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The Genetic Control and Cytoplasmic Expression of "Inducibility" in the Synthesis of β-galactosidase by *E. Coli*†

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A number of extremely closely linked mutations have been found to affect the synthesis of β-galactosidase in *E. coli*. Some of these (*z* mutations) are expressed by loss of the capacity to synthesize active enzyme. Others (*i* mutations) allow the enzyme to be synthesized constitutively instead of inducibly as in the wild type. The study of galactosidase synthesis in heteromerozygotes of *E. coli* indicates that the *z* and *i* mutations belong to different cistrons. Moreover the constitutive allele of the *i* cistron is recessive over the inducible allele. The kinetics of expression of the i^+ (inducible) character suggest that the *i* gene controls the synthesis of a specific substance which represses the synthesis of β-galactosidase. The constitutive state results from loss of the capacity to synthesize active repressor.

1. Introduction

Any hypothesis on the mechanism of enzyme induction implies an interpretation of the difference between "inducible" and "constitutive" systems. Conversely, since specific, one-step mutations are known, in some cases, to convert a typical inducible into a fully constitutive system, an analysis of the genetic nature and of the biochemical effects of such a mutation should lead to an interpretation of the control mechanisms involved in induction. This is the subject of the present paper.

It should be recalled that the metabolism of lactose and other β-galactosides by intact *E. coli* requires the sequential participation of two distinct factors:

(1) The galactoside-permease, responsible for allowing the entrance of galactosides into the cell.

(2) The intracellular β-galactosidase, responsible for the hydrolysis of β-galactosides.

Both the permease and the hydrolase are inducible in wild type *E. coli*. Three main types of mutations have been found to affect this sequential system:

(1) $z^+ \rightarrow z^-$: loss of the capacity to synthesize β-galactosidase;

(2) $y^+ \rightarrow y^-$: loss of the capacity to synthesize galactoside-permease;

(3) $i^+ \rightarrow i^-$: conversion from the inducible (i^+) to the constitutive (i^-) state.

The $i^+ \rightarrow i^-$ mutation always affects both the permease and the hydrolase. All these mutations are extremely closely linked: so far all independent occurences of each of these types have turned out to be located in the "*Lac*" region of the *E. coli* K 12 chromosome. However, the mutations appear to be *independent* since all the different phenotypes resulting from combinations of the different alleles are observed (Rickenberg, Cohen, Buttin & Monod, 1955; Cohen & Monod, 1957; Cohn, 1957).

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It should also be recalled that conjugation in *E. coli* involves the injection of a chromosome from a ♂ (Hfr) into a ♀ (F^-) cell, and results generally in the formation of an incomplete zygote (merozygote) (Wollman, Jacob & Hayes, 1956). Recombination between ♂ and ♀ chromosome segments does not take place until about 60 to 90 min after injection; moreover segregation of recombinants from heteromerozygotes occurs only after several hours, thus allowing ample time for experimentation.

In order to study the interaction of these factors, their expression in the cytoplasm and their dominance relationships, we have developed a technique which allows one to determine the kinetics of β-galactosidase synthesis in merozygotes of *E. coli*, formed by conjugation of ♂ (Hfr) and ♀ (F^-) cells carrying different alleles of the factors *z*, *y* and *i* (Pardee, Jacob & Monod, 1958). Before discussing the results obtained with this technique, we shall summarize some preliminary observations on the genetic structure of the "*Lac*" region in *E. coli* K 12.

2. Materials and Methods

(a) *Bacterial strains*

A ♂ (Hfr) strain (no. 4,000) of *E. coli* K 12 was used in most experiments. It was derived from strain 58,161 F^+, and was selected for early injection of the "*Lac*" marker (Jacob & Wollman, 1957). This strain is streptomycin sensitive (S^s), requires methionine for growth and carries the phage λ. A second Hfr strain (no. 3,000), isolated by Hayes (1953), was used in some experiments. This strain is S^s, requires vitamin B_1, and does not carry λ prophage. Other Hfr strains carrying mutations for galactosidase (*z*), inducibility-constitutivity (*i*), and permease (*y*) were isolated from the Hayes strain after u.v. irradiation. These markers were also put into ♀ (F^-) strains, by appropriate matings and selection of the desired recombinants.

A synthetic medium (M 63) was commonly used. It contained per liter: 13·6 g KH_2PO_4 2·0 g $(NH_4)_2SO_4$, 0·2 g $MgSO_4 . 7H_2O$, 0·5 mg $FeSO_4 . 7 H_2O$, 2·0 g glycerol, and KOH to make pH 7·0. If amino-acids were required, they were added at a concentration of 10 mg/l. of the L-form. For mating experiments, the above stock medium was adjusted to pH 6·3 and vitamin B_1 (0·5 mg/l.) was added prior to use. Aspartate (0·1 mg/ml.) was generally added at the time of mating, according to Fisher (1957).

(b) *Mating experiments*

The desired volume of fresh medium was inoculated with an overnight culture (grown in the same medium) to an initial density of approximately 2×10^7 bacteria/ml. This culture was aerated by shaking at 37°C in a water bath. Turbidity was measured from time to time; and when the density reached 1 to 2×10^8 bacteria/ml., the experiment was started. Usually small volumes of ♂ and ♀ bacteria were mixed in a large Erlenmeyer flask, with the ♀ strain in excess (e.g. 3 ml. ♂ plus 7 ml. ♀ in a 300 ml. flask). The mixed bacteria were agitated very gently so that the motion of the liquid was barely perceptible. From time to time samples were removed for enzyme assay and plating on selective media, usually lactose-B_1-streptomycin agar, for measurement of recombinants. Under these conditions, in a mating of ♂ z^+Sm^s by ♀ z^-Sm^r, up to 20 % of the ♂ population formed z^+Sm^r recombinants (as tested by selection on lactose-streptomycin agar). More often 5 to 10 % recombinants were found.

Streptomycin (Sm)† was used in many mating experiments, to block enzyme synthesis by z^+Sm^s ♂ cells. Controls showed that the synthesis of β-galactosidase was blocked in these strains immediately upon addition of 1 mg/ml. of Sm. Incorporation of ^{35}S from $^{35}SO_4^=$ as well as increase of turbidity were also suppressed by this treatment. This concentration of Sm had no effect on Sm-resistant (Sm^r) mutants. In some experiments, virulent phage (T6) was used to kill the ♂ cells, thus preventing remating.

† The following abbreviations are used in this paper:

Sm = streptomycin
IPTG = *iso*propyl-thio-β-D-galactoside
ONPG = *o*-nitrophenyl-β-D-galactoside
TMG = methyl-thio-β-D-galactoside

It should be noted that if streptomycin was added initially, it significantly reduced the number of recombinants (e.g., 75 % fewer colonies were formed on lactose-B_1-streptomycin plates after 80 min mating in the presence of 1 mg/ml. streptomycin) relative to mating in the absence of streptomycin; but the antibiotic had little effect on enzyme formation by zygotes if added at the commencement of the experiment or after the z^+ locus had been injected.

