THE L-RHAMNOSE GENETIC SYSTEM IN *ESCHERICHIA COLI* K-12

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IN 1961 JACOB and MONOD reviewed the general state of our knowledge concerning genetic regulatory mechanisms involved in protein synthesis. They cited evidence that the induction of the lactose utilizing enzymes in *Escherichia coli* was mediated through the inactivation of an internal repressor by the externally given inducer. Such a model of "negative control" implies that there is no other regulatory element required for the expression of structural genes once the repressor is removed (WILLSON *et al.* 1964). While crucial evidence was lacking in other systems, they speculated that this type of mechanism could account for all cases of enzyme induction and repression in bacteria. In repressible systems the repressor would be inactivated when the end product of the biosynthetic pathway (co-repressor) is removed.

Although this model has been supported in several cases (A regulator gene in which constitutive alleles are recessive to wild type has been found in the arginine system (MAAS and CLARK 1964), and operator regions have been demonstrated in the galactose (BUTTIN 1963), isoleucine-valine (RAMAKRISHNAN and ADELBERG 1965), and tryptophan (MATSUSHIRO *et al.* 1965) systems all in *E. coli.*) there are at least two genetic systems which are at variance with it. In the control of alkaline phosphatase synthesis in *E. coli*, GAREN and ECHOLS (1962) have presented evidence for the presence to two regulatory elements, R1 and R2. It was proposed that R1 produces an inducer which, in the presence of high concentrations of inorganic phosphate and the product of the second regulatory gene R2, is converted into a repressor. The inducer has a positive but yet unknown function in controlling the synthesis of alkaline phosphatase. In the L-arabinose gene-enzyme complex of *E. coli* B/r it has been quite conclusively shown that this system is under the positive control of a single regulatory gene *araC*, and that the role of the inducer is to mediate the production by gene *C* of an activator which facilitates gene expression (IRR and ENGLESBERG 1967; SHEPPARD and ENGLESBERG 1966, 1967).

In view of the variety of possible mechanisms for induction or repression and the paucity of systems in which adequate genetic data are available, it seemed useful to study the control of enzyme synthesis in other systems. The utilization of L-rhamnose by *E. coli* K-12 appeared to be a good choice for this purpose. The enzymes involved in L-rhamnose utilization in *E. coli* have been elucidated and characterized (CHIU and FEINGOLD 1964, 1965; TAKAGI and SAWADA 1964). This

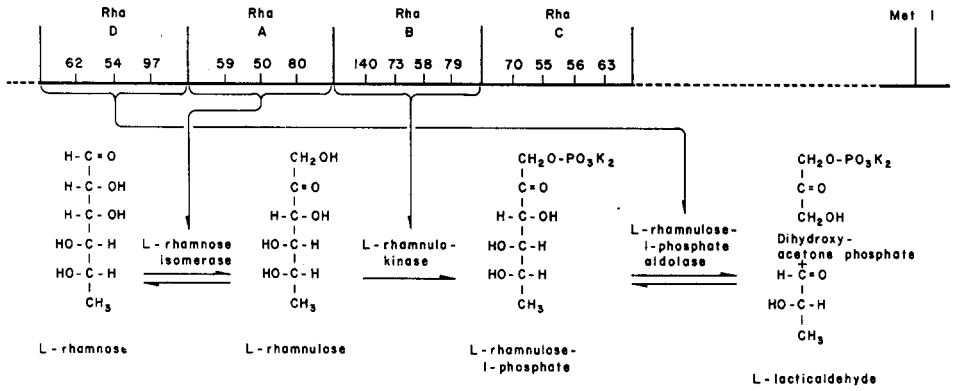

FIGURE 1.—The pathway for L-rhamnose metabolism with the enzymes involved and the genes corresponding to these enzymes. The order of the genes relative to the *metB1* marker is shown. Gene *C* is a regulatory gene controlling induction of these enzymes.

