mutation in either the *araA*, *araB*, or *araC* gene (4) or, less frequently, deletions of various lengths in the *ara* region (32). (In this paper the L-arabinose-resistant phenotype in *araD*⁻ mutants will be designated as Ara^r.) The likelihood of a class of Ara^r mutants that would contain an *ara-leu* deletion depends on the chance that the deoxyribonucleic acid (DNA) excised would not specify a vital (noncorrectable) function. They would have an Ara⁻ Ara^r phenotype plus a requirement for at least leucine.

A series of L-broth cultures of SB5003, inoculated with approximately 100 cells and incubated overnight, were streaked once across an EMBara plate; 10 subcultures were streaked per plate. The plates were incubated for 48 hr at 37 C. Since some growth of the parental strain occurs on this complex medium, Ara^r mutants arising from a single streak could have independent origin. Therefore, 5 to 15 Ara⁻ Ara^r colonies per streak were transferred to GT, GTL, and EMBara agar plates to screen them for a leucine or some other auxotrophic requirement, or both. Care was exercised in picking only the resistant colonies growing out from the original streak so as not to transfer the parental type which would mask any auxotrophic response. The Ara⁻ Ara^r auxotrophic mutants were purified on EMB agar, retested for the requirement, and transferred to nutrient agar slants. No more than one presumptive *ara-leu* deletion mutant was found per subculture.

Genetic mapping experiments. A "screening set" of Ara⁻ homogenotes, containing the mutant sites *araA2*, *araA54*, *araBa87*, *araB27*, *araC101*, and *araC19* (see Fig. 1 for location of mutant sites), was used to classify the L-arabinose-resistant mutants. A sample containing 10^8 cells from an exponentially growing L-broth culture of the homogenote was plated on a dry ATL plate, spread evenly with a glass rod, and allowed to dry. A drop of an L-broth culture of the mutant to be tested was spotted on the surface of these plates and on a control plate containing no homogenote. The plates were examined for patches of growth after incubation at 37 C for 1 and 2 days. Complementing strains displayed uniform growth after 1 day. Growth of recombinants from noncomplementing strains was evident after 2 days. The failure to observe any growth within a spot after 2 days was preliminary evidence for a deletion mutant. These presumptive deletion mutants were analyzed by more refined mating experiments with many different Ara⁻ homogenotes (see Fig. 1 for mutant sites) and Hfr33 *hisleuB1*. Overnight, nonaerated L-broth cultures of male and female strains were diluted 1:20 into fresh medium, and, after 2 hr, equal volumes of the appropriate exponentially growing cells were mixed. After 90 min, samples were plated on selective agar and the plates were incubated for 48 hr at 37 C. In the cross F′ × F⁻, we selected for Ara⁺ recombinants on ATL agar. It was assumed that the failure to obtain Ara⁺ recombinants with a specific Ara⁻ homogenote meant that a deletion encompassed that specific site. In the cross Hfr33 *hisleuB1* × F⁻ mutant, we selected for his⁺ Thr⁺ recombinants on GL agar and for His⁺ Leu⁺ recombinants on GT agar. The former served as a control for recombinant formation, and the latter tested for the extension of a deletion to include the *leuB1* site.

Transduction. All transductions were carried out with P1bt by the methods of Gross and Englesberg (14).

Preparation of extracts. Twelve-hour nutrient agar slants provided the inoculum for 200 ml of GlyTL medium, with or without L-arabinose, in 1-liter Erlenmeyer flasks. The cultures were incubated at 37 C with shaking. Exponentially growing bacteria were used throughout. Ice-chilled bacteria were harvested by centrifugation, washed once in 30 ml of either 1 mM ethylenediaminetetraacetic acid (EDTA), *p*H 7.4, for L-arabinose isomerase and L-ribulokinase assays or 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.2, for the α-isopropylmalate (α-IPM) synthetase assay, resuspended in 1.5 ml of the preceding solutions except that the 1 mM EDTA contained 1 mM glutathione, and disrupted with the Branson Sonifier. Debris was cleared after one 30-min centrifugation at 40,000 × *g*. All steps were carried out in the cold.