When galactosidase synthesis had to be induced in zygotes, *iso*propyl-thio-β-D-galactoside (IPTG) was used at 10^{-3}M, a concentration at which this inducer is known to be active even in the absence of permease (Rickenberg *et al.*, 1956).

(c) *Recombination studies*

The blender technique of Wollman & Jacob (1955) was used to determine the times of penetration of markers into the zygotes. It should be noted that this treatment reduces enzyme-forming capacity in zygotes by 30 to 60 %. Recombinant colonies, selected on appropriate selective media, were restreaked on the selector medium and replica plating was used to determine unselected characters. Tests for galactosidase synthesis (with or without induction) were performed on maltose-synthetic agar plates with or without IPTG, using filter paper impregnated with ONPG, according to Cohen-Bazire & Jolit (1953).

Transductions were performed with phage 363, according to Jacob (1955).

(d) *β-galactosidase assay*

For this enzyme assay, 1 ml. aliquots of culture were pipeted into tubes containing 1 drop of toluene. The tubes were shaken vigorously and were incubated for 30 min at 37°C. They were then brought to 28°C; 0·2 ml. of a solution of M/75 *o*-nitrophenyl-β-D-galactoside in M/4 sodium phosphate (pH 7·0) was added, and the tubes were incubated a measured time, until the desired intensity of color had developed. The reaction was halted by addition of 0·5 ml. of 1 M-Na_2CO_3, and the optical density was measured at 420 mμ with the Beckman spectrophotometer. A correction for turbidity could be made by multiplying the optical density at 550 mμ by 1·65 and subtracting this value from the density at 420 mμ. One unit of enzyme is defined as producing 1 mμ-mole *o*-nitrophenol/minute at 28°C, pH 7·0. The units of enzyme in the sample can be calculated from the fact that 1 mμ-mole/ml. *o*-nitrophenol has an optical density of 0·0075 under the above conditions (using 10 mm light-path).

(e) *Chemicals*

o-nitrophenyl-β-D-galactoside (ONPG), methyl-thio-β-D-galactoside (TMG) and *iso*propyl-thio-β-D-galactoside (IPTG) were synthesized at the Institut Pasteur by Dr. D. Türk. Other chemicals were commercial products.

3. Genetic Structure of the "Lac" Region

Figure 1 presents the structure of the "*Lac*" region, as it can be sketched from the data available at present. This complex locus, as established long ago by Lederberg (1947) and confirmed by the blender experiments of Wollman & Jacob (1955), lies at about equal distances from the classical markers *TL* and *Gal*. The closest known markers are *Proline* (left) and *Adenine* (or *T6*) (right). As shown in the map, the several (about 10) occurrences of the y^- mutation all lie together probably at the left of the segment, while the different z^- mutations and the i^- mutant are packed together at the other end. No attempt has been made to establish the order of individual y^- mutations. The order of the z^- mutations relative to each other and to the i^- marker is unambiguously established, as shown, except for the z_U^- mutation, whose position is largely undetermined. Several independent occurrences of the i^- mutation have been isolated. They all appear to be closely linked to the i_3^- marker, but they have not been mapped, for lack of adequate methods of selection i^+ recombinants. The evidence for this structure is briefly as follows:

(1) The frequency of recombination between *z* and *y* mutations is very low:

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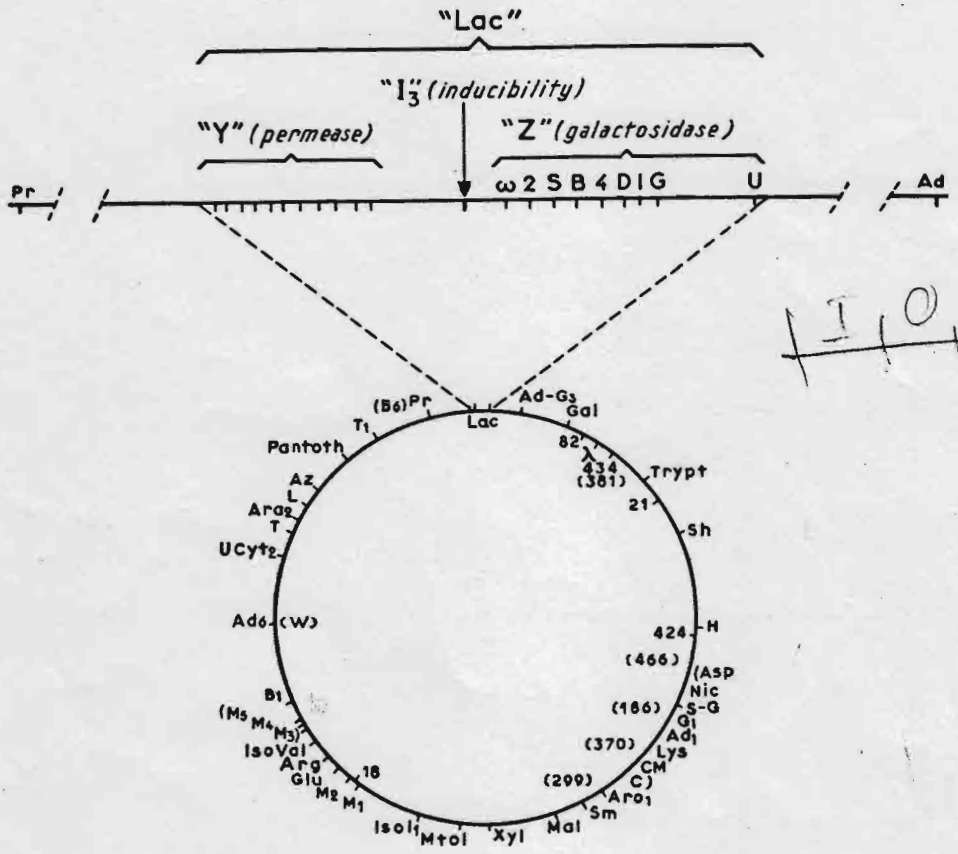


FIG. 1. Fine structure of the "*Lac*" segment.

The "*Lac*" segment is shown enlarged and positioned with respect to the rest of the *E. coli* K 12 linkage group for which the circular model (Jacob & Wollman, 1958) has been adopted.

roughly 1/100th of the frequency of recombination between *T1* and *Gal*. The frequency of recombination between individual *z* markers is about one order of magnitude lower.

