sidase and of the galactoside-permease, seemed to correspond to modifications of the hypothetical operator. It will be recalled that three distinct genes have been recognized in this system: (1) y, the gene for structure of the galactoside-permease; (2) z, the gene for structure of the β-galactosidase, of which certain alleles permit the synthesis of a modified, enzymatically-inactive protein, Cz; and (3) i, the regulator gene synthesizing a repressor specific for the system. These three genes are closely linked. It will further be recalled that bacteria which are diploid for the genes of this group can be obtained by transfer of sex factors (F) having incorporated the corresponding fragment of the bacterial genome (F-lac)[2].

Units of galactosidase and of Cz protein [*cf.* (3)] expressed as the percentage of the amount found for the allele present on the chromosome in induced bacteria.

nd, not detectable. The excess of the product of the z allele present on the factor F-lac seems to indicate the presence of several F-Lac factors per chromosome[2] [3].

Genotype		Non-induced Bacteria			Induced Bacteria		
Chromosome	*F-Lac*	*Galactosidase*	*Protein Cz*	*Permease*	*Galactosidase*	*Protein Cz*	*Permease*
$i^+o^+z^+y^+$		<1	—	nd	100	—	100
$i^+o^+z_4^-y^+$	$/Fi^+o^+z^+y^+$	<1	nd	nd	320	100	100
$i^+o^+z_4^-y^+$	$/Fi^+o^cz^+y^+$	36	nd	33	270	100	100
$i^+o^+z_1y^+$	$/Fi^+o^cz^+y^+$	110	nd	50	330	100	100
$i^+o^+z^+y_R^-$	$/Fi^+o^cz_1y^+$	<1	30	—	100	400	—
$i^+o^+z_1y^+$	$/Fi^+o^cz^+y_R^-$	60	—	nd	300	—	100

Starting with a diploid $i^+z^-/F^-i^+z^+$, constitutive mutants (o^c) have been isolated. By appropriate recombinations and transfers, the different diploid genotypes given in the table have been obtained. It will be noted the alleles z_1^- and z_4^- which were used permit the synthesis of inactive proteins (Cz_1, Cz_4) which can be measured in the presence of β-galactosidase by immunochemical methods[3]. The table shows that in bacteria heterozygous for o and for z, the permease as well as the galactosidase or the Cz protein are partially constitutive, but that only the allele of z or of y which is *cis* with respect to o^c is constitutively expressed, the *trans* allele remaining strictly inducible as in the genotype o^+/o^+. The constitutive mutation o^c is thus pleiotrophic and dominant, and its effect is only manifested in the *cis* position.

Starting with haploid wild-type bacteria, several other mutants have been isolated in which an apparently simple mutational event has led to the loss of ability to synthesize both the permease and the β-galactosidase. These mutants revert to the wild-type at a rate of 10^{-7} to 10^{-8}. They are recessive, and are complemented neither by z^- mutants nor by y^- mutants. Genetic analysis shows that these mutations (o^o) are extremely closely linked to the o^c mutations, and that they are situated between the loci z and i (themselves closely linked). The order of the loci in the Lac segment is: TL...Pro...y-z-o-i...Ad...Gal.

According to their characters, the mutations o^o and o^c seem to affect a genetic element which is not expressed by an *independent* cytoplasmic product. The remarkable properties of these mutations are inexplicable according to the "classical" concept of the gene for structure and distinguish them equally from mutations affecting the regulator gene, i. On the other hand, they conform to the predictions arising from the hypothesis of the operator. Several simple defective mutations, having a pleiotrophic, coordinated effect, and non-complementable, have been described for other bacterial systems, in particular for the metabolism of galactose[4]. We suggest that these mutations could affect an operator.

The hypothesis of the operator implies that between the classical gene, independent unit of biochemical function, and the entire chromosome, there exists an intermediate genetic organization. The latter would include the *units of coordinated expression* (*operons*), comprising an operator and the group of genes for structure which it coordinates. Each operon would be, through the intermediation of the operator, under the control of a repressor whose synthesis would be determined by a regulator gene (not necessarily linked to the group). The repression would be exercised either directly at the level of the genetic material, or at the level of "cytoplasmic replicas" of the operon. This hypothesis would explain the correlation which is very generally observed in bacteria between functional association and genetic linkage for the sequential enzyme systems. It has other verifiable consequences, notably that the enzymes of a sequence governed by the same operator should not be *separately* induced[6].