sugar is metabolized by three inducible enzymes (Figure 1). A region of the chromosome concerned with L-rhamnose utilization is linked to the *metB1* (methionine requiring) marker, indicating the feasibility of genetic mapping in this area. A strain of *E. coli* K-12 was available (F14 AB 1206) which carries this rhamnose region on an episome and might therefore be used in complementation analysis. In addition, earlier studies with other bacteria suggested that the genes involved in L-rhamnose utilization might exist as a genetic unit of coordinate expression. ENGLESBERG (1957) showed that as a result of a single mutational event *Pasteurella pestis* gained the ability to produce the first two enzymes involved in the L-rhamnose pathway. Evidence was presented that this multiple effect was not the result of sequential induction or repair of an L-rhamnose permease. Similarly, ENGLESBERG and BARON (1959) showed that a single step mutation in *Salmonella typhimurium* LT2 could result in the simultaneous loss of the same two enzymes. In light of the evidence they presented and more recent studies it appeared that the latter work might represent the first observation of a pleiotropic effect of a mutation in a genetic regulatory element.

This paper represents an initial attempt to define the L-rhamnose gene-enzyme complex in *E. coli* K-12.

MATERIALS AND METHODS

Organisms: The strains of *E. coli* K-12 employed are described in Table 1. Phage P1kc was obtained from C. YANOFSKY through E. ORIAS and was grown according to the method of LURIA, ADAMS and TING (1960).

Media: The mineral base used was described by GROSS and ENGLESBERG (1959). Carbohydrates (0.2% final conc.), amino acids (40 μg/ml final conc.) and thiamine (4 μg/ml final conc.) were added as indicated. Nutrient broth and casein hydrolysate were from Difco.

Isolation of L-rhamnose nonutilizing (Rha⁻) mutants: Strain P72 was grown overnight with shaking at 37°C in nutrient broth to the stationary phase. N-methyl-N-nitroso-N′-nitroguanidine was added to a final concentration ranging from 80 to 160 μg/ml and the culture was held without shaking at 37°C for 1 hour. After dilution samples were plated on Endo's agar plates containing 1% L-rhamnose. After two days at 37°C rhamnose utilizing colonies had turned red; the white

TABLE 1

Strains of bacteria employed

Strain	Source	Characteristics
P72	F. JACOB	*metB1* F+ revertant of Hfr P72
AB 1203	E. ADELBERG through D. KESSLER	*thi-1 ilv-7 arg-3 xyl-5 gal-2 lac-1 or 4* F⁻, resistant to phages T4 and T6
AB 1206	E. ADELBERG through S. FALKOW	*his-4 thi-1 pro-2* F'14, streptomycin resistant, F14 region deleted in endogenote
AB 1450	E. ADELBERG through T. GARTNER	*ilv-16 metB1 arg-1 his-1 thi-2 xyl-4 mal-1* F⁻, resistant to phages T6 and λ and to streptomycin

All bacteria are *E. coli* K-12. Symbols used in this paper conform to the recommendations of DEMEREC *et al.* (1966): three-letter, lower-case italicized symbols for genetic loci followed by an isolation number, and three letter symbols with the first capitalized to indicate phenotypes. Loci leading to nutritional requirements are: *met*, methionine; *ilv*, isoleucine and valine; *his*, histidine; *arg*, arginine; *thi*, thiamine; *pro*, proline. Loci for ability to utilize carbohydrates are: *xyl*, D-xylose; *lac*, lactose; *gal*, D-galactose; *mal*, maltose.

nonutilizing colonies were picked and purified on nutrient agar and saved. Some of these mutants have already been described (CHIU *et al.* 1967).

Transductions: All transductions were carried out with phage P1kc by the procedure of GROSS and ENGLESBERG (1959). For spot transductions two drops of L broth containing 5×10^{-3} M $CaCl_2$ were inoculated with strains to be tested and grown 6 hours at 37°C. Two drops of phage lysate were then added, and after an additional ½-hour incubation, the mixtures were spread onto agar plates.

Ordering of Rha⁻ mutants by reciprocal three factor crosses: met+ derivatives of rhamnose negative P72 strains were constructed by transduction with phage P1kc previously grown on a prototrophic P72 strain. Phage was then grown on these methionine positive strains through two cycles and was then used to transduce Met⁻ Rha⁻ strains to Rha+ with selection on mineral rhamnose methionine agar. The Rha+ recombinants were then replica plated to mineral rhamnose agar to score for the Met+ phenotype. Similar crosses were carried out using the same pair of rhamnose markers in reciprocal positions. The theory involved in the use of reciprocal three-factor crosses to order mutant sites is described by GROSS and ENGLESBERG (1959).