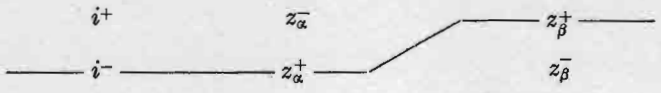
(2) When y^+z^+ recombinants are selected (by growth on lactose-agar) in crosses of the type:

$$y^+i^-z^- \times y^-i^+z^+$$

the i^+ marker remains associated with z^+ 85 % of the time.

(3) The frequency of cotransduction of *i* with *z* (selecting for z^+ alone) is very high (> 90 %), while the frequency for *i* and *y* is also high, although definitely lower (about 70 %). (These data are somewhat ambiguous, because of the heterogeneity of the clones resulting from a transduction.)

(4) The selection of z^+ recombinants in crosses involving different z^- mutants, and *i* as unselected marker, invariably results in about 90 % of the progeny being either i^- or i^+, depending on the particular z^- mutants used. Assuming this result to be due to the position (left or right) of *i* with respect to the *z* group:



a linear order can be established, without contradictions, for the eight markers shown. This however leaves an ambiguity as to whether *i* lies *between* the *y* and the *z* groups, or outside.

Let us emphasize that this sketch of the *Lac* region is preliminary and very incomplete, and that the results concerning the relationships of certain markers are not understood. For instance, the z_U^- marker recombines rather freely with all the other mutants shown (both *y* and *z*) yet, by cotransduction tests, it is closely linked to *i* (25 % cotransduction). It should also be mentioned that certain of the z^- mutants (z_ω^- ; z_3^-; z_G^-) have apparently lost the capacity to synthesize *both* the galactosidase *and* the permease. Yet these mutations do not seem to be deletions. We shall not attempt, here, to interpret this finding, since we shall center our attention on the interaction between the *i* marker and the *z* region.†

A question which should now be considered is whether we may regard the *z* region as possessing the specific structural information concerning the galactosidase molecule. The fact that so far all the independent mutations resulting in loss of the capacity to synthesize galactosidase were located in this region might not constitute sufficient evidence‡. However, it has been found by Perrin, Bussard & Monod (1959, in preparation) that several of the z^- mutants synthesize, instead of active galactosidase, an antigenically identical, or closely allied, protein. Moreover several of these mutant proteins are different from one another by antigenic and other tests. These findings appear to prove that the *z* region indeed corresponds to the "structural" genetic unit for β-galactosidase.

4. β-Galactosidase Synthesis by Heteromerozygotes

(a) *Preliminary experiments*

The feasibility and significance of experiments on the expression and interaction of the *z*, *y* and *i* factors depended primarily on whether *E. coli* merozygotes are physiologically able to synthesize significant amounts of enzyme very soon after mating. It was equally important to determine whether the mating involved any cytoplasmic mixing. These questions were investigated in a series of preliminary experiments.

Since the physical separation of *E. coli* zygotes from unmated or exconjugant parent cells cannot be achieved at present, test conditions must be set up, such that the zygotes only, but not the parents, can synthesize the enzyme. This is obtained when the following mating:

$$\sigma\ z^+y^+i^+Sm^s \times \varphi\ z_s^-y^+i^+Sm^r \qquad \text{(A)}$$

is performed in the presence of inducer (IPTG) and of 1 mg/ml. of streptomycin. The ♀ lack the z^+ factor; the ♂ are inhibited by streptomycin (cf. Methods); the zygotes are not, because they inherit their cytoplasm from the ♀ cells (see below

† Interaction of *i* with the *y* region is of course equally interesting, but since determinations of activity are much less sensitive with the galactoside-permease than with the galactosidase, we have used the latter almost exclusively.

‡ In addition to the mutants shown on Fig. 1, 20 other galactosidase-negative mutants, as yet unmapped, have been found to belong to the same segment by contransduction tests. None was found outside. Lederberg *et al.* (1951), however, have isolated some lactose-"non-fermenting" mutants (as tested on EMB-lactose agar) which are located at other points on the *E. coli* chromosome. In our hands, one of these mutants (Lac_3^-) formed normal amounts of both galactosidase and galactoside-permease (although it did form white colonies on EMB-lactose). Another one (Lac_7^-) formed reduced, but significant, amounts of both. A third (Lac_3^-) which is a galactosidase-negative, appears to belong to the "*Lac*" segment, by cotransduction tests.

pages 170 and 171), and because the type of ♂ used transfers the Sm^s gene to only a very small percentage of the cells. Under these conditions, enzyme is formed in the mated population with a time course and in amounts showing that the synthesis can be due only to zygotes having received the z^+ factor. Figure 2 shows the

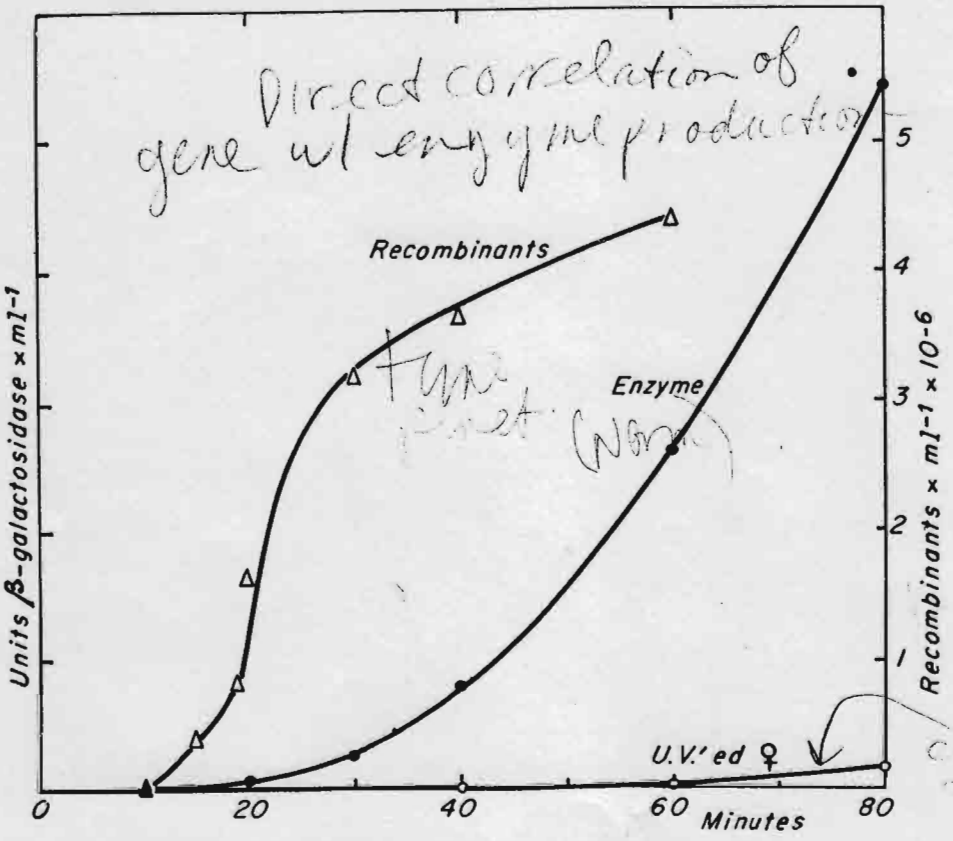


FIG. 2. Enzyme formation and appearance of recombinants in mating A.
Mating in presence of streptomycin (1 mg/ml.) and IPTG (10^{-3}M). A control with u.v.-treated ♀ cells (0·01 % survival) is shown. Recombinants (z^+Sm^r) selected by plating on Sm-lactose agar after blending (separate experiment with the same ♂ culture).

