

## Positive Control of Enzyme Synthesis by Gene C in the L-Arabinose System

ELLIS ENGLESBERG,<sup>1</sup> JOSEPH IRR,<sup>1</sup> JOSEPH POWER,<sup>1</sup> AND NANCY LEE<sup>1</sup>

*Department of Biology, University of Pittsburgh, Pittsburgh, Pennsylvania*

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### ABSTRACT

ENGLESBERG, ELLIS (University of Pittsburgh, Pittsburgh, Pa.), JOSEPH IRR, JOSEPH POWER, AND NANCY LEE. Positive control of enzyme synthesis by gene C in the L-arabinose system. *J. Bacteriol* **90**:946-957. 1965.—The L-arabinose gene complex consists of genes D, A, B, and C, linked in that order between the markers *thr* and *leu*, and an unlinked gene E. Genes D, A, B, and E are the structural genes for three inducible enzymes and permease, respectively. Gene C, with two mutant alleles, C<sup>-</sup> and C<sup>c</sup>, is the regulatory gene exhibiting positive and negative control. C<sup>-</sup> mutants are deficient and C<sup>c</sup> mutants are constitutive for all three enzymes and permease. Complementation analysis, employing sexual merozygotes (A<sup>-</sup>C<sup>+</sup> × A<sup>+</sup>C<sup>-</sup>), with six different C<sup>-</sup> mutants, demonstrates that C<sup>-</sup> is recessive to C<sup>+</sup> (positive control). A total of 61 C<sup>c</sup> mutants, isolated as clones resistant to D-fucose inhibition, are linked to the *leu ara* region of the chromosome, and the 22 C<sup>c</sup> mutants that were analyzed in detail mapped within the C gene among the C<sup>-</sup> mutant sites. C<sup>c</sup> mutants produce various but coordinate levels of the two enzymes measured, and permease. Complementation analysis (A<sup>-</sup>C<sup>c</sup> × A<sup>+</sup>C<sup>-</sup>, A<sup>-</sup>C<sup>c</sup> × A<sup>+</sup>C<sup>+</sup>) shows that C<sup>c</sup> is dominant to C<sup>-</sup> (positive control) and recessive to C<sup>+</sup> (negative control). Deletion mutants that extend into the C gene are L-arabinose permease-negative, thus supporting the positive regulatory role of the C gene. The name "activator gene" is proposed for genes of the C type to accentuate their positive role in gene expression. A working model consistent with these results is presented.

The C gene has been shown to be a new type of regulatory gene exhibiting positive control of the expression of genes A, B, D, and E, the structural genes for L-arabinose isomerase, L-ribulokinase, L-ribulose-5-phosphate 4-epimerase, and L-arabinose permease, respectively, in the L-arabinose gene complex (Gross and Englesberg, 1959; Englesberg and Killeen, 1959; Englesberg, 1961; Englesberg et al., 1962; Helling and Weinberg, 1963; Isaacson and Englesberg, 1964; Novotny and Englesberg, 1964). (Although no modified epimerase or permease protein has been detected, we are assuming that genes D and E are the structural genes for L-ribulose-5-phosphate 4-epimerase and L-arabinose permease, respectively, on the basis of physiological and enzymatic characteristics of the mutants concerned.) The C gene is linked to genes D, A, and B in the order DABC between the markers for a threonine (*thr*) and a leucine (*leu*) requirement. Gene E is unlinked to this area of the coli chromosome by transduction analysis, but is linked to *ser-A* and *arg-1* (Fig. 1) (Isaacson and Engles-

berg, 1964; Englesberg and Lieberman, *unpublished data*).

L-Arabinose-negative mutations in the C gene (C<sup>-</sup>) have a pleiotropic effect, leading to the inability to induce the synthesis of the enzymes listed above (Englesberg and Killeen, 1959; Englesberg, 1961) and the L-arabinose permease (Novotny and Englesberg, 1964). Complementation analysis has shown that the C gene (as defined by the C<sup>-</sup> alleles) is separate and distinct from genes A, B, and D. The C<sup>-</sup> allele, analyzed in mutant C-19, was found to be recessive to C<sup>+</sup> (Helling and Weinberg, 1963). This evidence, together with the finding that the C gene controls the expression of the unlinked L-arabinose permease gene, E gene (Novotny and Englesberg, 1964), suggested that the C gene produced a cytoplasmic product essential for the expression of the structural genes in the L-arabinose gene complex.

This is an example of positive control of gene expression and is in sharp contrast to the negative or repressor control as has been primarily elaborated in the  $\beta$ -galactosidase system (Jacob and Monod, 1961b).

<sup>1</sup> Present address: Department of Biological Sciences, University of California, Santa Barbara.

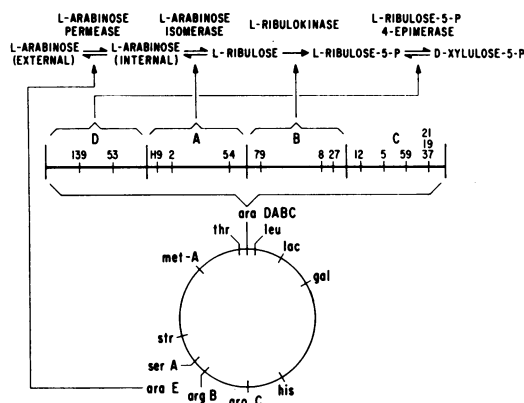


FIG. 1. *L*-Arabinose gene-enzyme complex.

In this paper, we shall show that complementation analysis with other  $C^-$  mutants confirms the above findings. In addition, we shall describe the isolation of constitutive mutants ( $C^c$ ) in this system, all of which map within the C gene among the  $C^-$  mutant sites (Englesberg, Irr, and Power, 1964). The results of an analysis of the interaction of  $C^c$  with  $C^-$  and  $C^+$  in the synthesis of *L*-arabinose isomerase are in agreement with what would be expected from the previous findings, but indicate, in addition, that the C gene exerts a negative as well as a positive control over gene expression. Since a regulator gene has been defined as producing a substance that only inhibits the expression of a structural gene (Jacob and Monod, 1961a), we propose the name "activator gene" for the C gene to accentuate its positive role in this transfer of information from structural genes to protein.

#### MATERIALS AND METHODS

**Nomenclature.** Genes A, B, C, D, and E are defined as follows: A, *L*-arabinose isomerase gene; B, *L*-ribulokinase gene; C, regulatory gene (activator type); D, *L*-ribulose-5-phosphate 4-epimerase gene; E, *L*-arabinose permease gene.

$A^+$ ,  $B^+$ ,  $D^+$ ,  $E^+$  or A, B, D, E indicates ability to produce the respective enzyme due to wild-type configuration of the gene involved.

$A^-$ ,  $B^-$ ,  $D^-$ ,  $E^-$ , usually followed by a number (B-8), indicates inability to produce the wild-type enzyme due to mutation in the respective structural gene. The number indicates the mutational site involved.

$C^+$ ,  $C^-$ , and  $C^c$  are genetic markers for inducibility (wild type), inability to induce, and constitutive formation, respectively, of the first three metabolic enzymes and permease in *L*-arabinose metabolism.  $C^-$  and  $C^c$  are usually followed by numbers ( $C^c5$ ) indicating the mutational site in the C gene.

Hfr indicates male type (Wollman, Jacob, and

Hayes, 1956);  $F^-$ , female type;  $str^s$  and  $str^r$ , genetic markers for sensitivity and resistance to streptomycin;  $ara^+$  and  $ara^-$ , ability and inability to utilize *L*-arabinose as sole carbon and energy source for growth; *leu*, leucine; *thr*, threonine; and a minus superscript indicates inability to grow in the absence of the particular amino acid.