Assay procedures. L-Arabinose isomerase (8) and L-ribulokinase (21) assays were performed as described previously. α-IPM synthetase was assayed as reported (7), except for a slight modification (communicated to us by H. E. Umbarger). The reaction mixture, in 0.5 ml, contained 70 μmoles of Tris-hydrochloride (*p*H 7.2), 70 μmoles of KCl, 5 μmoles of α-ketoisovaleric acid adjusted to *p*H 7.0 with KOH, 0.4 μmoles of acetyl coenzyme A, and 0.1 to 2 mg of extract protein. Protein was determined by the phenol reagent method (22). Results of enzyme assays are expressed as micromoles of product formed per hour per milligram of extract protein.

Chemostat experiments. Cultures were grown in 500-ml graduated cylinders at 37 ± 0.01 C. GlyTL medium was fed to the cultures by a peristaltic pump with a variable flow rate. A constant volume of 400 ml of aerated culture was maintained by siphoning off the excess medium into chilled reservoirs. After establishing the flow rate, the initial culture volume corresponding to at least one generation of growth was discarded. Subsequently, 400 ml of culture was collected and processed for enzyme assays. The medium was then fed at a slower rate, and the above procedure was repeated.

Chemicals. α-Ketoisovaleric acid and the trilithium salt of acetyl coenzyme A were purchased from Calbiochem. 5′,5′,5′-Trifluoroleucine (TFL) was obtained from H. E. Umbarger. L-Arabinose-*1*-^{14}C, obtained from Nuclear Research Chemicals, Inc., was used to prepare L-ribulose-*1*-^{14}C (21).

RESULTS

Growth requirements of mutants. Of a total of 32,100 Ara^- Ara^r colonies tested from 4,900 subclones of strain SB5003, 123 were unable to grow on GT agar. Of these 123 mutants, 93 required leucine (referred to as class I mutants), whereas the remaining 30 (referred to as class II mutants) grew only on complex medium. Some class II mutants were able to grow on GT agar supplemented with an additional amino acid. The distribution of these amino acid requirements is as follows: 9 Met^-, 3 Pro^-, 2 Phe^-, 1 Lys^-, 1 Arg^-, and 1 Met^- Leu^-. Thirteen mutants failed to grow when GT agar was supplemented with any amino acid or groups of amino acids with or without uracil. Vitamins, purines, and carbon sources other than glucose were not tested.

Genetic characterization of mutants. All class I mutants (Ara^- Ara^r Leu^-), with one exception, failed to give recombinants with one or more of the "screening set" homogenotes. The exception gave Ara^+ recombinants with all of the "screening set" homogenotes and reverted to Leu^+ when treated with the mutagen ethyl methane sulfonate. The Ara^r Leu^- phenotype of this strain was assumed to result from two independent mutational events. This double mutant was not studied further.

Additional mating experiments showed that none of the 92 class I mutants gave recombinants with the sites *araC19*, *araC3*, *araC5*, and *araC12*. Fifty-three mutants gave Ara^+ recombinants with at least one of the many Ara^- homogenotes used; the rest failed to give Ara^+ recombinants with any of the homogenotes (these deletions extend past the *araA2* site). More than half of the class I mutants failed to give Leu^+ recombinants with strain SB3002. All gave Thr^+ recombinants (control). Thus, the class I mutants contain deletions extending from some point in the leucine region to various points within or beyond the L-arabinose region. The ability to isolate these deletion mutants establishes that no essential functions (non-correctable) are specified by the DNA between *ara* and *leu*.

One class II mutant, a Met^- Leu^- auxotroph, also failed to give either Ara^+ recombinants with several F′ *araC*$^-$ homogenotes or Leu^+ recombinants with strain SB3002. This mutant thus carried an *ara-leu* deletion (designated 1165, see Fig. 1) and a lesion probably affecting methionine biosynthesis.

All other class II mutants, except one, gave Ara^+ recombinants with all of the Ara^- homogenotes tested. It was concluded that these mutants carried no extended deletion in the L-arabinose region, and that their auxotrophic requirement may result from a mutation distinct from that responsible for resistance to L-arabinose. The exception proved to be an Ara^- auxotroph containing a deletion beginning in *araB* and extending to the right of *araC* (but not into the leucine operon). These class II mutants were not studied further.