kinetics of galactosidase accumulation, compared with the appearance of z^+Sm^r recombinants, determined on aliquots of the same population (cf. Methods). The latter curve corresponds, as shown by Wollman & Jacob (1955), to the distribution of times of penetration of z^+ genes in the zygote population. It will be remarked that enzyme synthesis commences just within a few minutes after the first z^+ genes enter into zygotes. Assuming that the number of zygotes having received a z^+ gene is 4 to 5 times the number of recovered z^+Sm^r recombinants, and taking into account the fact that normal cells are on the average trinucleate (i.e., have three z^+ genes), the rate of enzyme synthesis per injected z^+ appears nearly normal.

This rapid expression of the z^+ factor poses the problem whether cytoplasmic constituents are injected from the ♂ into the zygote. This already appeared unlikely from the previous observations of Jacob & Wollman (1956). We reasoned that if there occurred any significant cytoplasmic mixing, such a mixing should allow the

♂ cells to feed the ♀ cells with any small metabolites which the ♂ had and the ♀ lacked. This condition is obtained in the following mating:

♂ z^+Sm^s *maltose*$^+$ × ♀ z^-Sm^r *maltose*$^-$

if it is performed in presence of maltose as sole carbon source, using a ♂ which virtually does not inject the *maltose*$^+$ gene. It results in a very strong inhibition of enzyme synthesis (and recombinant formation) showing that the ♂ cannot effectively

TABLE 1

Enzyme formation in nutritionally deficient zygotes

Deficiency	Rate of enzyme formation †			Mean % inhibition of recombinant formation
	Control	Deficient	Mean % inhibition	
Carbon source ‡	1·6	0·4	73	75
	0·66	0·20		
Arginine §	0·28	0·02	96	65
	0·36	0·01		

† Units of enzyme × hr^{-1}.

‡ ♂ z^+Sm^s *maltose*$^+$ × ♀ z^-Sm^r *maltose*$^-$ mated in presence of inducer and Sm with glycerol plus maltose (control) or maltose as sole carbon source.

§ ♂ z^+Sm^s Arg^+ × ♀ z^-Sm^r Arg^- mated in presence of inducer and Sm with and without arginine (10 μg/ml.).

feed the ♀. An even stronger effect is observed when the ♀ requires arginine, the ♂ not, and mating takes place in absence of arginine (again on condition that the Ar^+ gene is not injected by the ♂) (Table 1). These observations indicate that even small molecules do not readily pass from the ♂ into the ♀ cell during conjugation.†

It therefore appears that cytoplasmic fusion or mixing does not occur to an extent which might allow cross-feeding. That the contribution of the ♂ is exclusively genetic, and does not involve cytoplasmic constituents of a nature, or in amounts, significant for our purposes, is however only proved by the results of the opposite matings, which we shall consider in the next section.

(b) *Expression and interaction of the alleles of the z and i factors*

We should first consider which of the alleles of the *z* factors are dominant, and whether they all belong to a single cistron. Experiments of the type described above (mating A) were performed with each of the eight z^- mutants, used as ♀ cells, receiving a z^+ from the ♂. Enzyme was synthesized to similar extents in all cases, showing that the z^- mutants in question were all recessive. Each of the mutants was also mated (as ♂) to a z^- ♀. No enzyme was synthesized by any of these double recessive heterozygotes where the mutations were in the *trans* position.

† However such leakage may occur when the concentration of a compound is exceptionally high in the ♂. This happens when a ♂ with the constitution $z^-i^-y^+$ is used in the presence of lactose. The constitutive permease then may concentrate lactose up to 20 % of the cells' dry-weight (Cohen & Monod, 1957). Adequate tests have shown that this lactose does flow from the ♂ into a permease-less ♀ during conjugation.

$$\frac{z_\alpha^+ z_\beta^-}{z_\alpha^- z_\beta^+}$$

showing that all the (tested) z^- mutants belong to the same cistron as defined by Benzer (1957).

The next and most critical problem is whether the z and i factors also belong to the same unit of function (gene or cistron) or not. Let us recall that cells with the constitution z^+i^+ synthesize enzyme in presence of inducer only, while z^+i^- cells synthesize enzyme without induction, and z^-i^+ or z^-i^- cells do not synthesize enzyme under any condition. The extremely close linkage of z and i mutations suggests that they may belong to the same unit. If this were so, they would not be able to interact through the cytoplasm, but could act together only when in *cis* position within the same genetic unit. The heterozygote, z^+i^+/z^-i^- would then be expected not to synthesize galactosidase constitutively.

In order to test this expectation, the following mating:

$$\text{♂}\, z^+i^+ \times \text{♀}\, z^-i^- \qquad \text{(B)}$$

was performed *in absence of inducer*. The ♂ cannot synthesize enzyme, because they are i^+. The ♀ cannot because they are z^-. The zygotes, however, do synthesize enzyme (Fig. 3): during the first hour following mating the synthesis is, if anything, even more rapid and vigorous than when both parents are i^+ and inducer is used, as in mating (A).