**Strains of bacteria.** Mutants derived from *Escherichia coli* B/r are: *araA*-2, *araB*-8, *araC*-12, *araC*-5 (Gross and Englesberg, 1959; Englesberg, 1961; Lee and Englesberg, 1962); *araD*-139, *araD*-53 (Englesberg et al., 1962); *araA*-H9, *araA*-54, *araB*-79, *araB*-27, *araC*-37, *araC*-47, *araC*-59 (Cribbs and Englesberg, 1964); *araC*<sup>c</sup>1 to *araC*<sup>c</sup>50, constitutive mutants isolated from B/r *leu*<sup>-</sup>; *araD*-53,204 and *araD*-53,201 are deletion mutants to be described in Results. Various competent females ( $F^-$ ) were constructed from these strains as described below (see *Complementation*). The bacterial strains were constructed in *thr leu* and in either *thr leu*<sup>-</sup> or *thr leu*<sup>-</sup> genetic backgrounds (Gross and Englesberg, 1959).

*E. coli* B/r (hs) produces a heat-stable *L*-ribulokinase; *araC*<sup>c</sup>60 to *araC*<sup>c</sup>70 are constitutive mutants isolated from B/r (hs).

*E. coli* K-12, Hfr KH600 *leu*<sup>-</sup> *str*<sup>s</sup>, has the entire threonine, arabinose, leucine region replaced by that of *E. coli* B/r (Helling and Weinberg, 1963), and was employed in the construction of males with the appropriate arabinose markers as described below (see *Complementation*).

**Transduction.** Phage P1bt lysates (Boyer, Englesberg, and Weinberg, 1962), were used for transduction (Gross and Englesberg, 1959).

**Media.** The following media were used: mineral base with the carbon and energy sources and amino acids added as indicated (Gross and Englesberg, 1959); *L*-broth and EMB (Gross and Englesberg, 1959); nutrient agar and nutrient broth (Difco).

**Enzyme assays.** *L*-Arabinose isomerase and isocitric dehydrogenase were determined by the methods of Cribbs and Englesberg (1964), and *L*-ribulokinase by the method of Lee and Englesberg (1963).

**Complementation.** Appropriate arabinose mutations were transferred from *E. coli* B/r, where they were originally isolated, by transduction into KH600 males, and genotypes of the males were then verified by progeny tests (Helling and Weinberg, 1963). The females in these experiments were prepared from *E. coli* B/r by crossing into this strain the region of the K-12 chromosome linked to *thr* that controls K-12 modification and restriction (Boyer, 1964).

The actual procedures for complementation analysis were essentially those of Helling and Weinberg (1963), except that the male to female ratio of 1:1 was used and mating was performed on a slow-shaking water bath at 37°C.

In each cross, the female was *str*<sup>r</sup> and the male *str*<sup>s</sup>. After 50 min of mating, streptomycin was added (to 0.5 mg/ml). In some experiments, where indicated, *L*-arabinose was added after 10 min

at a final concentration of 0.4%. Samples were then removed at stated times, and extracts were prepared for L-arabinose isomerase assays. The amount of protein present in the mating mixture was determined by turbidity measurements in a previously calibrated Klett-Summerson colorimeter [1 Klett unit (42 filter) = 1.18  $\mu\text{g}/\text{ml}$  of protein (Lowry et al., 1951)]. The amount of protein per milliliter was multiplied by the specific activity of the cell-free extract to obtain units of enzyme present per milliliter of culture. Controls of males and females alone were maintained under the same conditions. The units of isomerase per milliliter in a mixture of the same composition of male and female as in the mating mixture were calculated from the separate control cultures and given as the control values in the results.

We assayed for isomerase activity in these crosses because the assay procedure for this enzyme was the simplest. Since genes BAD act as a genetic unit of coordinate expression, similar results would probably have been obtained if kinase (Helling and Weinberg, 1963; Lee and Englesberg, 1963) and epimerase (Lee and Englesberg, 1963) activities had been determined. The coordination of kinase and isomerase activities in the constitutive mutants, as presented in this paper, further supports this conclusion.

*Cell suspension for permease assay and for preparation of cell-free extracts.* L-Arabinose permease, L-arabinose isomerase, and L-ribulokinase, and, as controls, glucose permease and isocitric dehydrogenase, were determined on the same culture with each of 22 constitutive mutants in the following manner. Overnight cultures of each of the constitutive mutants grown in a mineral glycerol (0.1%) liquid medium with aeration were used to inoculate 200 ml of mineral glycerol (0.2%) medium contained in 500-ml Erlenmeyer flasks to approximately  $6 \times 10^7$  cells per milliliter. The cultures were aerated by agitation at 37 C and removed to an ice bath during the exponential phase of growth when the turbidity reached approximately  $5.4 \times 10^8$  cells per milliliter. A 15-ml amount of each culture was removed for permease assay, and the remainder was employed for the preparation of cell-free extracts (Englesberg, 1961). The 15-ml sample was centrifuged in the cold, and the cells were suspended in 7.5 ml of suspension fluid ( $\text{KH}_2\text{PO}_4$  -  $\text{K}_2\text{HPO}_4$ , pH 7.0, 1.0%;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01%), recentrifuged, and resuspended in the suspension fluid at a turbidity equivalent to 156  $\mu\text{g}$  (dry weight) of bacteria per ml. This suspension was employed for both permease assays.

Since a few of the constitutive cultures are unstable (there appears to be a high selection pressure in some cases for  $\text{C}^+$  and  $\text{C}^-$ ), each culture employed in the assays for permease and metabolic enzymes was streaked on nutrient agar, and the colonies appearing on this medium were assayed by use of the filter-paper technique for their ability to utilize L-arabinose constitutively. A maximum of 9% "reversion" from  $\text{C}^-$  was found

with  $\text{C}^8$ , and appropriate corrections were made in the assays.

*Detection of constitutive mutants by a filter-paper assay.* Whatman no. 2 filter paper (diameter, 8.7 cm) was impregnated with 1 ml of a solution containing 2% L-arabinose, 0.0025 M  $\text{MnCl}_2$ , and 1.0% streptomycin, and was air-dried. The streptomycin was added to prevent induction during the assay. The surface of a nutrient agar plate containing colonies to be tested was partially dried by exposure to flowing air at 37 C for 1 hr. The impregnated filter paper was placed over the nutrient agar and pressed down lightly to insure contact with the individual colonies. The lids were placed back on the dishes, and the dishes were incubated for 4 hr at 37 C. The filter paper was then removed and air-dried. To detect L-arabinose utilization by the individual colonies, the filter paper was dipped briefly into a developing solution containing equal quantities of 1 M NaOH in methanol and 2% triphenyltetrazolium chloride in methanol. The paper was hung in an oven at 100 C and removed as soon as a uniform red color developed. The position of the individual colonies can be readily noted due to the adherence of a portion of the colony on the filter paper. Non-constitutive variants, unable to utilize L-arabinose under these conditions, yielded a red color similar to the background, whereas constitutive colonies left a white spot, indicating complete utilization of the L-arabinose. As a control, each agar plate assayed in this manner was previously inoculated at a defined area with a  $\text{C}^+$  and  $\text{C}^-$  culture.

*L-Arabinose permease.* D-Xylose-1- $\text{C}^{14}$  accumulation was employed as a measure of the L-arabinose permease (Novotny and Englesberg, 1964). The membrane-filter technique was used (Hoffee and Englesberg, 1962). A 2-ml amount of a previously washed and standardized cell suspension brought to 25 C was added to 0.5 ml of a reaction mixture consisting of the following at the given final concentrations: D-xylose-1- $\text{C}^{14}$ ,  $10^{-3}$  M, specific activity,  $1.75 \times 10^5$  counts per min per  $\mu\text{mole}$ ; chloramphenicol, 50  $\mu\text{g}/\text{ml}$ ; and glycerol, 0.1%. The reaction mixture was in a test tube at approximately an  $18^\circ$  angle from the horizontal in a New Brunswick shaking water bath at 25 C. Incubation was continued for 10 min to insure that the cells had reached the steady state of accumulation, and two 1-ml samples were removed and filtered onto each of two membrane filters. Each filter was washed with 1 ml of the suspending fluid (as previously described) maintained at 25 C. The membrane filters were air-dried, mounted with Vaseline on planchets, and counted in a Nuclear-Chicago Micromil thin-window Geiger counter. At least 1,000 counts were determined for each sample. Counts due to diffusion, based upon cells containing 80% water, were subtracted. Results of duplicate assays were averaged and expressed as micromoles of D-xylose accumulated per gram (dry weight) of cells.