(1) F. Jacob and J. Monod, *Comptes rendus, 249*, 1959, p. 1282.
(2) F. Jacob and E. A. Adelberg, *Comptes rendus, 249*, 1959, p. 189.
(3) D. Perrin, A. Bussard and J. Monod, *Comptes rendus, 249*, 1959, p. 778.
(4) H. M. Kalckar, K. Kurahashi and E. Jordan, *Proc. Nat. Acad. Sc., 45*, 1959, p. 1776.
(5) H. W. Rickenberg, G. N. Cohen, G. Buttin and J. Monod, *Ann. Inst. Pasteur, 91*, 1956, p. 829.
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LETTER TO THE EDITOR

A Deletion Analysis of the *Lac* Operator Region in *Escherichia coli*

Much of the information on the relation of the structure of a genetic region to its control and expression comes from studies on the enzymes involved in lactose metabolism in *Escherichia coli* (Jacob & Monod, 1961). Three enzymic activities, β-galactosidase (*z*), galactoside permease (*y*) and galactoside transacetylase, are specified by the *lac* region of the chromosome. The level of these enzymes is co-ordinately controlled by a cytoplasmic repressor produced by the *i* gene which is closely linked to the structural genes for the enzymes. The site of action of the repressor has been determined by the isolation of mutants (o^c) which have lost the sensitivity to repressor, and which thus produce β-galactosidase constitutively in the presence of repressor. These mutants are very closely linked to z^- mutations mapping at the beginning of the *z* gene. Another class of mutants, o^0, also mapping in this area, shuts off the production of all three enzymic activities of the *lac* region. It has been proposed that o^0 and o^c mutations together define the operator, not only as the site of action of the repressor, but also as the initiation site for the transcription of messenger-RNA from the structural genes. A group of genes thus controlled is termed an operon.

In this paper it will be shown that the operator, defined as the site of repression of the operon, does not lie in the same region in which o^0 mutants are found. This conclusion has been reached from a study of various deletion mutants of the *lac* genes. Mutants carrying deletions of different segments of the *lac* region have been isolated from o^0 mutants by selection of z^-y^+ revertants on melibiose–agar (Beckwith, 1963, 1964). Two o^0 strains, o^0_2 (i^-o^0) and o^0_{118} (i^+o^0), have been studied. Some of the z^-y^+ revertants result from deletions within the *lac* region; the remainder arise as a result of mutations at unlinked suppressor loci. The two classes of revertants can be easily distinguished from each other, since only the deletions abolish the basal level of β-galactosidase activity present in the o^0 mutants (Table 1) and do not further revert to lac^+ phenotype. With the exception of M112, these revertants could be shown to contain deletions by their failure to give lac^+ recombinants with other lac^- point mutants (Fig. 1).

All of the deletions, except M12 and M112, include all four o^0 mutants tested (Fig. 1). The extent of these deletions was further determined by examining them for deletion of the *i* gene, the nearest known marker to the operator end of the operon. Since most of the revertants were derived from an i^-o^0 strain, it was necessary to use genetic techniques to determine the presence or absence of the *i* gene. The mutants were crossed with Hfr strains of genetic constitution i^+z^- (with o^0_2 revertants) or i^-z^- (with o^0_{118} revertants), and the inducibility of the lac^+ recombinants was tested. As Jacob & Wollman (1961) have demonstrated, negative interference

in the *lac* region is extensive, so that a high proportion of recombinants resulting from crossovers between two *lac*$^-$ mutants will have a second crossover in the same region. Thus, if the *i* gene is still present in these deletions, a fraction of the *lac*$^+$ recombinants from such crosses will have incorporated the *i* character of the revertant strain as a result of a second crossover. The conclusions from these experiments are illustrated in Fig. 1. The proportion of *lac*$^+$ recombinants having the *i* character of the o^0 mutant from which the deletions were derived was high (15 to 28%) in M15, M111 and M12 (M112 was not tested). In the others, no such recombinants were found; in particular, 250 *lac*$^+$ recombinants from M116 were tested and none was found to exhibit the i^- phenotype of the $o^0{}_2$ mutant. Thus, some of the strains still carry an intact *i* gene, while others have deletions extending either into or beyond the *i* gene.

TABLE 1

Strain	β-Galactosidase	Permease	Repressibility
o^0_{118} i^+	0·001	<1	–
o^0_2 i^-	0·001	<1	–
o^0_2-M31 i^-	0·001	15	–
-M12	0	70	yes
-M15	0	90	yes
-M41	0	25	–
-M111	0	90	yes
-M112	0	110	–
-M116	0	25	no
o^0_{118}-M22 i^+	0	25	–
-M23	0	25	–

All activities are in terms of per cent of the amount produced by a constitutive strain. The permease was assayed by incubating the bacteria for 25 min with 2×10^{-3} M-orthonitrophenyl-β-D-galactoside, glycerol-synthetic medium and 1 μg/ml. chloramphenicol. Ordinarily, 25 ml. of bacterial culture with a density of 3×10^9 cells/ml. was used. After incubation the cells were centrifuged and resuspended in 2 ml. cold 5% trichloroacetic acid. The trichloroacetic acid precipitate was centrifuged and the supernatant made basic with 0·35 ml. 6N-KOH. The basic solution was heated in a boiling-water bath for 3 min, thus hydrolysing the orthonitrophenyl-β-D-galactoside to orthonitrophenol. The orthonitrophenol concentration was measured colorimetrically at 420 mμ and a correction of $1{\cdot}75 \times$ O.D.$_{550\,m\mu}$ was subtracted from this reading. The mutant, o^0_2, was used as a control. This assay can only be used with mutants which lack β-galactosidase activity. All of the revertants, with the exception of o^0_2-M31, carry deletions in the *lac* region.