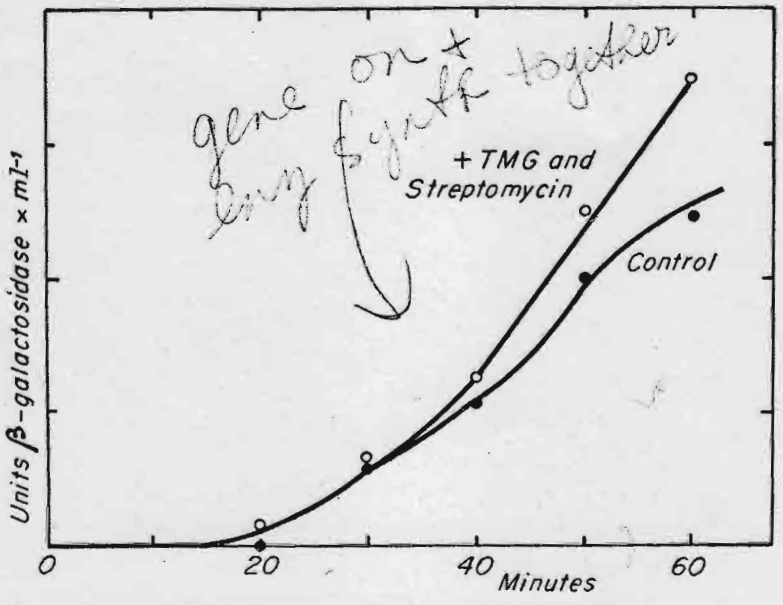


FIG. 3. Enzyme formation during first hour in mating B.
Mating under usual conditions. To an aliquot streptomycin (0·8 mg/ml.) was added at 20 minutes, and TMG at 25 minutes, to allow comparison of synthesis with and without inducer.

Such a mating therefore allows immediate and complete interaction of the z^+ from the ♂ with the i^- from the ♀. The possibility that the interaction depends upon actual recombination yielding z^+i^- in *cis* configuration is excluded because: (a) the synthesis begins virtually immediately after injection whereas genetic recombination is known (Jacob & Wollman, 1958) not to occur until 60 to 90 min after injection; (b) the factors z and i are so closely linked that recombination is an exceedingly rare

event (less than 10^{-4} of the zygotes) while the rate of enzyme synthesis is of an order indicating that most or all of the zygotes participate.

The possibility should also be considered that, rather than taking place through the cytoplasm, the interaction requires actual *pairing* of the homologous chromosome segments. This is excluded by the fact that the following mating:

when performed in the *absence* of inducer, yields no trace of enzyme, at any time after mixing, although conjugation and chromosome injection occur normally as shown by adequate controls involving other markers. The zygotes obtained in matings B and C are genetically identical, except that the wild type alleles (z^+i^+) are in relative excess (about 3 to 1) in (B), while the mutant alleles are in similar excess in (C). This quantitative difference cannot account for the absolute contrast of the results of the reciprocal matings, one allowing vigorous constitutive synthesis, the other none at all. This can only be attributed to the fact that the cytoplasm of the zygote is entirely furnished by the ♀ cell, with no significant contribution from the ♂. Therefore the $i^- \rightarrow z^+$ interaction must be considered to take place through the cytoplasm.

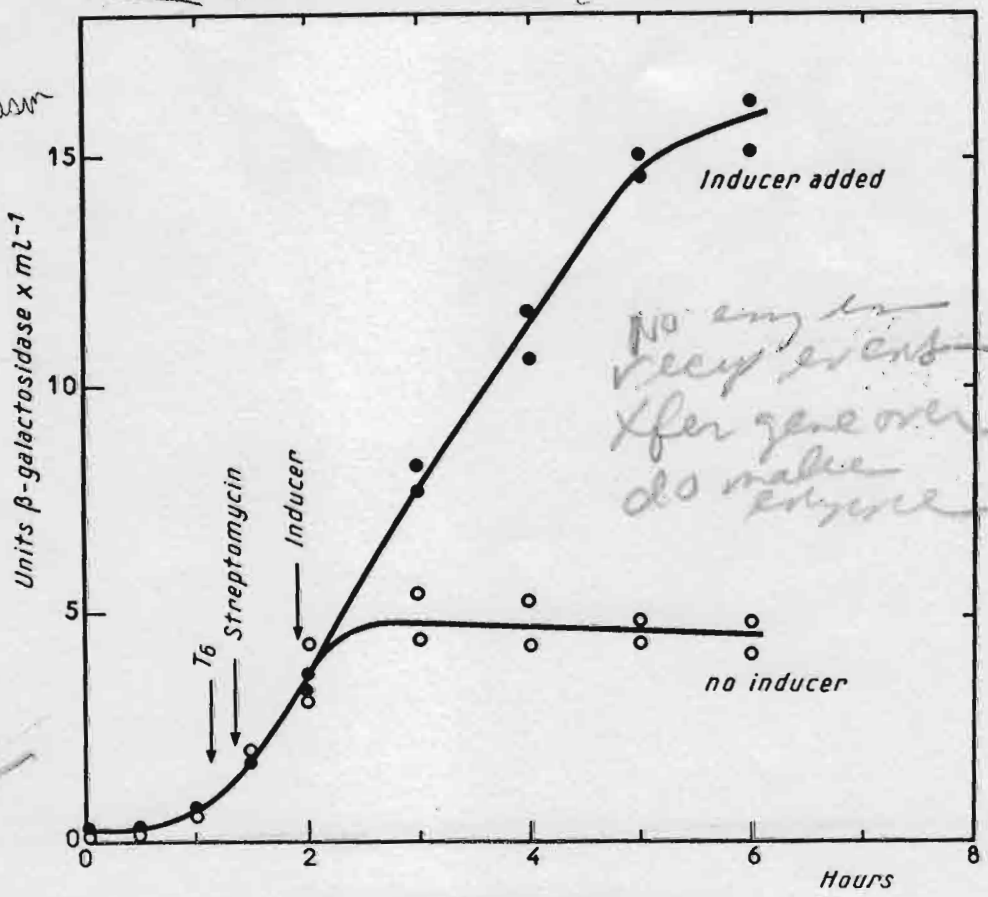


FIG. 4. Enzyme formation in mating D.
Mating performed under usual conditions in quadruplicate in absence of inducer. At times indicated, a suspension of phage T6 (20φ/B final concentration) and streptomycin (1 mg/ml.) were added to all of the cultures and TMG (2×10^{-3}M) was added to two of them (black circles) while the other two (white circles) received no addition.

This result may also be expressed by saying that the i factor sends out a cytoplasmic message which is picked up by the z gene, or gene products. Postulating, as we must, that this message is borne by a specific compound synthesized under the control of the i gene, we may further assume that one of the alleles of the i gene provokes the synthesis of the message, while the other one is inactive in this respect. If these assumptions are adequate, one of the alleles should be absolutely dominant over the other, but the dominance should become expressed only gradually when the cytoplasm of the zygotes came from the recessive parent, while it should be expressed immediately when the cytoplasm came from the dominant parent.