*Glucose permease.* The glucose permease was assayed by use of  $\alpha$ -methyl glucoside- $\text{C}^{14}$  (Engles-

berg, Watson, and Hoffee, 1961). The procedure was similar to that described for the L-arabinose permease, except that  $\alpha$ -methyl glucoside- $C^{14}$  at  $10^{-3}$  M (final concentration), specific activity,  $8.1 \times 10^4$  counts per min per  $\mu$ mole, was employed in place of D-xylose.

## RESULTS

**Isolation of constitutive mutants ( $C^c$ ).** D-Fucose inhibits the growth of *E. coli* B/r in mineral L-arabinose medium, and selection occurs for mutants that are constitutive for the three metabolic enzymes and permease involved in the initial steps in L-arabinose metabolism. Beginning with a *leu*<sup>-</sup> parental strain, we isolated 50 independent L-arabinose constitutive mutants ( $C^c1$  to  $C^c50$ ) from late growth in mineral L-arabinose (0.04%)–D-fucose (0.08%) (MAF)–leucine liquid medium, in the following manner. An overnight nutrient broth culture of *E. coli* B/r *leu*<sup>-</sup> was diluted into fresh nutrient broth to yield  $10^8$  viable cells per milliliter. The broth suspension was divided into 50 separate 1-ml portions and was incubated overnight. Fifty tubes containing 2.5 ml of MAF-leucine were inoculated with 0.05 ml of these overnight cultures; the tubes were incubated at 37 C until they became turbid (2 to 7 days), and the contents were streaked onto nutrient agar. Colonies that appeared were tested by picking and streaking onto MAF-leucine agar containing 0.1% L-arabinose and 0.2% D-fucose. One colony from each of the original cultures that grew on MAF-leucine agar was further purified on nutrient agar; it was then transferred to nutrient agar slants and deeps for storage after verifying that each of the isolates were *leu*<sup>-</sup> and resistant to fucose inhibition.

We also isolated 11 independent constitutive mutants ( $C^c60$  to  $C^c70$ ) directly from colonies that appeared on MAF agar plates. These plates were originally smeared with approximately  $10^8$  *E. coli* B/r (hs) cells from nutrient broth cultures started from small inocula as described above.

**Mapping of the constitutive mutants.** By use of the linkage of leucine to L-arabinose (approximately 50% cotransduction of leucine and any arabinose negative markers in that area), it was possible to determine whether the  $C^c$  mutant sites map within this L-arabinose region of the chromosome. Phage grown on the wild-type B/r was crossed to each of the 50  $C^c$  *leu*<sup>-</sup> ( $C^c1$  *leu*<sup>-</sup> to  $C^c50$  *leu*<sup>-</sup>) mutants. We selected for *leu*<sup>+</sup> and scored each of the *leu*<sup>+</sup> transductants appearing on mineral glucose plates for  $C^c$  by replica plating onto MAF, upon which  $C^+$  fails to produce visible growth. Among the *leu*<sup>+</sup> transductants analyzed in each cross, we found approximately equal

numbers of  $C^+$  and  $C^c$  recombinants, indicating that each of the  $C^c$  mutant sites analyzed is loosely linked with *leu* and, therefore, may map within the arabinose region of the chromosome located next to *leu*.

A more definitive mapping of 22  $C^c$  mutant sites ( $C^c1$  to  $C^c11$ ,  $C^c60$  to  $C^c70$ ) was performed with reference to  $C^-$  sites distributed throughout the C gene ( $C^-5$ ,  $C^-12$ ,  $C^-59$ ,  $C^-37$ ), and a B mutant site (B-8) closely linked to the C gene. (Mapping of the  $C^c$  sites in this manner was indicated on the basis of results of preliminary experiments which showed that the  $C^c$  sites tested were more closely linked to  $C^-$  mutant sites than to mutant sites in the other arabinose genes.)

The order of the  $C^-$  and the B-8 mutant sites had been previously established by three-factor crosses (Gross and Englesberg, 1959; Cribbs and Englesberg, 1964). The relative distances between the four reference  $C^-$  mutant sites and B-8 were determined by the per cent of *ara*<sup>+</sup> to *leu*<sup>+</sup> recombinants per unit volume of the transduction mixture, as determined by plating on mineral arabinose-threonine-leucine plates (selection for *ara*<sup>+</sup>) and on mineral glucose-threonine plates (selection for *leu*<sup>+</sup>). The distances given (Fig. 3) are the averages of the distances determined by reciprocal crosses. The following is a typical cross: phage *araC*-12  $\times$  bacterium *araC*-5 *thr*<sup>-</sup> *leu*<sup>-</sup>; phage *araC*-5  $\times$  bacterium *araC*-12 *thr*<sup>-</sup> *leu*<sup>-</sup>.

The position of the  $C^c$  mutant sites within the C gene was ascertained by determining the recombination frequencies between each of the  $C^c$  mutant sites and some or all of the five *ara*<sup>-</sup> sites (Fig. 2), in the following manner:  $C^c1$  to  $C^c11$ , originally all *leu*<sup>-</sup>, were converted to *leu*<sup>+</sup> by transduction with phage grown on the *ara*<sup>+</sup> prototroph, and nonlysogenic *leu*<sup>+</sup> constitutive transductants were isolated. Phage were grown on each of the *leu*<sup>+</sup> constitutive mutants and crossed to  $C^-12$ ,  $C^-5$ , and  $C^-59$ , or  $C^-37$ , *leu*<sup>-</sup>

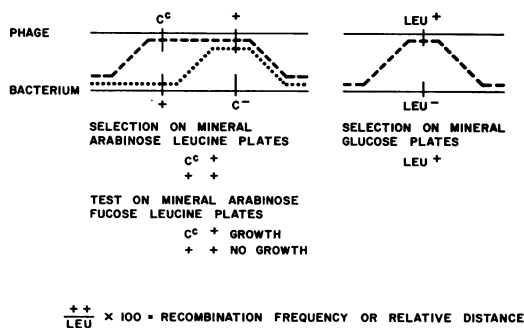


FIG. 2. Mapping of constitutive mutants.

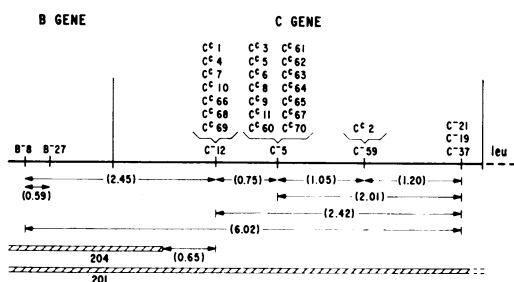


FIG. 3. Map of the *C* gene. The figures in parenthesis represent the average of reciprocal distance measurements (see Results). The order of B-8, C-12, C-5, C-19, C-21 (Gross and Englesberg, 1959) and B-27 (Cribbs and Englesberg, 1964) has been established by reciprocal three-factor crosses. The distance between B-8 and B-27 was established by Cribbs and Englesberg (1964). C-59 has been erroneously placed to the right of the cluster of mutants C-19, C-21, C-37, etc. (Cribbs and Englesberg, 1964). Reciprocal three-factor crosses and distance measurements that we have performed place C-59 unambiguously to the left of C-37 and to the right of C-5. C-37 is placed at the same site as C-19 and C-21, since it fails to yield any recombinants with these two mutants.