Preparation of cell free extracts: The mutant strains were reisolated immediately before use to minimize the number of revertants. They were grown in mineral base containing 1% casein hydrolysate plus 0.4% L-rhamnose as an inducer for three to four generations. The cultures were then harvested as described in CRIBBS and ENGLESBERG (1964). A 1.5 ml volume of the final cell suspension was broken by three 10-second pulses with a Branson sonifier model S75 using a micro tip and 2 amperes current.

Enzyme assays: L-rhamnose isomerase was measured in a reaction mixture containing per ml: 50 μmoles L-rhamnose, 2.5 μmoles $MnCl_2$, 125 μmoles of pH 7.6 glycylglycine buffer and crude extract. At various times 0.1 ml aliquots were removed and assayed for L-rhamnulose production using the cysteine-carbazole method with the calibration factor determined by PALLERONI and DOUDOROFF (1956) for D-rhamnulose. The *in vivo* L-rhamnose isomerase assay is similar except that 300 μmoles of pH 8.0 sodium borate is used as a buffer and as a trapping agent for L-rhamnulose and whole cells are employed. L-rhamnulokinase and L-rhamnulose 1-phosphate aldolase were measured by the one step methods of CHIU and FEINGOLD (1964 and 1965) using extracts which had been precipitated overnight with 0.05 M $MnCl_2$. According to these authors, this treatment removes interfering nicotinamide adenine dinucleotide oxidase activity without any loss of the two enzymes being measured. All specific activities are reported as μmoles product per hour per mg protein. Protein was estimated by the method of LOWRY *et al.* (1951) using crystalline bovine serum albumin (California Corp. for Biochemical Research) as a standard.

Matings: Overnight nutrient broth cultures of male and female strains were diluted with an

equal portion of fresh broth and incubated for 1 hour at 37°C with shaking. Mating was then carried out for 1 hour with a mixture of equal volumes of each parent culture at 37°C.

Growth experiments: Mutants were grown overnight in 0.2% casein hydrolysate mineral medium. In the morning they were diluted into duplicate tubes of fresh 0.15% casein hydrolysate mineral base one with and one without 0.2% L-rhamnose. Measurements of turbidity were carried out as described by GROSS and ENGLESBERG (1959). After stationary phase was reached, an 0.1 ml aliquot of the rhamnose containing culture was assayed for accumulation of the intermediate L-rhamnulose.

Construction of partial diploids: The F14 strain AB 1206 is unusual in several respects (PITTARD and RAMAKRISHNAN 1964). It has about 10% of the length of the *coli* chromosome as an episome carrying *arg*, *met*, *rha* and *ilv* genes. The corresponding section of the chromosome is deleted. Therefore, strains carrying *metB1* and various *rha* markers on this episome could be constructed by transduction. F14 diploids are very unstable. To obtain partial diploid strains these F14 donors were crossed with *met*+ derivatives of AB 1450 carrying various *rha* markers and plated onto mineral glucose histidine thiamine agar. This selected for strains possessing the *metB*+ allele on the chromosome and the *arg*+ and *ilv*+ alleles on the episome and eliminated most classes of recombinants which are auxotrophic for one of these amino acids. Subsequent picking to mineral rhamnose histidine thiamine agar gave growth in 24 hours in the case of diploids with complementing pairs of *rha* mutations. With noncomplementing pairs growth was negative in 24 hours, but by 48 hours Rha+ recombinant colonies began to appear. Approximately one fourth of all colonies picked were completely Rha− and were presumably *arg*+ *met*+ *ilv*+ recombinants. Figure 2 illustrates these three responses.