A summary of the termination points of the *ara-leu* deletions is given in Table 2. Only *araC-leu* deletion mutants and *araB-leu* deletion mutants containing an intact *leuA* gene (gave Leu^+ recombinants with Hfr33 *hisleuB1*) were selected for further study. The length of these deletions is illustrated in Fig. 1.

Transduction experiments. In these *ara-leu* deletion mutants, the position on the chromosome where the *ara* and *leu* ends have joined should behave as a point lesion in transduction experiments, i.e., Ara^- and Leu^- should cotransduce 100% of the time. This prediction was tested in the following manner with several of the deletions. The *thr*$^-$ *araD139* markers in the *ara-leu* deletion mutants were first converted to *thr*$^+$ *araD*$^+$ by transduction with P1bt grown on the wild type strain UP1000. Subsequently, phage was grown on the *thr*$^+$ *araD*$^+$ *ara-leu* deletion mutants and was used as donors in transduction experiments with a Thr^- recipient. As a control, phage grown on strain SB5078, containing point mutations *araC101* and *leuB1*, was crossed with the same Thr^- recipient. (The phages were recycled three times on the donor strains to avoid any rare transducing particle that would still carry the wild-type *thr ara leu* regions.) Thr^+ transductants were selected and scored for Ara^- and Leu^-. There was 100% cotransduction of Ara^- and Leu^- with the five deletion mutants tested, as compared to only 33% cotransduction in the control cross (Table 3).

Complementation analysis. In the preliminary spot tests with the *araA* and *araB* "screening set" homogenotes, a positive complementation response (a patch of growth in 24 hr) was given only by strains containing deletions 1109, 1165, 1204, and 1216. It should be noted that the left end of deletions 1165, 1204, and 1216 terminates within the C gene as defined by two *araC*$^-$ point mutations. On the other hand, on the basis of mapping data obtained with the Ara^- homogenotes, one cannot determine whether deletion 1109 terminates within or to the left of *araC*. Stable merodiploids of strains SB1509, SB1604, and SB1616, containing deletions 1109, 1204, and 1216, respectively, were constructed by mating with an *araA2* homogenote, grown in a GlyTL medium with and without inducer, and were assayed for their content of isomerase and kinase.

Haploid strains were grown, and enzyme content was determined in a similar manner. The results showed that the *araA* gene *cis* to these deletions was expressed only in the merodiploid strain and was inducer-dependent (Table 4). The fact that genes *araA* and *araB cis* to deletion 1109 are activated in merodiploids containing a C^+ allele in the *trans* position is further evidence (32; J. Irr and E. Englesberg, Bacteriol. Proc., p. 54, 1967) for the positioning of the controlling sites for the structural genes *araA*, *araB*, and *araD* to the left of gene *araC* and therefore for the exclusion of gene *araC* from the BAD operon. (The relatively high uninduced level of isomerase activity in the merozygote containing deletion 1109 will be discussed later.) These results also confirm one of the essential features of the positive control model of *ara* gene expression. Deletions in the *araC* gene produce a pleiotropic negative phenotype (C^-) and the C^+ allele is dominant (*cis* and *trans*) to C^-; therefore, the *araC* gene produces a product essential for *araBAD* gene expression (12, 15, 31, 32).

TABLE 2. *Summary of termination points of ara-leu deletions*

leu Region	*araC*	Between *araC101* and *araB27*	*araB*	Between *araBa87* and *araA54*	*araA*	Left of *araA2*
Left of *leuB1*....	0	2	12	2	4	11
Right of *leuB1*...	2	1	16	6	8	28
Not tested.....	1					

TABLE 3. *Cotransduction frequency of Leu and Ara in ara-leu deletion mutants*[a]

Donor	Pertinent marker	Selected marker (*thr*$^+$)	Unselected marker	
			ara$^-$	*leu*$^-$
SB5078	*araC101 leu B1*	682	24	8
SB1631	Δ1119	777	12	12
SB1634	Δ1170	767	13	13
SB1632	Δ1125	863	17	17
SB1628	Δ1100	737	18	18
SB1630	Δ1110	676	24	24

[a] P1bt was grown on the indicated donor strains each having the phenotype Ara$^-$ Leu$^-$. As a control, phage was grown on SB5078, a strain containing point mutations *araC101* and *leu B1*. SB5010 was the recipient. All Thr$^+$ transductants on GL agar were picked to GL (a control plate), G, and AL agar to test for the presence of the unselected markers.