mutants. Crosses were made with B-8 when recombination frequencies indicated that a *C*<sup>c</sup> mutant site might be to the left of C-12. Selection was made for *leu*<sup>+</sup> on mineral glucose agar and for *ara*<sup>+</sup> on mineral L-arabinose leucine agar. The *ara*<sup>+</sup> recombinants were of two types: wild type (+ +) or constitutive (*C*<sup>c</sup> +). To determine the recombination frequency between a *C*<sup>c</sup> site and a *C*<sup>-</sup> or B<sup>-</sup> site, it is necessary to know the number of wild-type recombinants. This was determined by testing each of the *ara*<sup>+</sup> recombinants for ability to grow on MAF agar. Constitutives grow, whereas wild type is inhibited on this medium. The per cent wild type/*leu*<sup>+</sup> recombinants gives the recombination frequency or relative distance between the mutant sites involved. We settled for scoring a minimum of 800 *ara*<sup>+</sup> recombinants for each cross because of the difficulties of the mapping procedure. There is no positive selection for wild type (inducibility), and *C*<sup>c</sup> sites are closely linked to the *C*<sup>-</sup> sites. Since recombination frequencies were low, and it was not possible to perform reciprocal crosses, which are crucial for any fine structure mapping, the constitutive mutant sites were merely grouped next to the *C*<sup>-</sup> mutant sites with which they gave the smallest recombination frequencies (Fig. 3). Of the 22 constitutive mutants, 18 mapped between the two distal *C*<sup>-</sup> mutant sites. C<sup>c</sup>8 and C<sup>c</sup>70 failed to give any wild-type recombinants with C-5, and, based upon the num-

ber of *ara*<sup>+</sup> recombinants assayed, we estimated that C<sup>c</sup>8 and C<sup>c</sup>70 are less than 0.02 and 0.1 recombination units away from C-5, respectively. The great majority of the *C*<sup>c</sup> mutants mapped

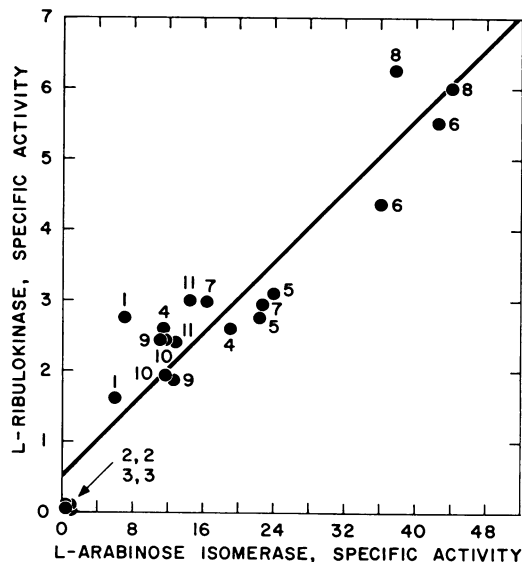


FIG. 4. L-Arabinose isomerase versus L-ribulokinase. The numbers denote particular constitutive mutants isolated from *Escherichia coli* B/r. Assays are from duplicate extracts prepared from the same cell suspension. The straight line was determined by the method of least squares; data from the wild type were not included. Specific activity = micromoles of product formed per hour per milligram of protein.

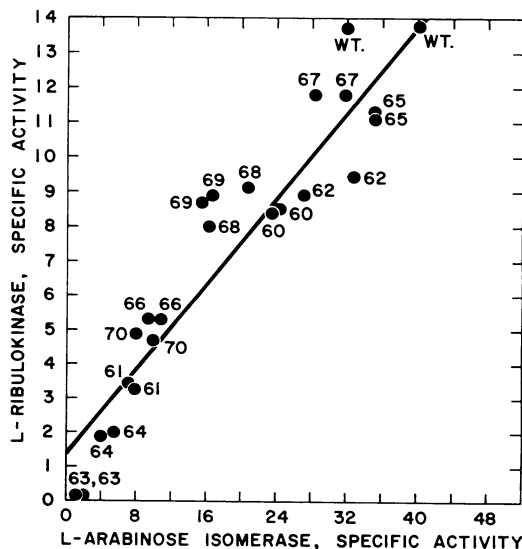


FIG. 5. L-Arabinose isomerase versus L-ribulokinase. As in Fig. 4, except constitutive mutants were derived from *Escherichia coli* B/r (hs).

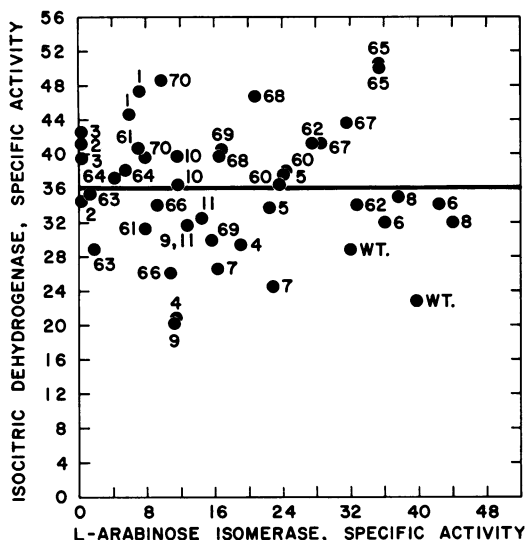


FIG. 6. *L*-Arabinose isomerase versus isocitric dehydrogenase (as in Fig. 4 and 5).

close to C<sup>-</sup>5. On the basis of additive distances from each of the C<sup>-</sup> markers, four C<sup>c</sup> mutant sites that map in the vicinity of C<sup>-</sup>12 may map to the left of C<sup>-</sup>12. C<sup>c</sup>4 was perhaps the farthest to the left of C<sup>-</sup>12 by a distance of 0.47. Since the distance between the closest B mutant site (B<sup>-</sup>27) and C<sup>-</sup>12 was 1.85 recombination units, and on the basis of phenotypic characteristics, we can, with some justification, assume that these four mutants also map within the confines of the C gene.

*Enzyme and permease levels in mutants resistant to D-fucose inhibition.* Mineral glycerol grown cells in exponential phase were assayed for *L*-arabinose isomerase, *L*-ribulokinase, and *L*-arabinose permease, and, as controls, the same preparations were assayed for glucose permease and isocitric dehydrogenase. The C<sup>c</sup> mutants have wide differences in constitutive levels of isomerase, kinase, and permease (approximately a 100-fold difference between the highest and lowest constitutive level of isomerase and kinase and approximately a 16-fold difference between the highest and lowest constitutive level of *L*-arabinose permease). A plot of constitutive *L*-arabinose isomerase versus *L*-arabinose permease and *L*-ribulokinase specific activities indicates that they are coordinate (Fig. 4, 5, 6, 7, and 8). Since genes BAD act as a genetic unit of coordinate expression (Lee and Englesberg, 1963), we presume that epimerase activity would also vary coordinately.

Isocitric dehydrogenase and glucose permease levels varied at a maximum of 2.5, and there

was no coordination between these levels and *L*-arabinose isomerase levels.

The coordination between *L*-arabinose isomerase and *L*-arabinose permease is interesting, since the isomerase gene (gene A) and the permease gene (gene E) are unlinked. This finding demonstrates that coordination of enzyme activities alone cannot be taken as evidence for linkage of the genes concerned, or for the production of a multicistronic message from a series of linked genes. The slope of the lines representing isomerase versus kinase activity of the C<sup>c</sup> mutants 1 to 11 and 60 to 70 are noticeably different. This difference is, no doubt, due to the heat stability of the kinase in C<sup>c</sup>60 to C<sup>c</sup>70 mutants, since each of these mutants was isolated from a parent strain having a heat-stable kinase. This difference in kinase, probably due to a difference in the B gene, obviously does not interfere with coordinate control between isomerase and kinase exercised by the C gene.