Analysis of diploid structure: After purification of partial diploid strains by twice isolating single colonies on mineral glucose histidine thiamine agar, diploid structure was verified by allowing segregation to occur during growth for approximately 20 generations in nutrient broth. Single colonies were then grown on nutrient agar, and Rha−, Met− or Arg− segregants were

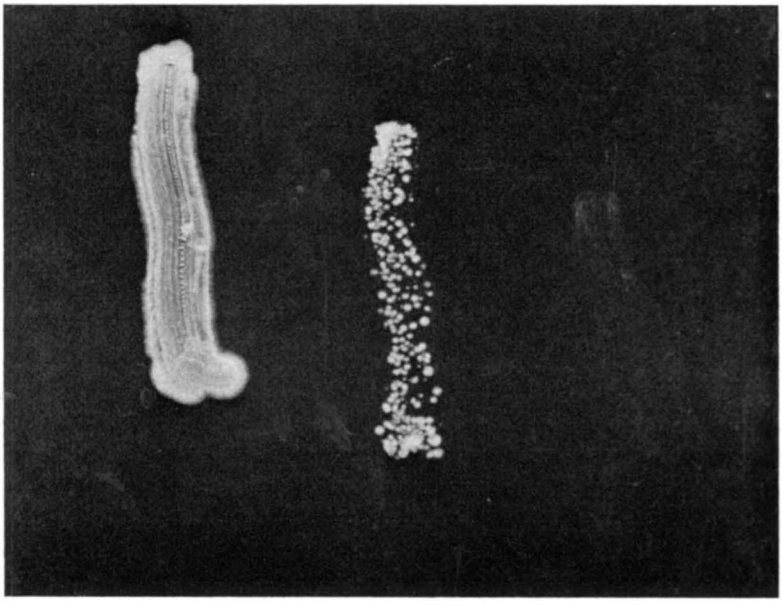

FIGURE 2.—Complementation responses after growth on rhamnose histidine thiamine agar for 48 hours. At left a complementing diploid shows heavy growth. In the middle a noncomplementing diploid shows recombinant colonies coming up from a background of nongrowing cells. On the right a haploid recombinant is completely unable to grow.

detected by replica plating. In some cases the *rha* marker present in these segregants was determined by progeny tests using spot transductions with phage carrying both parental markers. Almost always the *rha* marker found could be predicted by whether the Met phenotype of the segregant was that of the male or female parent since the *metB1* marker is so closely linked to *rha*. Owing to the scarcity of Met$^-$ segregants, the presence of the original male *rha* marker was sometimes demonstrated by mating the diploid to F$^-$ AB 1203, obtaining Rha$^-$ segregants from the new diploid which should contain only the original episomal *rha* marker, and progeny testing these segregants.

RESULTS

Grouping of rhamnose negative mutants: Growth experiments were performed on all of the mutants isolated. Those cultures which showed a much slower growth rate than the wild-type culture in casein hydrolysate were discarded. They probably contained secondary mutations since the nitrosoguanidine used is such a powerful mutagen. As with L-arabinose negative mutants (GROSS and ENGLESBERG 1959) all other L-rhamnose negative mutants could be placed into one of the four classes described in Table 2. Several mutants from each class were selected for further study.

Ordering of mutant sites: All of the *rha* mutants could be cotransduced with *metB1* at a frequency of about 20%, showing that all the mutant sites are located on the same small segment of the chromosome. They were ordered within this region by reciprocal three-factor crosses. Data for these crosses are shown in Figure 3. The mutant sites in the most probable map order from farthest to nearest *metB1* are arranged left to right and top to bottom. Accordingly, all crosses requiring simply a double crossover event to produce a *rha*$^+$ *met*$^+$ recombinant are entered below and to the left of the diagonal. These frequencies are higher than those for crosses requiring at least quadruple crossovers to give *rha*$^+$ *met*$^+$ recombinants whose frequencies are located on the opposite side of the diagonal. Values for reciprocal crosses are located in mirror positions across the diagonal from each other. These typically show a twofold difference of about 20% versus 10%. Estimates of the two sigma values from the number of colonies tested for each cross indicate that the differences between reciprocal crosses for all pairs of mutants placed next to each other on the map are significant at the 95% confidence level except for the pair (*rhaB140*, *rhaB73*). In addition the values for the pairs (*rhaA59*, *rhaA50*) and (*rhaB58*, *rhaB79*), while signifi-

TABLE 2

Characteristics of rhamnose negative mutants

Group	Rhamnose inhibition	Rhamnulose accumulation
rhaA	slight	none
rhaB	slight	high
rhaC	none	none
rhaD	severe	high

Rhamnose inhibition occurs after adding L-rhamnose to cells growing on casein hydrolysate as described in METHODS. Slight inhibition is about a 10% decrease in growth rate, while severe inhibition is a growth rate approaching zero before the casein hydrolysate is exhausted. Rhamnulose accumulation was measured at the end of growth using the cysteine-carbazole method taking the culture growing wihout rhamnose as a blank.