The results with six of the eight noncomplementing deletion mutants are summarized in Table 5. All of the haploid mutant strains, except SB1570 (which contains deletion 1170), contained higher basal levels of L-arabinose isomerase activity than the wild type. None had L-ribulokinase activity. Growth in the presence of inducer had no effect on the isomerase or kinase levels. In merodiploids $A^-B^+I^+O^+C^+/A^+$ *ara-leu* deletion, isomerase activity was the same as that found with the haploid deletion mutants; the presence of inducer had no effect. Kinase activity, on the other hand, increased considerably in the merodiploids in the presence of inducer, presumably as a result of activation by the *araC* gene product of the kinase structural gene *cis* to the C^+ allele.

Deletions 1114, 1100, 1125, and 1162 (also 1133 and 1110) all extend to the left of *araB27* and therefore have excised the controlling sites for the L-arabinose operon. Although deletion 1119 does not include mutant site *araB27*, it probably encompasses *araI* and extends into the *araB* gene

TABLE 4. *Analysis of complementing stable merodiploids*[a]

Strain	Pertinent markers	Isomerase		Kinase		Per cent segregation (Ara$^-$/total × 100 inducer)
		− inducer	+ inducer	− inducer	+ inducer	
UP1001	Wild type	<1	100	<0.1	7	
UP1009	A^-	<1	<1	<0.1	43	
SB1604	Δ1204	<1	<1	<0.1	<0.1	
SB3532	$A^-B^+I^+O^+C^+/A^+B^+I^+O^+$ Δ1204	<1	58	<0.1	13	<1
SB1616	Δ1216	<1	<1	<0.1	<0.1	
SB3533	$A^-B^+I^+O^+C^+/A^+B^+I^+0^+$ Δ1216	<1	31	<0.1	6	20
SB1509	1109[b]	1	1	0.1	0.1	
SB3523	$A^-B^+I^+O^+C^+/A^+B^+I^+$ Δ1109	2.5	78	0.1	15	12

[a] Bacteria were grown in GlyTL medium ± inducer (L-arabinose). Before harvesting, a sample from the diploid cultures was diluted and plated on EMBara to determine the amount of segregation.

[b] See text for evidence that Δ1109 extends into *araO*.

TABLE 5. *Analysis of noncomplementing stable merodiploids*[a]

Strain	Pertinent markers	Isomerase		Kinase		Per cent segregation (Ara⁻/total × 100 inducer)
		− inducer	+ inducer	− inducer	+ inducer	
UP1001	Wild type	<1	100	<0.1	7	
UP1009	A⁻	<1	<1	<0.1	43	
SB1519	Δ1119	5	7	<0.1	<0.1	
SB3527	A⁻B⁺I⁺O⁺C⁺/A⁺ Δ1119	6	6	<0.1	36	3.9
SB1570	Δ1170	<1	<1	<0.1		
SB3531	A⁻B⁺I⁺O⁺C⁺/A⁺ Δ1170	<1	<1	<0.1	11	15
SB1525	Δ1125	7	7	<0.1	<0.1	
SB3538	A⁻B⁺I⁺O⁺C⁺/A⁺ Δ1125	7	7	<0.1	23	19
SB1562	Δ1162	6	5	<0.1	<0.1	
SB3530	A⁻B⁺I⁺O⁺C⁺/A⁺ Δ1162	7	6	<0.1	32	2.5
SB1500	Δ1100	1	2	<0.1	<0.1	
SB3521	A⁻B⁺I⁺O⁺C⁺/A⁺ Δ1100	1	2	<0.1	15	19
SB1514	Δ1114	6	6	<0.1	<0.1	
SB3526	A⁻B⁺I⁺O⁺C⁺/A⁺Δ1114	10	8	<0.1	14	29

[a] See footnote *a* to Table 4.

since the strain containing this deletion is not complemented by C⁺ and lacks kinase activity. Evidence presented below is consistent with the interpretation that deletion 1170 also encompasses *araO* and *araI*. These data thus account for the failure of activator to turn on *ara* gene expression in these deletion mutants and confirm previous findings as to the essential nature of the region between *araB* and *araC* for the control of genes *araBAD* by the inducer, L-arabinose, and the *araC* regulatory gene product (32).