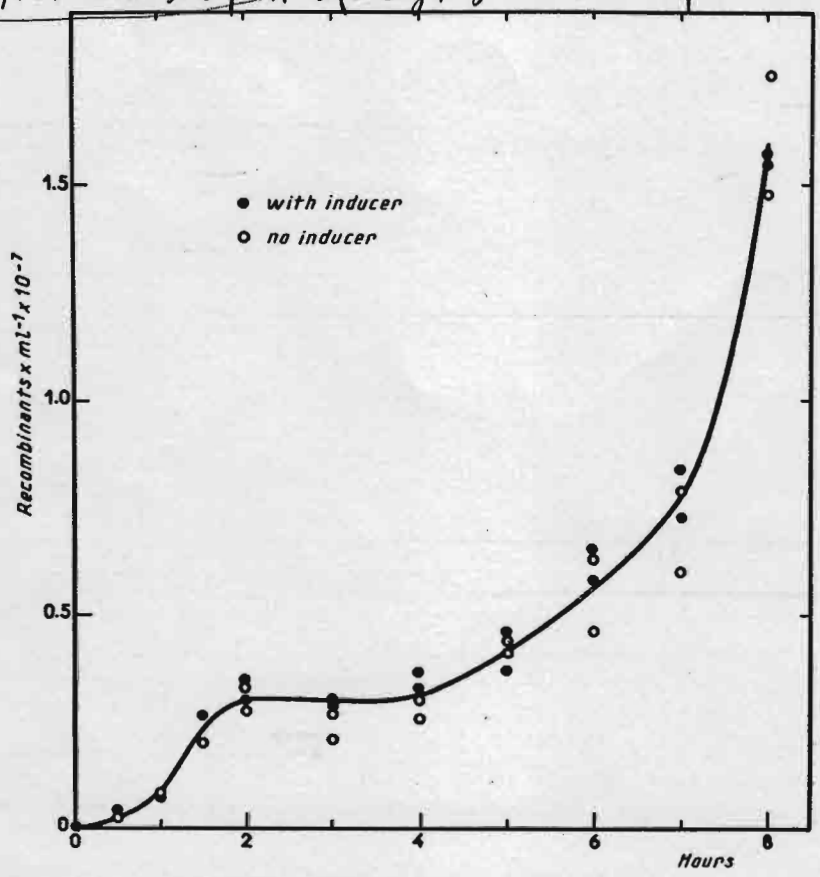


FIG. 5. Recombinant appearance in mating D.

Formation of z^+Sm^r recombinants tested by plating aliquots of the four cultures used in the experiment above (Fig. 4) on lactose-Sm agar. Portions of the culture were diluted 1000-fold and shaken vigorously at 100 minutes to prevent further mating. The increase up to the second hour is due to increasing numbers of zygotes. The increase after the fourth hour is due to *multiplication* of segregants (Wollman, Jacob & Hayes, 1956).

The fact that in matings of type (C) no enzyme is synthesized, even several hours after mating, means that the constitutive (i^-) allele from the ♂ is never expressed. This suggests that the dominant allele is the inducible (i^+). If so, the i^+ should eventually become expressed in matings of type (B)—i.e., the zygotes, initially constitutive (since their cytoplasm comes from the i^- parent), should eventually become inducible. To test this prediction, the following mating was performed:

$\sigma^{\pi} z^+i^+Sm^s\ T6^s \times \varphi\ z^-_2 i^-_3 Sm^r\ T6^r$ (D)

and the synthesis of enzyme, in the absence and in the presence of inducer, was followed over several hours (in order to block induction of the ♂ and remating, a mixture of streptomycin and T6 phage was used). Figure 4 shows that, in the absence of inducer, enzyme synthesis stops about 90 min (or earlier) after entry of the z^+i^+ genes into the ♀ cells. When inducer is added at this stage, enzyme synthesis is resumed, showing that the initially constitutive z^+i^-/z^-i^- zygotes have not been inactivated, but have become inducible.

It should be asked whether this conversion to inducibility, rather than occurring in the heterozygotes, might not correspond to the segregation of homozygous $z^+i^+Sm^rT6^r$ recombinants with concomitant disappearance of the heterozygotes. This is excluded because the earliest homozygous recombinants only appear 2 hr after the time when constitutive synthesis ceases† (Fig. 5).

From these observations we may conclude that the constitutive (i^-) allele is inactive, while the i^+ is dominant, provoking the synthesis of a substance responsible specifically for the inducible behaviour of the galactosidase enzyme-forming center.

5. Discussion and Conclusions

(1) The conclusions which can be directly drawn from the evidence presented above may be summarized as follows:

The synthesis of β-galactosidase and galactoside-permease in *E. coli* is controlled by three extremely closely linked genes (cistrons), *z*, *i* and *y*. The *z* gene determines, in part at least, the structure of the galactosidase protein molecule. The *y* gene probably does the same for the permease molecule, but there is no evidence on this point. The *i* gene in its active form controls the synthesis of a product which, when present in the cytoplasm, prevents the synthesis of β-galactosidase and galactoside-permease, unless inducer is added externally (inducible behaviour). When the *i* gene-product is absent or inactive as a result of mutation within the gene, no external inducer is required for β-galactosidase and galactoside-permease synthesis (constitutive behaviour). The *i* gene product is very highly specific, having no effect on any other known system.

(2) While proving that the interaction of the *i* [redacted] *z* factors involves a specific cytoplasmic messenger, the data presented here do not, by themselves, give any indication as to the mode of action of this compound. Two alternative models of this action should be considered.

According to one, which we shall call the "inducer" model, the activity of the galactosidase-forming system‡ requires the presence of an inducer, both in the constitutive and in the inducible organism. Such an inducer (a galactoside) is synthesized by *both* types of organisms. The i^+ gene controls the synthesis of an enzyme which destroys or inactivates the inducer: hence the requirement for external inducer in the wild type. The i^- mutation inactivates the gene (or its product, the enzyme) allowing accumulation of endogenous inducer. This model accounts for the dominance of inducibility over constitutivity, and for the kinetics of conversion of the zygotes.

† It may also be recalled that, according to Anderson & Maze (1957), heterozygosis prevails for many generations in the descendants of *E. coli* zygotes.

‡ By this term we designate the system of all cellular constituents *specifically* involved in galactosidase synthesis. This includes the *z* gene and its cytoplasmic products.

According to the other, or "repressor", model the activity of the galactosidase-forming system is inhibited in the wild type by a specific "repressor" (probably also involving a galactosidic residue) synthesized under the control of the i^+ gene. The inducer is required only in the wild-type as an *antagonist* of the repressor. In the constitutive (i^-), the repressor is not formed, or is inactive, hence the requirement for an inducer disappears. This model accounts equally well for the dominance of i^+ and for the kinetic relationships.