Percent Met+ among Rha+ recombinants

Rha- marker in Met+ phage

Rha- marker in Met+ bacteria	D 62	D 54	D 97	A 59	A 50	A 80	B 140	B 73	B 58	B 79	C 70	C 55	C 56	C 63
D 62		13												
D 54	20		8											
D 97	28	27		12		13								
A 59			21		11	11								
A 50				16		8		9						
A 80			31	13	19		13	9						
B 140						22		12		11				
B 73					31	29	18		12	10				
B 58								25		18				
B 79								28	25		6			
C 70										34		6		
C 55										34	28		12	12
C 56												23		12
C 63												14	21	

FIGURE 3.—Results of reciprocal three-factor crosses of the type (donor phage) *rha-1 met*+ × (recipient bacteria) *rha-2 met-1* and (donor phage) *rha-2 met*+ × (recipient bacteria) *rha-1 met-1*. Numbers represent the frequency of incorporation of the unselected *met*+ marker among *rha*+ recombinants in the crosses indicated. Crosses were carried out in strain P72 except for those involving *rhaB58* as a recipient where a derivative of strain AB 1450 was used.

cantly different, are not in the typical range. Therefore, the data may not really justify the positioning of these particular mutant sites. However, in all three of these cases unambiguous results have been obtained in crosses with other mutants further away, so that at most only two or three contiguous sites have been placed out of their correct order.

CRIBBS and ENGLESBERG (1964) have reported that in many cases where mutant sites are very close together the phenomenon of high negative interference reduces differences in reciprocal three-factor crosses. Recombination frequencies between the three ambiguously ordered pairs show that they are indeed very close together and therefore high negative interference may be the source of difficulty in mapping them.

Most important, all of the mutants in each of the four groups seem to map together. This indicates that the four groups lie in four distinct genetic units which are very closely linked and perhaps contiguous on the chromosome as represented in Figure 1.

Complementation: To determine whether these four genetic units also correspond to four units of function, complementation tests were performed. The results (Table 3) indicate the existence of three cistrons corresponding to groups

TABLE 3

Complementation between rhamnose negative mutants

rha marker on episome	*rha* marker on chromosome													
	D62	D54	D97	A59	A50	A80	B140	B73	B58	B79	C70	C55	C56	C63
D54	—	..	—	+	+*	+*	+	..	+	+	+*	+	+*	+
A50	+	+	+	—*	..	—*	+	+*	+*	+	..	+*	+*	+
B73	..	..	—	—*	..	—	—*	..	—*	..	+*	+	..	+*
C55	..	..	..	..	..	..	..	..	..	+	+*	..	..	—
C56	..	..	..	..	..	..	..	..	..	+*	+*	—*	..	—*
C63	..	+*	+*	..	+*	+*	+*	+	+*	+	+*	..	—*	..

Strains diploid for the *rha* region were constructed using F'14 as described in the text. A + indicates growth on L-rhamnose in 24 hours, while a — indicates failure to grow during this period. All + strains give rise to Rha⁻ segregants, and all — strains give rise to Rha⁺ recombinants. In addition, for those strains marked * both parental *rha* markers have been segregated from the diploid and identified by progeny tests.

A, B, and D. The failure of *rhaB73* when on the episome to complement *rhaA* and *rhaD* mutants does not contradict this conclusion since independent evidence indicates that this mutant is a polarity mutant. Enzymatic analysis to be presented shows that all three rhamnose enzymes are present in low amounts in a *rhaB73* haploid strain. Furthermore, the diploids in question do appear to grow slowly on rhamnose although not enough to be scored positive in 24 hours. When on the chromosome, *rhaB73* does complement *rhaA50*, but the growth is noticeably less than normal.