*Complementation analysis* (A<sup>-</sup>C<sup>+</sup> × A<sup>+</sup>C<sup>-</sup>). It has been previously demonstrated that C<sup>+</sup> is dominant to C<sup>-</sup> (Helling and Weinberg, 1963). These experiments were performed in a K-12 cross with just one C<sup>-</sup> mutant, C<sup>-</sup>19. This mutation

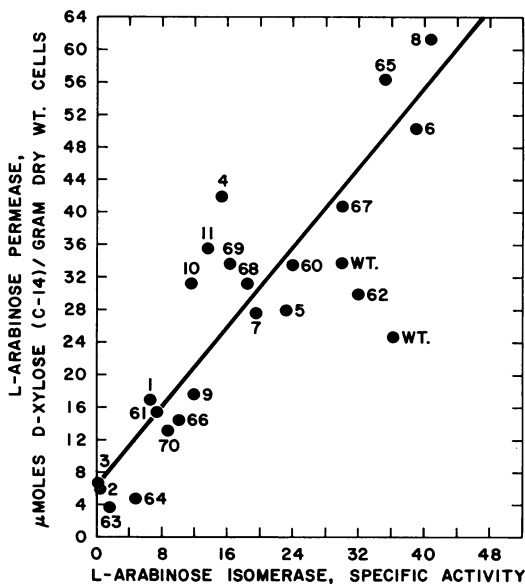


FIG. 7. *L*-Arabinose isomerase versus *L*-arabinose permease. The numbers denote particular constitutive mutants isolated from *Escherichia coli* B/r and B/r (hs). Points represent the average of permease assays from duplicate samples of the permease reaction mixture and the average of kinase activities of the duplicate extracts. The straight line was determined by the method of least squares; data from the wild type were not included.

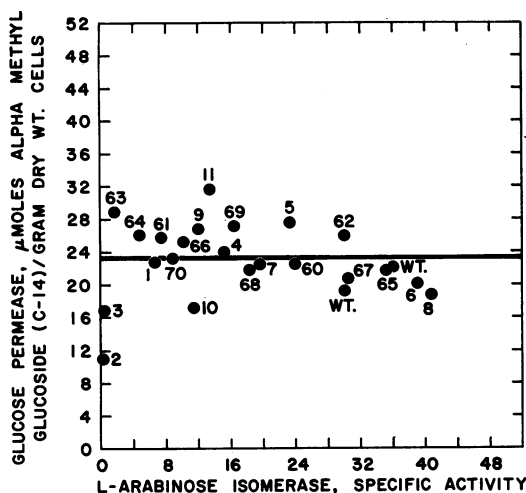


FIG. 8. *L*-Arabinose isomerase versus glucose permease (as in Fig. 7).

was initially isolated in B/r and transferred into K-12. The following experiments were conducted with five additional  $C^-$  mutants, this time with  $F^-$  B/r females (see Materials and Methods). In each case, merozygotes formed between *araA*<sup>-2</sup> males, and the  $C^-$  females produced substantially more *L*-arabinose isomerase than the controls (Table 1). Thus, all  $C^-$  mutants tested behave in the same manner as  $C$ -19:  $C^+$  is dominant to  $C^-$ . The isomerase structural gene on the same strand as the *araC*<sup>-</sup> mutations is functional if an *araC*<sup>+</sup> gene is present in the same cell.

In a second cross ( $A^-C^+ \times A^+C^-$ ), merozygotes from  $A^-C^+$  males and  $C^-$  B/r females, in the absence of the inducer *L*-arabinose, produced 6- to 30-fold more *L*-arabinose isomerase than the respective controls (Table 2). The results of these two types of crosses,  $A^-C^+ \times A^+C^-$  and  $A^-C^+ \times A^+C^+$ , are compatible and point to the same conclusion. The *C* gene produces a product required for the expression of the isomerase structural gene. The isomerase structural gene on the same strand as the *araC*<sup>-</sup> mutations is functional and can be activated if an *araC*<sup>+</sup> gene plus *L*-arabinose is present, or, in the case of a constitutive mutant, the presence of the  $C^+$  allele alone is sufficient. The results are exactly what one would have anticipated if control was purely positive.

In a third cross ( $A^-C^+ \times A^+C^+$ ), merozygotes from  $A^-C^+$  males and  $A^+C^+$  B/r females, in the absence of *L*-arabinose, failed in every case to yield enzyme activity significantly higher than determined in the unmated controls. This is the first evidence for the production of a repressor substance by the *C* gene.

**Deletion mutants.** If the *C* gene does produce an activating substance, i.e., if it does exert positive control over the expression of genes A, B, D, and E, a deletion in the *C* gene should produce an *ara*<sup>-</sup> phenotype. On the other hand, if control is only negative, a deletion should produce a constitutive phenotype. Deletions were isolated in the following manner. Growth of *araD*<sup>-53</sup> is inhibited by *L*-arabinose, and it is a simple matter to isolate hundreds of spontaneous *L*-arabinose-resistant mutants on EMB-*L*-arabinose agar plates. The great majority of these

TABLE 1. *L*-Arabinose isomerase production by merozygotes formed with *araA*<sup>-2</sup> males (Hfr) and different *araC*<sup>-</sup> females ( $F^-$ )\*

$C^-$ marker in $F^-$ parent	Units of <i>L</i> -arabinose isomerase/ml	
	Mating	Control
<i>araC</i> <sup>-</sup> 12	3.4	0.7
<i>araC</i> <sup>-</sup> 5	1.5	<0.1
<i>araC</i> <sup>-</sup> 37	1.8	0.1
<i>araC</i> <sup>-</sup> 47	2.2	0.8
<i>araC</i> <sup>-</sup> 59	0.8	0.1

\* The Hfr in each case was KH600 *araA*<sup>-2</sup> *str*<sup>s</sup>, and the females were B/r *araC*<sup>-</sup> *str*<sup>r</sup> (see Materials and Methods). The inducer *L*-arabinose was added 1 hr after the initiation of mating, and extracts were prepared from samples after 2 additional hr of incubation. Units of isomerase = micromoles of ribulose produced per hour.

TABLE 2. *L*-Arabinose isomerase production by merozygotes formed from *araA*<sup>-</sup>  $C^+$  males (Hfr) and *araC*<sup>-</sup> or *araC*<sup>+</sup> females ( $F^-$ )\*

Hfr	$F^-$	<i>L</i> -Arabinose isomerase activity/ml	
		Mating	Control
<i>araA</i> <sup>-2</sup> $C^+$ 8	<i>araC</i> <sup>-</sup> 37	1.0	<0.1
<i>araA</i> <sup>-</sup> H9 $C^+$ 11	<i>araC</i> <sup>-</sup> 3	0.6	0.1
<i>araA</i> <sup>-2</sup> $C^+$ 60	<i>araC</i> <sup>-</sup> 5	1.8	0.06
<i>araA</i> <sup>-2</sup> $C^+$ 62	<i>araC</i> <sup>-</sup> 5	1.7	0.06
<i>araA</i> <sup>-2</sup> $C^+$ 65	<i>araC</i> <sup>-</sup> 5	1.7	0.06
<i>araA</i> <sup>-2</sup> $C^+$ 8	$C^+$ (wild type)	0.2	0.1
<i>araA</i> <sup>-2</sup> $C^+$ 8	$C^+$ (wild type)	0.1	0.1
<i>araA</i> <sup>-2</sup> $C^+$ 60	$C^+$ (wild type)	0.08	0.12
<i>araA</i> <sup>-2</sup> $C^+$ 62	$C^+$ (wild type)	0.07	0.12
<i>araA</i> <sup>-2</sup> $C^+$ 65	$C^+$ (wild type)	0.01	0.12

\* The Hfr in each case was KH600 *araA*<sup>-</sup>  $C^+$  *str*<sup>s</sup>, and the females were B/r *araC*<sup>-</sup> *str*<sup>r</sup> or B/r *araC*<sup>+</sup> *str*<sup>r</sup>. No *L*-arabinose was added in these experiments. Extracts were prepared from cells 3 hr after the initiation of mating. See Table 1 for further details.

mutants are double L-arabinose-negative. They contain the original *araD*<sup>-53</sup> mutant site and, in addition, another mutation in the A, B, or C genes (Englesberg et al., 1962). A group of 200 L-arabinose-resistant mutants of *thr*<sup>-</sup> *araD*<sup>-53</sup> *leu*<sup>-</sup>, isolated on EMB-L-arabinose plates, were screened for deletions in the A, B, and C genes by crossing each of them with phage grown previously on *araA*<sup>-H9</sup>, *araA*<sup>-54</sup>, *araB*<sup>-87</sup>, *araB*<sup>-8</sup>, *araC*<sup>-12</sup>, and *araC*<sup>-37</sup>. Two deletion mutants were isolated that are of interest to this study. *AraD*<sup>-53,201</sup> failed to yield any *ara*<sup>+</sup> recombinants with any of the phage employed, although *leu*<sup>+</sup> recombinants were plentiful. *Ara*<sup>+</sup> recombinants were isolated with crosses with *araD*<sup>-139</sup>; thus, it appears that the deletion extends from the *araC*<sup>-37</sup> mutant site in the C gene through the B and A genes and possibly further into the D gene. A second deletion, *araD*<sup>-53,204</sup>, gave recombinants with both *araC*<sup>-37</sup> and *araC*<sup>-12</sup>, but failed to yield recombinants with B or A mutants. Crosses with *araD*<sup>-53,204</sup> and *araC*<sup>-12</sup>, in which the per cent of *ara*<sup>+</sup> recombinants to *leu*<sup>+</sup> recombinants were scored, placed the right end of the 204 deletion 0.65 map units to the left of C<sup>-12</sup>, which may place it within the C gene.