If the o^0 mutations lie in the repressor-sensitive site, then it would be expected that revertants which carry deletions of this region would no longer respond to repressor. Several of the deletions were tested for their repressibility in the presence of the wild-type i^+ gene. Diploids with the genetic constitution $i^- z^{del}\, y^+/\text{F-}i^+ z^- y^\pm$ were constructed and tested for the repressibility of the permease. Deletion M15, which deletes all o^0 mutations, is still sensitive to *i*-product repression (Table 1). In addition, M12, which is in a sense complementary to M15, since it deletes the rest of the *z* gene and not the o^0 mutations, is also repressible. These results indicate that no part of the *z* gene as defined by z^- and o^0 mutations is involved in the sensitivity to repressor. It might be argued that the permease gene has its own repressor-sensitive site. Such an interpretation, however, is eliminated by the finding that in a

revertant, M116, carrying an extensive deletion including the *i* gene, the permease is no longer repressible. Revertant M116 deletes the operator (*repressor-sensitive site*) while M15 does not. Thus the operator must lie beyond the furthest known *lac*⁻ mutation (o^0_{84}) at the beginning of the *z* gene. If the operator is still part of the structural gene, then it must be either extremely small or unusually insensitive to mutational alteration. Alternatively, since no z^- or o^0 mutations have been found in this region, it is possible that the operator is a separate locus from the structural gene (see Fig. 1).

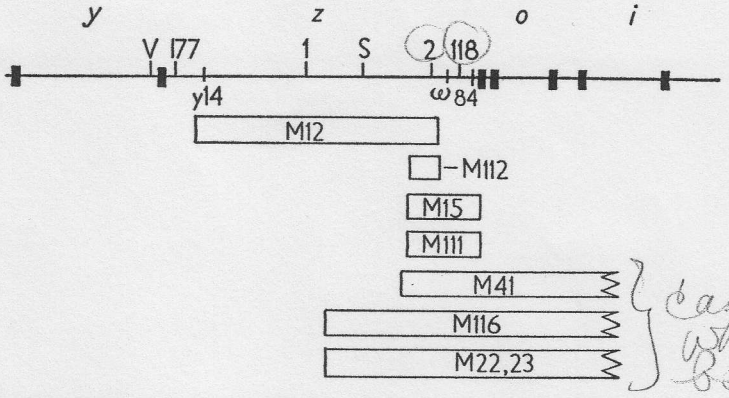

FIG. 1. *The extent of deletions in the* lac *region.* The deletions were mapped using the z^- and y^- markers indicated here. Mutant 177 maps outside deletion M12 (Jacob, personal communication). Revertant M112 gives recombinants with all *lac*⁻ mutants tested to date. The deletion mutants, M22 and M23, were isolated from o^0_{118}; the rest were derived from o^0_2. Mutants ω, 118, 2 and 84 have all been classified o^0.

The finding that revertants M15 and M111, which retain the intact operator, delete all o^0 mutations without strongly affecting the rate of permease production indicates that the o^0 mutations do not define a site essential for the initiation of the transcription of messenger-RNA from the operon. This site, like the operator, must lie beyond the furthest known *lac*⁻ mutation at the beginning of the *z* gene. There is no evidence, at present, to distinguish the initiation site from the operator. The deletions which remove the operator and the *i* gene (Fig. 1) should also delete the initiation site, and it must then be proposed that, in these strains, the permease is connected to an adjacent operon with its own initiator site. The characteristic lower rate of permease production (25%) in these extended deletions suggests that the permease gene has come under the control of a new initiation site and/or operator, functioning more slowly than the comparable sites of the *lac* operon. Such a mechanism has been proposed in the case of revertants of a histidine o^0 mutant (Ames, Hartman & Jacob, 1963).

It appears that o^0 mutations do not map in the operator nor do they define a site essential for the transcription of messenger-RNA from the operon. Therefore, it seems quite likely that they are only extreme examples of polar mutants found in the *z* gene (Franklin & Luria, 1961; Jacob & Monod, 1961), and that they lie within the structural gene for β-galactosidase. Evidence has already been presented which indicates that the effect of o^0 mutations is to inhibit the process of translation of operon messenger-RNA information into protein (Beckwith, 1964).