The *rhaC* mutants define a fourth cistron consisting of *rhaC55*, *rhaC56* and *rhaC63* and a possible fifth cistron consisting of *rhaC70* alone. One mutant does not seem adequate to define a new cistron here because it is possible that intracistronic complementation is occurring. For the time being, I will refer to all the *rhaC* mutants as belonging to one gene while recognizing the possibility that they may actually occur in two separate citrons.

Enzymatic defects of the mutants: Rhamnose induced extracts of all the Rha⁻ Met⁺ P72 strains were prepared and assayed for the three rhamnose utilizing enzymes (Table 4). Since conditions of induction were not completely gratuitous, levels in the Rha⁺ strain should not be quantitatively compared to those of the mutants. Group D mutants are all missing only the L-rhamnulose 1-phosphate aldolase, so this gene probably contains the structural information for this enzyme. All of the group B mutants are missing the L-rhamnulokinase, and this gene is probably the structural gene for this enzyme. *rhaB73* and *rhaB79* are in addition missing the aldolase. However, since the isomerase levels are low in these mutants and since *rhaB79* does complement an aldolaseless mutant, it seems likely that they are polarity mutants and that low levels of aldolase are actually present but are beneath the limit of detection of the assay. The mapping and complementation data indicate that these two mutants are in the same cistron as *rhaB58* and *rhaB140* which are missing only the kinase. The *rhaA* mutants, except for *rha50*, are missing just the L-rhamnose isomerase so this group must represent the isomerase gene. *rhaA50* definitely belongs in this group because it maps between the other two mutants and does not complement either of them,

TABLE 4

Enzyme levels present in cell free extracts

rha mutant	Specific activity (μmoles product/hr/mg protein)		
	Isomerase	Kinase	Aldolase
Rha+	46	10	0.9
A50	39	23	2.0
A59	< 0.1	12	1.1
A80	< 0.2	30	3.7
B58	160	< 0.8	3.9
B73	7.5	< 1.0	< .05
B79	3.9	< 0.5	< .03
B140	175	< 0.6	4.1
C55	1.3	< 0.7	< .03
C56	0.7	< 0.7	< .03
C63	0.8	< 0.6	< .03
C70	< 0.1	< 0.7	< .03
D54	27	4.6	< .03
D62	56	9.1	< .05
D97	44	9.2	< .05

Extracts were prepared from Met+ Rha- P72 strains, and enzymes were assayed as described in METHODS.

while it does complement all other mutants tested. It is possible that this mutant gene codes for a form of the isomerase which is sensitive to some intracellular inhibitor that becomes diluted or lost during the extraction procedure. Such a mutant enzyme has been found in the tryptophan synthetase of *Neurospora crassa* by SUSKIND and KUREK (1959). In support of this hypothesis is the fact that no isomerase activity could be detected when the *in vivo* assay was used to measure this enzyme under conditions where one-sixth of the wild-type level could be easily detected, whereas *in vitro rhaA50* extracts have about half the isomerase level of the wild type. Also, when extracts are heated to 55°C for 6 minutes in 0.01 M glycylglycine buffer at pH 7.6, over 90% of the wild-type isomerase activity is lost while only 20% is lost with *rhaA50*. This indicates a difference in the structure of the mutant enzyme.

Group C mutants all have the same enzymatic makeup; the levels of all three enzymes are extremely low or undetectable. This suggests that they are mutations in a gene concerned with the expression of the other three genes rather than directly specifying the structure of one of the enzymes.

DISCUSSION

In *E. coli* K-12 three genes (*rhaA*, *rhaB* and *rhaD*) have been shown to be closely linked and to be involved in the formation of the three enzymes needed for L-rhamnose utilization, presumably as structural genes. Mutants deficient for an enzyme utilizing L-lacticaldehyde or for an L-rhamnose permease would probably not have been picked up by the methods used for obtaining mutants since

they should grow on 1% rhamnose, so other genes in this system may well exist.