Enzymatic analysis of cell-free extracts of both deletion mutants prepared from cells grown in a casein hydrolysate-L-arabinose medium (Cribbs and Englesberg, 1964) showed them to be devoid of L-arabinose isomerase and L-ribulokinase activity, as would be expected, since both deletions included genes A and B. However, both deletion mutants were also deficient in L-arabinose permease determined by gene E, which is not included in the deletion.

#### DISCUSSION

Based upon the analysis of the  $\beta$ -galactoside gene-enzyme complex (Jacob and Monod, 1961a, b; Pardee, Jacob, and Monod, 1959), it is generally inferred that the transfer of all information from deoxyribonucleic acid (DNA) to the final synthesis of enzyme molecules is under negative (repressor) control, with no further specific genetic element intervening in the regulation (Willson et al., 1964). This system of control, as initially conceived, was composed of essentially three elements: one or more structural genes, and an operator (O) and a regulator gene (i). The concept of the operator was derived from an analysis of two types of mutants in this system: Lac O<sup>-</sup> (operator-negative) and Lac O<sup>c</sup> (operator-constitutive). Both types were originally thought to map at the end of the *z* gene, the  $\beta$ -galactosidase structural gene, and exert a pleiotropic effect. O<sup>-</sup> mutants are phenotypically  $\beta$ -galacto-

sidase-,  $\beta$ -galactoside permease-, and  $\beta$ -galactoside transacetylase-negative. O<sup>c</sup> mutants produce all three enzymes constitutively. In complementation studies, both types of mutation exert a dominant effect on the control of the adjacent structural genes (*z*, *y*, *Ac*) for the above enzymes (*cis* dominant), but exert no effect on the same genes in another strand of DNA (*trans* recessive). Some revertants of O<sup>-</sup> mutants, closely linked to the original O<sup>-</sup> site, produced an altered  $\beta$ -galactosidase molecule (Jacob and Monod, 1961b). From these results, together with evidence from complementation studies with *i* gene mutants, Jacob and Monod (1961b) proposed that the end of the *z* gene, the operator region, was the site for repressor attachment and for initiation of messenger ribonucleic acid (mRNA) synthesis. [In a recent paper, Jacob, Ullman, and Monod (1964) have separated these two functions. The site for repressor attachment (operator region) is now thought to be outside the *z* cistron and is defined solely by O<sup>c</sup> mutants, whereas the site for initiation of transcription, now called the promoter area (P), remains part of this gene.]

According to the model proposed by Jacob and Monod (1961a, b), the O<sup>-</sup> and O<sup>c</sup> phenotypes were the result of changes in the DNA in the operator region, preventing transcription and repressor attachment, respectively. Attardi et al. (1963) were not able to detect any Lac mRNA synthesis by Lac O<sup>-</sup> mutants, thereby offering support for this hypothesis. However, the finding of O<sup>-</sup>-like mutations in the middle of various structural genes which exert a pleiotropic negative effect in a polar direction (dual effect or polarity mutants; Englesberg, 1961; Franklin and Luria, 1961; Jacob and Monod, 1961b; Lee and Englesberg, 1963; Ames and Hartman, 1963), together with the finding that it is possible to obtain unlinked suppressors to Lac O<sup>-</sup> mutations (Beckwith, 1963; Orlas and Gartner, 1964; Schwartz, 1964), indicates that the effect of O<sup>-</sup> mutations may be at the level of messenger reading rather than at the level of transcription. This leaves only O<sup>c</sup> mutants to define the operator region.

The regulator gene (*i*) was defined on the basis of an analysis of two types of mutations: *i*<sup>-</sup> (constitutive) and *i*<sup>s</sup> (super-repressed). The former are constitutive, whereas the latter are negative for the three enzymes in the system. In complementation analysis, *i*<sup>-</sup> is recessive to *i*<sup>+</sup>, (Pardee et al., 1959; Jacob and Monod, 1961b), and *i*<sup>s</sup> is dominant to *i*<sup>-</sup> and *i*<sup>+</sup> (Jacob and Monod, 1961b; Willson et al., 1964). On the basis of these experiments, negative control by a repressor substance was formulated. According to



this hypothesis, the *i* gene in the wild-type configuration produces a repressor. The repressor has two sites of attachment. The inducer reacts with the repressor at one site and, by an allosteric effect, turns it into an inactive substance. In the absence of the inducer, the other site of the repressor is free to react with the operator region. It is this reaction that prevents transcription. The *i*<sup>s</sup> mutants produce a repressor which has lost the site for inducer attachment, and thereby cannot be neutralized. Constitutive *i*<sup>-</sup> mutants fail to produce repressor or *any biologically active* entity. In the absence of an active repressor, the transfer of information flows freely from genes *z*, *y*, and *A*. Is this the only type of control of gene expression found in microorganisms?

Similar types of experiments which, in the  $\beta$ -galactosidase gene complex, indicated a negative control system have led to defining a system that exhibits both a negative and positive control in the *L*-arabinose gene complex.

The regulator gene in the *L*-arabinose system, gene *C*, is adjacent to, but functionally distinct from, gene *B*. Mutations in gene *C* are of two types: *C*<sup>-</sup> (phenotypically kinase-, isomerase-, epimerase-, and permease-negative) and *C*<sup>c</sup> (constitutive for all three enzymes and permease).

Is this *C* region the operator region of the *L*-arabinose gene complex? Are *C*<sup>-</sup> and *C*<sup>c</sup> mutants, which define this region, similar to *Lac* *O*<sup>-</sup> and *Lac* *O*<sup>c</sup> mutants in the  $\beta$ -galactosidase system?

The fact that *C*<sup>-</sup> mutants are deficient in *L*-arabinose permease by itself would rule out the possibility that the *C*<sup>-</sup> mutants are similar to *Lac* *O*<sup>-</sup> mutants, since the *L*-arabinose permease gene is unlinked to the *C* region of the chromosome. Whether *Lac* *O*<sup>-</sup> mutants, as originally defined by Jacob and Monod (1961b), really exist, or whether they are really polarity mutants, control by these mutants probably rests either upon continuous transcription or translation in a polar fashion of an intact DNA or mRNA sequence. The fact that the *E* gene for the *L*-arabinose permease is approximately one-half the total length of the chromosome away from the *C* gene makes this an impossibility. The *C* gene would then have to control not only *L*-arabinose metabolism but something like one-half of the metabolic activities of the cell.

Complementation analysis with temporary merozygotes furnishes further evidence that the *C* region is not the operator region of the *L*-arabinose gene complex. Six *C*<sup>-</sup> mutants defining the *C* region were shown to be recessive to *C*<sup>+</sup>. In particular, in the cross *A*-*C*<sup>+</sup>  $\times$  *A*<sup>+</sup>*C*<sup>-</sup>, the temporary merozygotes produce *L*-arabinose isomerase, demonstrating that *C*<sup>-</sup> is comple-

mented by mutants in the *A* gene and, therefore, that the isomerase gene on the strand of DNA containing the *C*<sup>-</sup> mutation is activated by *C*<sup>+</sup>. *C*<sup>+</sup> must be producing some diffusible product required to activate the *C*<sup>-</sup> strand, whereas the operator region in the  $\beta$ -galactosidase system apparently does not produce such a product. If this mutation were of an *O*<sup>c</sup> type, no isomerase would be produced in this cross.