Derepression studies with araB-leu deletion mutants. Genetic data have established that this set of deletions ends within the leucine operon leaving intact the *leuA* gene and presumably the leucine controlling elements. [They all gave Leu⁺ recombinants with *leuB1* (Fig. 1)]. The increased basal level of L-arabinose isomerase found in these deletion mutants, as compared to the wild type (Table 5), is probably the result of linking the *araB* gene to the leucine operon which, under the growth conditions employed, is partly derepressed. Thus, it appears that in these deletions *araA* is part of the *leu* operon. To investigate this possibility further, a number of double mutants containing an *ara-leu* deletion and a leucine regulatory mutation were constructed. Several independent spontaneous TFL-resistant mutants (7) of strain SB5003 were isolated and purified on GT agar supplemented with 5mM TFL. All of the *araB-leu* deletions (Fig. 1) were transduced into one of the TFL-resistant mutants, SB5021, containing the regulatory mutation *leu-6*. The *leu-6* mutation (Table 6) caused approximately a 100-fold increase in α-IPM synthetase activity in the parental strain (compare SB5003 and SB5021) and about a 20- to 40-fold increase in all strains tested carrying a deletion. This mutation also caused a 7- to 10- fold increase in L-arabinose isomerase activity in the double mutants, but had no effect on isomerase activity in the parental strain SB5021.

Derepression of *ara* and *leu* gene products was also observed in chemostat experiments. α-IPM synthetase and L-arabinose isomerase activities were depressed in mutants containing deletions 1125, 1119, and 1170 when leucine, but not glycerol, was the limiting supplement (Fig. 3A, B, and C). When threonine was limiting, a slight increase in isomerase activity was observed. The derepressed levels of α-IPM synthetase were similar for the three mutants; however, the activity of L-arabinose isomerase was approximately 10-fold less in the strain with deletion 1170, as compared to the strains with deletions 1119 and 1125.

The values for α-IPM synthetase and L-arabinose isomerase in Table 6 would seem to indicate noncoordinate expression (compare the deletion mutant with the double mutant, *leu-6* + deletion); however, in the chemostat experiments, at least two of three of these deletion mutants tested (△1125 and △1170; Fig. 3A, B, C) showed a moderate degree of coordinate expression. This discrepancy remains to be clarified. Nevertheless, we imagine that the linking of these two regions by these *araB-leu* deletions results in the synthesis of a new single polycistronic mRNA for the remaining genes of the *ara* and *leu* operons.

These studies firmly establish that the *araA* gene in the *araB-leu* deletion mutants, containing deletions 1119, 1170, 1125, 1162, 1100, 1133, and

TABLE 6. *Effect of a leucine regulatory mutation on the rate of expression of the araA gene*[a]

Strain	Pertinent markers[b]	L-Arabinose isomerase	α-IPM synthetase
UP1001	Wild type	<1 (100)	
SB5003	*araD139*	<1	0.13 (1.2)
SB5021	*araD139 leu-6*	<1	12
SB1519	Δ1119	8	0.76
SB1646	Δ1119 *leu-6*	30	23
SB1634	Δ1170	<1	0.73
SB1654	Δ1170 *leu-6*	4	13
SB1525	Δ1125	7	0.60
SB1647	Δ1125 *leu-6*	64	13
SB1562	Δ1162	5	0.62
SB1649	Δ1162 *leu-6*	29	23
SB1500	Δ1100	2	0.68
SB1643	Δ1100 *leu-6*	9	15
SB1510	Δ1110	3	0.59
SB1644	Δ1110 *leu-6*	39	18
SB1533	Δ1133	2	0.61
SB1648	Δ1133 *leu-6*	28	21
SB1514	Δ1114	8	0.90
SB1645	Δ1114 *leu-6*	44	20

[a] Bacteria were grown in a GlyTL medium. The induced level of isomerase for wild type and the derepressed level of α-IPM synthetase for SB5003 (the parental strain of SB5021 and the ara-leu deletion mutants) are given in parentheses.

[b] In addition to the pertinent markers listed, the genotype of all, except two, of the SB strains in this table is *thr⁻ araD139 D-ara-5 str^r2*. The exceptions are SB1634 and SB1654, which have the genotype *D-ara-5 str^r2*.