A regulatory gene, defined by the *rhaC* negative mutants, which controls the expression of these three structural genes is located near them. The evidence that the *C* mutants are in a regulatory gene is that they simultaneously affect all three enzymes. Such effects might also be shown by a severe polarity mutant. However, if this were the case, such mutants would not be expected to complement mutants in the other genes as has been found. It is also possible that the *C* gene produces a subunit common to all three of the enzymes. Although cases of two enzymatic activities possessed by the same polypeptide are known (e.g. imidazole glycerol phosphate dehydrase and histidinol phosphate phosphatase in histidine biosynthesis, LOPER 1961), no examples of triple specificity are yet known; so this explanation seems improbable. Finally, it is possible that the *rhaC* mutants might actually be multiple mutations in the rhamnose region because of the potency of the mutagen used. Three facts argue against this. First, all of the mutant strains used except for *rhaB58* have been observed to revert to Rha$^+$ during the course of these experiments. Multiple mutants would be very unlikely to revert. Second, most of the three factor crosses gave typical high and low reciprocal values. This could not be the case with multiple mutants unless they were very closely linked, and in this case their effects should probably be confined to just one gene. Lastly, the ability of the group C mutants to complement mutants in all of the other groups show that the *A*, *B* and *D* genes are still intact in the *rhaC* strains.

The assumption that the *C* gene is a regulatory gene predicts that the *C* region should be the site of constitutive mutations. However, no mutants of this type have yet been isolated.

Assuming that the *C* gene is involved in regulation as it seems to be, then it might well produce an allosteric protein undergoing an allosteric transition in the presence of L-rhamnose as MONOD, CHANGEUX and JACOB (1963) have postulated for regulatory gene products in general. According to the theory of MONOD, WYMAN and CHANGEUX (1965) the protein would then consist of several subunits. This means that there should be a good possibility for intracistronic complementation to occur among mutants affecting this protein. For this reason the complementation between *rhaC70* and all of the other group *C* mutants may be simply intracistronic complementation, and two separate *C* cistrons may not actually exist. Resolution of this question must await analysis of more mutants in this region. Also, enzymatic analysis of the complementing diploids might reveal the low level of functioning usually associated with intracistronic complementation.

Because the *rhaC* mutants are recessive to the wild-type allele, they appear to represent the loss of an internal activator rather than the production of a superrepressor (see ENGLESBERG *et al.* 1965, for a discussion of this point). Alternatively, the *C* gene might control the production of an enzyme which converts rhamnose to some other form which is needed to destroy a repressor formed by an as yet undiscovered regulator gene. If this is the case, then it should be possible to revert the Rha$^-$ phenotype of *rhaC* mutants by obtaining constitutive mutants in the

postulated regulator gene. Preliminary results on 200 *rhaC* revertants show no constitutives among them. Further studies should provide a definitive answer as to the existence of such a gene.

MARKOWITZ and ROSENBAUM (1965) have recently pointed out a danger in the use of dominance tests to deduce the mode of action of a regulatory gene. If four subunits, for example, are associated into the final product in a random manner, then 7/8 of all the molecules of that particular regulatory protein in a diploid cell will be hybrids composed of a mixture of subunits from both of the two alleles present. Since there is no way to predict the properties of these hybrids *a priori*, the nature of the regulating elements present in the cell may be largely unknown. Therefore, the *rhaC* mutants could conceivably be producing a superrepressor which is only active when all of its subunits are of the mutant type. In a diploid with a *rhaC*$^+$ allele so little superrepressor could be produced that the negative phenotype would appear falsely recessive. Use of deletion mutants can solve this problem by completely eliminating the possibility of producing hybrids. Since the *rhaC* mutants have properties most analogous to the pleiotropic negative mutants in the L-arabinose regulatory gene (ENGLESBERG *et al.* 1965) and since use of deletion mutants have quite clearly established that the role of the product of that gene is to act as an internal activator (SHEPPARD and ENGLESBERG 1966, 1967), I favor the interpretation that the function of the *rhaC* gene is also to produce an internal activator of structural gene expression.

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SUMMARY

Three genes which seem to be structural genes for L-rhamnose isomerase, L-rhamnulokinase and L-rhamnulose 1-phosphate aldolase have been identified and mapped in a cluster near the *metB1* marker on the *E. coli* K-12 chromosome. The activity of these genes appears to be regulated by a system of positive control involving the product of an "activator" gene located in the same cluster. This activator may be composed of two nonidentical peptide subunits coded for by two adjacent cistrons.

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