(3) The "repressor" hypothesis might appear strictly *ad hoc* and arbitrary were it not also suggested by other facts which should be briefly recalled. That the synthesis of certain constitutive enzyme systems may be specifically inhibited by certain products (or even substrates) of their action, was first observed in 1953 by Monod & Cohen-Bazire working with constitutive galactosidase (of *E. coli*) (1953*a*) or with tryptophan-synthetase (of *A. aerogenes*) (1953*b*), and by Wijesundera & Woods (1953), and Cohn, Cohen & Monod (1953) independently working with the methionine-synthase complex of *E. coli*. It was suggested at that time that this remarkable inhibitory effect could be due to the displacement of an internally-synthesized inducer, responsible for constitutive synthesis, and it was pointed out that such a mechanism could account, in part at least, for the proper adjustment of cellular syntheses (Cohn & Monod, 1953; Monod, 1955). During the past two or three years, several new examples of this effect have been observed and studied in some detail by Vogel (1957), Yates & Pardee (1957), Gorini & Maas (1957). It now appears to be a general rule, for bacteria, that the formation of sequential enzyme systems involved in the synthesis of essential metabolites is *inhibited* by their end product. The convenient term "repression" was coined by Vogel to distinguish this effect from another, equally general, phenomenon: the control of enzyme *activity* by end products of metabolism.

(4) The facts which demonstrate the existence and wide occurrence of repression effects justify the basic assumptions of the repressor model. They do not allow a choice between the two models. Further considerations make the repressor model appear much more adequate:

(a) The repressor model is simpler since it does not require an independent inducer-synthesizing system.

(b) It predicts that constitutive mutants should, as a rule, synthesize more enzyme than induced wild-type. This appears to be the case for such different systems as galactosidase, amylomaltase (Cohen-Bazire & Jolit, 1953), glucuronidase (Stoeber, 1959, unpublished data), galactokinase of *E. coli* and penicillinase of *B. cereus* (Kogut, Pollock & Tridgell, 1956).

(c) The inducer model, if generalized, implies that internally synthesized inducers (Buttin, unpublished) operate in all constitutive systems. This assumption, first suggested as an interpretation of repression effects, has not been vindicated in recent work on repressible biosynthetic systems (Vogel, 1957; Gorini & Maas, 1957; Yates & Pardee, 1957). In contrast, the synthesis of numerous inducible systems has been known for many years (Dienert, 1900; Stephenson & Yudkin, 1936; Monod, 1942) to be inhibited by glucose and other carbohydrates. The recent work of Neidhardt & Magasanik (1957) has shown this glucose effect to be comparable to a non-specific repression and these authors have suggested that glucose acts as a preferential metabolic source of internally synthesized repressors. If this is so, and if our repressor model is correct, the conversion of glucose into specific galactosidase-repressor should be blocked in the constitutives. Accordingly the galactosidase-forming system of the

mutant should be largely insensitive to the glucose effect while other inducible systems should retain their sensitivity. That this is precisely the case (Cohn & Monod, 1953) is a very strong argument in favor of the repressor model.

(5) If adopted and confirmed with other systems, the repressor model may lead to a generalizable picture of the regulation of protein syntheses; according to this scheme, the basic mechanism common to all protein-synthesizing systems would be inhibition by specific repressors formed under the control of particular genes, and antagonized, in some cases, by inducers. Although the wide occurrence of repression effects is certain, the situation revealed with the present system, namely a genetic "complex" comprising, besides the "structural" genes (*z*, *y*) a repressor-making gene (*i*) whose function is to block or regulate the expression of the neighboring genes is, so far, unique for enzyme systems. But the formal analogy between this situation and that which is known to exist in the control of immunity and zygotic induction of temperate bacteriophage is so complete as to suggest that the basic mechanism might be essentially the same. It should be recalled that according to Jacob & Wollman (1956), when a chromosome from a λ-lysogenic ♂ of *E. coli* is injected into a non-lysogenic ♀, the process of vegetative phage development is started, which involves as an essential, probably as a primary, step the synthesis of specific proteins. When the reverse mating (♂ non-lysogenic × ♀ λ-lysogenic) is performed, zygotic induction does not occur; nor does vegetative phage develop when such zygotes are superinfected with λ particles. The λ-lysogenic cell is therefore immune against manifestations of prophage or phage potentialities, *and the immunity is expressed in the cytoplasm* (Jacob, 1958–59). Moreover the immunity is strictly specific, since it does not extend to other, even closely related, phages. The formation, under the control of a phage gene, of a specific repressor, able to block synthesis of proteins determined by other genes of the phage, would account for these findings.

(6) Implicit in the repressor model are two critical questions, which for lack of evidence we have avoided discussing, but which should be explicitly stated in conclusion. These questions are:

(a) What is the chemical nature of the repressor? Should it be considered a primary or a secondary product of the gene?

(b) Does the repressor act at the level of the gene itself, or at the level of the cytoplasmic gene-product (enzyme-forming system)?

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The Operon: A Group of Genes Whose Expression is Coordinated by an Operator

By François Jacob, David Perrin, Carmen Sanchez and Jacques Monod

The analysis of different bacterial systems leads to the conclusion that in the synthesis of certain proteins (enzymatic or viral) a double genetic determinism intervenes involving two genes with distinct functions: one (the gene for structure) responsible for the structure of the molecule, and the other (the regulator gene) governing the expression of the former through the intermediary action of a repressor[1]. The regulator genes which have so far been identified show the remarkable property of exercising a *pleiotrophic coordinated effect*, each governing the expression of several genes for structure, closely linked together, and corresponding to protein enzymes belonging to the *same biochemical sequence*. To explain this effect, it seems necessary to invoke a new genetic entity, called "operator," which would be: (a) adjacent to a group of genes and would control their activity; and (b) would be sensitive to the repressor produced by a particular regulator gene[1]. In the presence of the repressor, the expression of the group of genes would be inhibited through the intermediation of the operator. This hypothesis leads to some distinctive predictions concerning the mutations which could affect the structure of the operator. In effect:

(1) Certain mutations affecting an operator would be manifested by the loss of the capacity to synthesize the proteins determined by the group of linked genes "coordinated" by that operator. These simple mutations would behave like physiological deletions, and would not be complemented by any mutant in which one of the genes for structure of the sequence had been altered.

(2) Other mutations, for example involving a loss of sensitivity (affinity) of the operator for the corresponding repressor, would be manifested by the constitutive synthesis of the proteins determined by the coordinated genes. These constitutive mutations, unlike those which result from the inactivation of regulator genes, would be *dominant* in a diploid heterozygote, but their effect would only be manifested for the genes which were in the *cis* position with respect to the mutated operator.

We have studied certain mutations which, affecting the metabolism of lactose in *Escherichia coli* K-12 and acting simultaneously on the synthesis of β-galacto-

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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.