1114, is subject to leucine control and that the leucine controlling sites are to the right of the *leu* structural genes (Fig. 1). These findings also require that the *leu* and *ara* genes must be located on the same strand of DNA. A similar conclusion was reached concerning the *purE* and *lac* regions in *E. coli* K_{12} (20).

Since it is possible to obtain levels of L-arabinose isomerase activity in some *araB-leu* deletion mutants which are similar to those obtainable in the wild type, one improbable but often voiced alternative model to positive control is eliminated. This model proposes that the *araC* gene provides an essential subunit for all four enzyme molecules (including permease) in the L-arabinose system.

Extension of deletions 1170 and 1119 past the araI region. Data presented earlier are consistent with the notion that deletions 1170 and 1119 leave *leuA* and *leuO* intact (Fig. 1, 3C; Table 6), probably excise *araI* and *araO*, and may terminate in *araB*. To ascertain further whether *araI* may be present in some form in the strains containing these deletions, an attempt was made to transduce an *araI^c* site into strains containing these deletions. [*araI^c* mutations restore the Ara⁺ phenotype to mutants unable to produce a functional activator. They act only *cis* and produce low level constitutive rates of synthesis of the *araBAD* gene products (E. Englesberg et al., Proc. Nat. Acad. Sci. U. S. A., *in press*; E. Englesberg et al., J. Mol. Biol., *in press*)]. With *araI^c1 Δ766* as donor and the deletion mutants as recipients, Ara⁺ transductants were selected on AL agar and then tested for the Leu phenotype. If the *araI* region is left intact by an *ara-leu* deletion, some Ara⁺ transductants will retain the *ara-leu* deletion, i.e., they will be Leu⁻. If, however, the *araI* region is encompassed by the deletion, all Ara⁺ transductants will be Leu⁺. The results in Table 7 showing that no Ara⁺ Leu⁻ transductants were found (<0.006%) are consistent with the view that deletions 1170 and 1119 extend past the *araI* region. On the basis of these results, we presume that the low level of L-arabinose isomerase, produced as a result of the *leu-6* mutation (Table 6) or leucine starvation in the chemostat (Fig. 3) with the strain containing deletion 1170, may be the result of a polarity effect produced by a nonsense codon generated by the deletion (1, 5, 6, 24–29, 33–35).

Derepression studies with complementing ara-leu deletion mutants. In chemostat experiments with leucine as the limiting supplement, no increase in L-arabinose activity was detected with strains containing deletions 1204 and 1216. Since the left end of these deletions terminated within gene *araC*, these are the results that would be predicted on the basis of evidence that gene *araC* is not part of the *araBAD* operon (see above). However, these experiments cannot serve as a further test of this prediction. We have not been able to detect any α-IMP synthetase activity in these strains, and it is possible, therefore, that deletions 1204 and 1216 extend through the *leuA* gene and excise the region containing the controlling sites for the *leu* operon.

In contrast to the results with deletions 1204 and 1216, the strain containing deletion 1109, terminating somewhere between *araB27* and *araC101*, showed a small but definite increase in L-arabinose isomerase activity in chemostat experiments, specifically with leucine as the limiting supplement. Deletion 1109 also produced an α-IMP synthetase deficiency; therefore, it also must extend at least into the *leuA* gene. As a result of deletion 1109, the *araA* gene, and presumably the entire *araBAD* operon, although still sensitive to L-arabinose induction (in the presence of a *trans*-acting C⁺ allele), is now placed under the control of leucine and is therefore also part of