*C*<sup>c</sup> mutants are constitutive for the inducible enzymes and permease in *L*-arabinose metabolism. These mutants differ from *Lac* *O*<sup>c</sup> mutants. *C*<sup>c</sup> is dominant to *C*<sup>-</sup>. In the cross *A*-*C*<sup>c</sup>  $\times$  *A*<sup>+</sup>*C*<sup>-</sup>, *L*-arabinose isomerase is produced constitutively, indicating that *C*<sup>c</sup> produces a product which is able to activate the *C*<sup>-</sup> strand in the absence of *L*-arabinose. If *C*<sup>c</sup> were the same type of allele as *Lac* *O*<sup>c</sup>, no *L*-arabinose isomerase activity would be produced, since *O*<sup>c</sup> is only *cis* dominant. We must, therefore, conclude that the *C* region is not the operator region of the *L*-arabinose system.

Is the *C* gene a regulatory gene of the *i* type? In the merozygotes produced by the cross *A*-*C*<sup>c</sup>  $\times$  *A*<sup>+</sup>*C*<sup>+</sup>, no *L*-arabinose isomerase is formed in the absence of *L*-arabinose; *C*<sup>+</sup> (inducibility) is dominant to *C*<sup>c</sup>. In this respect, the *C* gene is similar to the *i* gene in the  $\beta$ -galactosidase system, but here the similarity ceases. No *i*<sup>s</sup>-like mutants have been uncovered in the *C* gene, and no *C*<sup>-</sup>-like mutants have been found in the *i* gene. *C*<sup>-</sup> is distinct from *i*<sup>s</sup> since *C*<sup>-</sup> is recessive to *C*<sup>+</sup> and *C*<sup>c</sup>, whereas *i*<sup>s</sup> is dominant to *i*<sup>+</sup> and *i*<sup>-</sup>. Using analogous reasoning, as employed in deriving the negative control model, we arrive at the following. The dominance of *C*<sup>+</sup> over *C*<sup>c</sup>, in the absence of inducer, indicates that the *C*<sup>+</sup> gene produces a repressor. However, the fact that both *C*<sup>+</sup> and *C*<sup>c</sup> are dominant over *C*<sup>-</sup> indicates that *C*<sup>-</sup> does not produce a repressor; therefore, *C*<sup>c</sup>, in the absence of *L*-arabinose, and *C*<sup>+</sup>, in the presence of *L*-arabinose, produce a cytoplasmic substance which is required to activate the transfer of information from the *L*-arabinose structural genes to protein. We shall call this substance "activator." This is an example of positive control—a switching-on mechanism that is necessary for gene expression. Although by an elaborate series of *ad hoc* postulates it may be possible to explain these results on the basis that the *C* gene is solely a repressor gene, the experiments with the deletion mutants offer further evidence supporting the activator function of the *C* gene. A deletion in the *i* gene and in general in any regulatory gene of a negative control system leads to constitutive enzyme synthesis. The deletions in the *C* gene lead to failure of expression of the unlinked *E* gene.

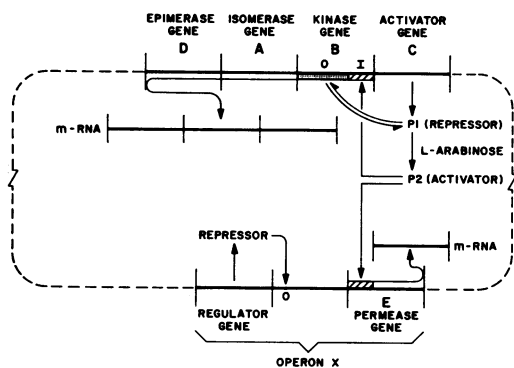


FIG. 9. Model for regulation in the *L*-arabinose system.

The R1 gene in the alkaline phosphatase system (Garen and Echols, 1962a, b) bears a resemblance to the C gene in that both exert positive control over gene expression. That such control exists in the alkaline phosphatase system is based mainly upon the finding that phosphatase-negative mutants (C mutants) that may map within the R1 gene are recessive to wild type. In addition, under conditions of nonrepression (low concentration of phosphate), most constitutive mutants (R1<sup>-</sup>) produce less phosphatase than the parent strain, and this trait is recessive to wild type. There are, however, certain differences between the R1 and C genes. Although negative mutants in both genes are recessive to wild type, many of the mutants in the R1 gene are dominant to R1<sup>-</sup> (constitutive), whereas all negative mutants in the C gene (C<sup>-</sup>) so far tested are recessive to C<sup>+</sup>. In addition the R1 gene acts in conjunction with another regulatory gene (R2), whereas the C gene appears, so far, to be the only regulatory gene (if we exclude the B gene) in the *L*-arabinose system.

We shall consider one model (Fig. 9) to explain the results presented and to guide us in future experiments. We propose that the C gene produces a product P1, the repressor, presumably a protein. P1 has two active sites: one for attachment to an operator region in the B gene and the other for *L*-arabinose. By an allosteric transition, *L*-arabinose converts P1 to P2, the activator. This system is so far somewhat similar to that proposed for the  $\beta$ -galactosidase system. It differs in that instead of the repressor being converted into an inert substance, we propose that, in the *L*-arabinose system, it is converted to a *biologically necessary* entity, P2. P2 activates the BAD region and the E gene for transcription at initiation sites of attachment (I), which we propose are at the beginning of the B and E genes. (There is

little, if any, evidence at present to suggest where P1 and P2 act in the transfer of information from gene to protein. However, for the sake of simplicity, the model shows all the control at the transcription level.) C<sup>-</sup> alleles fail to produce a substance that has any repressor function or that can be converted into an activator by *L*-arabinose. Thus, C<sup>+</sup> is dominant to C<sup>-</sup> (in the presence of *L*-arabinose). In merozygotes A<sup>-</sup>C<sup>+</sup>  $\times$  A<sup>+</sup>C<sup>-</sup>, in the presence of *L*-arabinose, the C product, P1, is converted to P2, which activates the strand containing the C<sup>-</sup> allele. C<sup>c</sup> mutant sites, all of which map within the C gene, alter the amino acid sequence of the C product so that altered proteins (P3, P4, P5, etc.) are formed by the different constitutive mutants. These proteins may function partially or fully as activators in the absence of *L*-arabinose. (Some may still have some weak repressor capabilities.) In merozygotes between C<sup>c</sup> and C<sup>-</sup> (A<sup>-</sup>C<sup>c</sup>  $\times$  A<sup>+</sup>C<sup>-</sup>), in the absence of *L*-arabinose, C<sup>-</sup> does not produce repressor or activator, whereas C<sup>c</sup> produces activator and, therefore, activates the BAD and E region for protein synthesis. In merozygotes A<sup>-</sup>C<sup>c</sup>  $\times$  A<sup>+</sup>C<sup>+</sup>, in the absence of *L*-arabinose, C<sup>+</sup> produces repressor and C<sup>c</sup> produces activator, but no *L*-arabinose enzymes are produced. Although such merozygotes were produced with hyperconstitutives, and enzyme assays were conducted for periods up to 3 hr after mating, no significant enzyme activity was detected in the merozygotes over that of the controls. Therefore, we propose that P1 and P2 or P3, etc., act at different sites and that only *L*-arabinose can neutralize the function of repressor, P1.

Since the different constitutive mutants produce various but coordinate levels of isomerase, kinase (and probably epimerase), and permease, we propose that the initiation sites in the B and E genes must be genetically similar to respond in this manner.