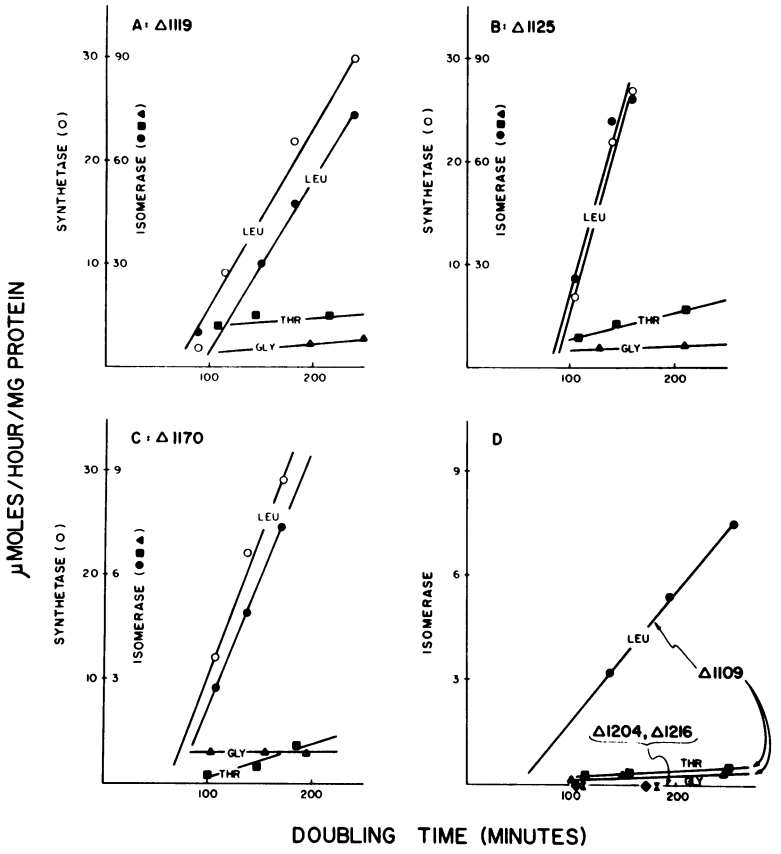

FIG. 3. *Effect of substrate limitation on expression of ara and leu gene products. Exponentially growing bacteria were harvested by centrifugation, concentrated, and added to the chemostat. Strains used had the genotype of SB5003 in addition to the specified ara-leu deletion. The substrate limited in the chemostat is indicated (GLY, THR, or LEU).*

the *leu* operon. To effect such a change, deletion 1109 may extend out of gene *araC*, excising the stop signal(s) to transcription that probably exists between genes *araB* and *araC* while leaving the area sensitive to the L-arabinose activator (*araI*) intact.

Deletion 1109: evidence for an araI site. Evidence presented above indicates that deletion 1109 does not extend into *araI*. This predicts that a strain containing deletion 1109 should be able to revert to Ara$^+$ as a consequence of an *araI^c* type mutation. To test this possibility, clones of such a strain were treated with ethyl methane sulfonate, plated on AL agar, and incubated for several days at 37 C. Several Ara$^+$ revertants were isolated and purified. The mutant sites of three revertants were mapped by transduction, using, as a donor, phage grown on SB1085 which contains deletion 766. We selected Leu$^+$ transductants and tested them for the ability to utilize L-arabinose. If the Ara$^+$ phenotype is not a consequence of an *araI^c* type mutation, then all Leu$^+$ transductants will be Ara$^-$. Ara$^+$ transductants were found (Table 8); thus, we conclude that the *araI* region is left intact by deletion 1109.

If the *ara* region is a functional part of the *leu*

TABLE 7. *Transductional evidence for the absence of the araI region in strains carrying deletions 1119 and 1170*[a]

Strain	Relevant genotype	Selected marker (*ara*$^+$)	Unselected marker (*leu*$^-$)	Per cent unselected marker (*leu*$^-$/*ara*$^+$ × 100)
SB1631	Δ1119	1707	0	<0.06
SB1634	Δ1170	1613	0	<0.06

[a] Phage P1bt grown on strain SB2078 was used to transduce an *araI^c* mutation into the indicated recipient strains by selecting for Ara$^+$ transductants on AL agar. The Leu phenotype of the transductants was determined by streaking on A agar.

operon, as the chemostat experiments indicate, we might expect to isolate a *leu* constitutive mutant from a strain with the genotype deletion 1109 + *araI*^{c} *1228*, which would grow faster on AL agar. Such mutants have not yet been found.

Extension of deletion 1109 into the ara operator region. Two lines of evidence defined a defective *ara* operator. The first involves the absence of a severe epistatic effect that a C^{+} allele has on expression of the *araA* gene *cis* to an *araI*^{c} mutation in merodiploids of the type $A^{-}B^{+}I^{+}O^{+}C^{+}/A^{+}B^{+}I^{c}$ deletion *OC*. The second line of evidence involves the high basel level of *araA* gene expression in merodiploids of the type $A^{-}B^{+}I^{+}O^{+}C^{+}/A^{+}B^{+}I^{+}$ deletion *OC*, as compared with the very low level of expression in a haploid strain of the type $A^{+}B^{+}I^{+}$ deletion *OC* (E. Englesberg et al., Proc. Nat. Acad. Sci. U.S.A., *in press*). When a strain carried deletion 1109 and an *araI*^{c} mutation, the presence of a C^{+} allele had no effect on *araA* gene expression (Table 9). Furthermore, the basal level of expression of the *araA* gene *cis* to deletion 1109 was increased in the presence of a C^{+} allele (Table 4, bottom line). Thus, this deletion excises *araO*.

DISCUSSION

Our experiments demonstrate that in *ara-leu* deletion mutants the structural genes of the L-arabinose operon, an inducible operon under positive control, may be placed under the control of the leucine operon, a repressible operon. The data indicate that, in order for the *ara* genes to be regulated exclusively by the control elements of the leucine operon, the deletion must eliminate that part of the region between *araB27* and *araC101* that contains all of the known controlling sites of the L-arabinose operon. The mutants of this type thus far isolated contain deletions that abolish L-ribulokinase activity. All of these deletions, with the possible exception of one, are judged by genetic and physiological criteria to end within the *araB* gene. It is likely that *ara* genes, in a strain containing an *ara-leu* deletion terminating in *araI* which would destroy the initiator site, would also be under the exclusive control of the leucine operon. (This follows from the characteristics of a strain containing deletion 1109 discussed below.) Mutants containing either type of deletion probably produce a single species of mRNA which contains information for both *ara* and *leu* gene products.

The *ara* genes in strains containing deletion 1109 are susceptible to potential regulation by both L-leucine and L-arabinose. These strains do not have a functional *ara* operator but do have a functional *ara* initiator. The fact that activation by L-arabinose of the genes *cis* to this deletion in merodiploids of the type C^{+}/Δ1109 is normal is consistent with the idea that, if there is an L-arabinose promoter site (17, 19, 30) distinct from *araI*, it lies somewhere between *araO* and *araB*. Under conditions of activation with a C^{+} allele in the *trans* position and L-arabinose as the inducer, the species of *ara* mRNA made is presumed to be similar to that made by a strain with an intact *ara* region.

The leucine-specific derepression of the *ara* genes in a strain containing deletion 1109 establishes that the leucine-controlling elements are functionally intact. Under conditions of leucine derepression, we assume that a single species of mRNA is made that begins in the leucine region and continues through *araI* and *araBAD*. Thus, it appears that *araI* can be transcribed under these conditions; whether *araI* can be transcribed in a strain with an intact *ara* region remains to be determined. It seems likely that the low level of

TABLE 8. *Genetic mapping by transduction of araI*^{c} *mutations in a strain carrying deletion 1109*[a]

Strain	Relevant genotype	Selected marker (*leu*$^{+}$)	Unselected marker (*ara*$^{+}$)	Per cent unselected marker (*ara*$^{+}$/*leu*$^{+}$ × 100)
SB2064	Δ1109 *ara-1228*	828	12	1
SB2065	Δ1109 *ara-1229*	880	12	1
SB2066	Δ1109 *ara-1230*	725	3	0.4

[a] Phage P1bt grown on SB1085 was used to transduce the indicated recipient strains to Leu^{+} on G agar. The Ara phenotype of the transductants was determined by streaking on A agar.

TABLE 9. *Test for operator region in strain containing deletion 1109*[a]

Strain	Pertinent markers	L-Arabinose isomerase	Per cent segregation (Ara^{-}/total × 100)
SB2064	I^{c}1228 Δ1109	10	
DC3000	$A^{-}B^{+}I^{+}O^{+}C^{+}/A^{+}B^{+}I^{c}$1228 Δ1109	9	11

[a] Bacteria were grown in a GlyL medium. Before harvesting, a sample from the diploid culture was diluted and plated on EMBara to determine the amount of segregation.

ara gene expression under conditions of maximal leucine derepression may be a general feature of *araO-leu* deletions which leave an intact *araI*.

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