This model also provides an explanation for the dual effects of mutations in the B gene. Mutations in this kinase structural gene affect a coordinate increase or decrease in inducible levels of isomerase, epimerase, and kinase protein. Evidence indicates that mutation in the A gene has no effect on levels of epimerase activity. Thus, modulation as an explanation for the polarity mutants in the histidine system (Ames and Hartman, 1963) may not apply in this case. To explain our results, we further propose that most of the B gene, except that portion adjacent to the C gene (I or initiator region), serves as the site for P1 attachment, i.e., the operator. One can explain the dual effect on the basis that changes in base sequence in the O region of the B gene

affect its affinity for P1, thereby modifying the equilibrium between the components of this system.  $B \text{ gene-O-P1} \rightleftharpoons P1 \rightleftharpoons P1 + L\text{-arabinose} \rightarrow P2$ . In some cases, mutation leads to a looser, and in other cases to a tighter, attachment to B. The rate at which P1 is removed from the B gene may determine the rate at which the message BAD is formed. Of course, since the permease gene is unlinked to BAD, its rate of synthesis is not so affected by mutation in the B gene (Novotny and Englesberg, 1964).

What is the biological significance of this control system that combines both a negative and a positive controlling element? We propose that such a system is required in coordinate control involving genes in different operon systems. In the L-arabinose system, the permease gene is unlinked to the other structural genes, and the C gene is apparently adjacent to gene B in the BAD genetic unit of coordinate expression. Since there is selective advantage in the bacteria for adjacent linkage of genes controlling a particular biochemical pathway, we have assumed that the L-arabinose permease gene is part of another control system or operon (operon X), and that positive control, in the form of P2, is required to remove the E gene from the control exerted by this other system. Similarly, in the CBAD area of the chromosome, message synthesis of the C gene must occur and stop at B in the absence of activator. We propose that P2 also functions to activate the BAD strand.

Why is the repressor needed at all? The activator-repressor system may function as a double lock. Activator may be similar to a master key, perhaps being less specific and thereby controlling a number of regions on the chromosome, whereas P1 is the specific element removable only by the specific inducer L-arabinose.

It should be realized that this proposed model may have no more than a limited conceptual utility. However, it makes several predictions that can be experimentally verified. For instance, it proposes that gene E is not subject to control by repressor P1; therefore, in merozygotes of  $E^-C^+ \times E^+C^+$ , constitutive permease synthesis should occur. It also predicts that permease may be induced by another inducer besides L-arabinose. Further, it predicts the following types of mutants that so far we have not obtained:  $C^-$  *cis* and *trans* dominant—a mutation producing a repressor that cannot be converted into an activator;  $I^-$ —a mutation in the initiator area which fails to respond to activator and is *cis* dominant for isomerase, kinase, and epimerase, but still produces an inducible permease.

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#### LITERATURE CITED

- AMES, B., AND P. HARTMAN. 1963. The histidine operon. Cold Spring Harbor Symp. Quant. Biol. **28**:349-356.
- ATTARDI, G., S. NAONO, J. ROUVIÈRE, F. JACOB, AND F. GROS. 1963. Production of messenger RNA and regulation of protein synthesis. Cold Spring Harbor Symp. Quant. Biol. **28**:363-372.
- BECKWITH J. 1963. Restoration of operon activity by suppressor. Biochim. Biophys. Acta **76**:162-164.
- BOYER, H. 1964. Genetic control of restriction and modification in *Escherichia coli*. J. Bacteriol. **88**:1652-1660.
- BOYER, H., E. ENGLESBERG, AND R. WEINBERG. 1962. Direct selection of L-arabinose negative mutants of *Escherichia coli* B/r. Genetics **47**:417-425.
- CRIBBS, R., AND E. ENGLESBERG. 1964. L-Arabinose negative mutants of the L-ribulokinase structural gene affecting the levels of L-arabinose isomerase in *Escherichia coli*. Genetics **49**:95-108.
- ENGLESBERG, E. 1961. Enzymatic characterization of 17 L-arabinose negative mutants of *Escherichia coli*. J. Bacteriol. **81**:996-1006.
- ENGLESBERG, E., R. L. ANDERSON, R. WEINBERG, N. LEE, P. HOFFEE, G. HUTTENHAUER, AND H. BOYER. 1962. L-Arabinose-sensitive, L-ribulose 5-phosphate 4-epimerase-deficient mutants of *Escherichia coli*. J. Bacteriol. **84**:137-146.
- ENGLESBERG, E., J. IRR, AND J. POWER. 1964. Evidence for a new type of regulatory gene in the L-arabinose gene enzyme complex. Bacteriol. Proc., p. 19.
- ENGLESBERG, E., AND N. L. KILLEEN. 1959. Mixed gene loci controlling L-arabinose isomerase and L-ribulokinase activities in *Escherichia coli* B/r. Genetics **44**:508-509.
- ENGLESBERG, E., J. WATSON, AND P. HOFFEE. 1961. The glucose effect and the relationship between glucose permease, acid phosphatase and glucose resistance. Cold Spring Harbor Symp. Quant. Biol. **26**:261-276.
- FRANKLIN, N. C., AND S. E. LURIA. 1961. Transduction by bacteriophage P1 and the properties of the lac genetic region in *E. coli* and *S. dysenteriae*. Virology **15**:299-311.
- GAREN, A., AND H. ECHOLS. 1962a. Properties of

- two regulating genes for alkaline phosphatase. *J. Bacteriol.* **83**:297-300.
- GAREN, A., AND H. ECHOLS. 1962b. Genetic control of induction of alkaline phosphatase synthesis in *E. coli*. *Proc. Natl. Acad. Sci. U.S.* **48**:1398-1402.
- GROSS, J., AND E. ENGBERG. 1959. Determination of the order of mutational sites governing L-arabinose utilization in *Escherichia coli* B/r by transduction with phage P1bt. *Virology* **9**:314-331.
- HELLING, R. B., AND R. WEINBERG. 1963. Complementation studies of arabinose genes in *Escherichia coli*. *Genetics* **48**:1397-1410.
- HOFFEE, P., AND E. ENGBERG. 1962. Effect of metabolic activity on the glucose permease of bacterial cells. *Proc. Natl. Acad. Sci. U.S.* **48**:1759-1765.
- ISAACSON, D., AND E. ENGBERG. 1964. L-Arabinose inhibition of growth and isolation of L-arabinose permeaseless, cryptic mutants of *Escherichia coli* B/r. *Bacteriol. Proc.*, p. 113-114.
- JACOB, F., AND J. MONOD. 1961a. Genetic regulatory mechanisms in the synthesis of proteins. *J. Mol. Biol.* **3**:318-356.
- JACOB, F., AND J. MONOD. 1961b. On the regulation of gene activity. *Cold Spring Harbor Symp. Quant. Biol.* **26**:193-211.
- JACOB, F., A. ULLMAN, AND J. MONOD. 1964. Le promoteur, élément génétique nécessaire à l'expression d'un opéron. *Compt. Rend.* **258**:3125-3128.
- LEE, N., AND E. ENGBERG. 1962. Dual effects of structural genes in *Escherichia coli*. *Proc. Natl. Acad. Sci. U.S.* **48**:335-348.
- LEE, N., AND E. ENGBERG. 1963. Coordinate variations in induced syntheses of enzymes associated with mutations in a structural gene. *Proc. Natl. Acad. Sci. U.S.* **50**:696-702.
- LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- NOVOTNY, C., AND E. ENGBERG. 1964. The L-arabinose permease of *Escherichia coli*, B/r. *Proc. Intern. Congr. Biochem.*, 6th, New York **3**:46.
- ORIAS, E., AND T. K. GARTNER. 1964. Suppression of a class of rII mutants of T4 by a suppressor of a lac "operator negative" mutation. *Proc. Natl. Acad. Sci. U.S.* **52**:859-864.
- PARDEE, A. B., F. JACOB, AND J. MONOD. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of  $\beta$ -galactosidase by *E. coli*. *J. Mol. Biol.* **1**:165-178.
- SCHWARTZ, N. 1964. Suppression of a lac  $O^o$  mutation in *Escherichia coli*. *J. Bacteriol.* **88**:996-1001.
- WILLSON, C., D. PERRIN, M. COHN, F. JACOB, AND J. MONOD. 1964. Non-inducible mutants of the regulator gene in the "lactose" system of *Escherichia coli*. *J. Mol. Biol.* **8**:582-592.
- WOLLMAN, E. L., F. JACOB, AND W. HAYES. 1956. Conjugation and genetic recombination in *Escherichia coli*. *Cold Spring Harbor Symp. Quant. Biol.* **21**:141-162.