Units of permease [*cf.* (5)] in percentage of the amount found 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 (oc)	nd	33 (oc)	270	100	100
$i^-o^+z_1^-y^+/Fi^+o^cz^+y^+$		110 (oc)	nd	50 (oc)	330	100	100
$i^+o^+z^+y_R^-/Fi^+o^cz_1^-y^+$		<1	30	—	100	400	—
$i^+o^+z_1^-y^+/Fi^+o^cz^+y_R^-$		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^0) 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_2^0 (i^-o^0) and o_{118}^0 (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_2^0 revertants) or i^-z^- (with o_{118}^0 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

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	26	–

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.

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.

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 $i^- z^{del} y^+ / F\text{-}i^+ z^- y^\pm$ 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).

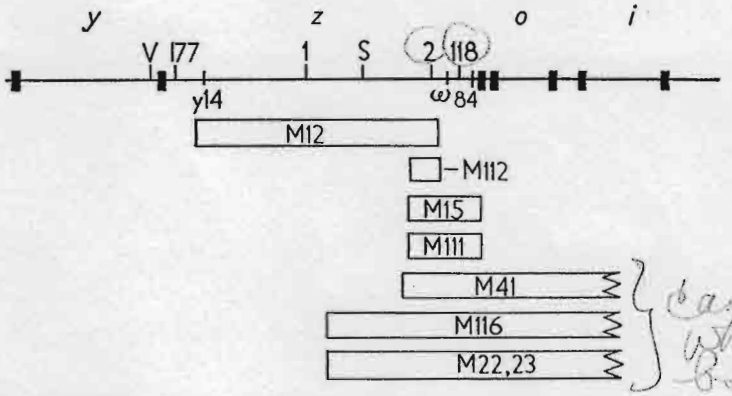

FIG. 1. *The extent of deletions in the* lac *region.* The deletions were mapped using the z^- and y^- markers indicated here. Mutant 177 maps outside deletion M12 (Jacob, personal communication). Revertant M112 gives recombinants with all *lac*⁻ mutants tested to date. The deletion mutants, M22 and 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).

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Restoration of operon activity by suppressors

The genes clustered in the *lac* region of the *Escherichia coli* chromosome are under the control of a single operator region adjacent to the gene for β-galactosidase (EC 3.2.1.2). o^0 mutants, located in this region, shut off the activities of all three genes in the operon, β- galactosidase (*z*), permease (*y*), and galactoside transacetylase[1]. It has been shown that these mutants, including the one described below, do not produce any detectable messenger-RNA from the operon[2]. Since according to this result there are no cytoplasmic gene products from the operon in mutants of this type, the ways in which reversion to the lac^+ state can occur should be limited. It is the purpose of this note to describe experiments which test this expectation by determining the nature of revertants of such a mutant.

We have previously reported the isolation of phenotypically z^+y^+ revertants of an o^0 mutant for the lactose operon[3]. The isolation and characterisation of a new type of revertant of this same mutant is reported here. By the use of melibiose, an α-galactoside which requires permease but not β-galactosidase for its metabolism[4], it was possible to select for phenotypically z^-y^+ revertants. The o^0 mutant used, 2320, was an i^- strain (produces no repressor) obtained from Dr. F. JACOB. More than 50 % of the revertants isolated using melibiose produced little or no β-galactosidase. These phenotypically z^-y^+ revertants are not the result of a mutation at another locus producing a new type of permease, since mutants deleted in the *lac* region do not revert in this manner. After purification, the revertants were assayed for permease by a new method involving the measurement of *o*-nitrophenyl-β-D-galactoside uptake. This method will be described in detail elsewhere[5]. In Table I are listed some of the properties of the "z^-y^+" revertants which were isolated. The very occurrence of this class of revertant indicates that they are not true back-mutants and that suppressors are involved.

Genetic studies were carried out to determine whether any of the suppressors in these revertants were located outside the *lac* region. In interrupted mating experiments, various wild-type Hfr donors were mated with these F^- revertants. The

TABLE I

CHARACTERISTICS OF z^-y^+ REVERTANTS OF 2320

All activities and the levels of CRM production are in terms of % of the amount produced by a fully constitutive strain, 33co.

Revertant number	*β-Galactosidase*	*Permease*	*CRM* production*	*Generation time** (min)*
M12	0	72	0	80
M15	0	91	10	80
M22	$3 \cdot 10^{-3}$	2	0	85
M31	$3 \cdot 10^{-3}$	18	0	95
M32	$3 \cdot 10^{-3}$	5	—	80
M35	$3 \cdot 10^{-3}$	0–1	—	—
M41	0	26	—	80
M42	$3 \cdot 10^{-3}$	12	0	110
M43	$3 \cdot 10^{-3}$	7	0	105
2320	$3 \cdot 10^{-3}$	<1	0	80

* CRM, material cross-reacting with β-galactosidase antibody, was determined according to the method of M. Cohn and D. Perrin as described by MASTERS AND PARDEE[6].

** Generation times are in minutes on glycerol–minimal medium.

time of entry of the wild-type allele of the suppressor locus was determined by scoring the appearance of su^0o^0 recombinants (the original o^0 mutant). These melibiose$^-$ recombinants could be distinguished from the revertants themselves on tetrazolium plates. In addition to the "z^-y^+" revertants, three "z^+y^+" revertants[3] (28, 1.0 %; 26, 1.5 % and 16, 60 % β-galactosidase), were mapped by this technique.

The 10 revertants which were examined fell into 4 classes according to suppressor location (Fig. 1). 1, two of the "z^+y^+" strains, 26 and 28, carry suppressors near the

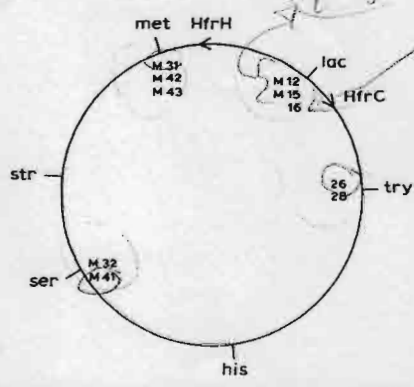

Fig. 1. Location of suppressors on *E. coli* chromosomal map. The map positions of the suppressors are rough estimates except for the three internal suppressors in the *lac* region.

tryptophan marker. 2, the suppressors in M32 and M41 map near the serine marker. 3, the wild-type suppressor loci for M31, M42 and M43 enter close to the methionine marker. 4, three suppressors, in M12, M15 and 16, are located within the *lac* region. None of these gave su^0o^0 recombinants with the Hfr donors used.

Two of this last group, M12 and M15, were studied further. Several Hfr strains carrying mutations either in the *z* or *y* cistrons were mated with M12 and M15, and

the frequency of z^+y^+ recombinants from the "z^-y^+" revertants was determined. By this method it was determined that the reversion in M15 is at the same site or very close to the o^0 mutation. In contrast, the reversion in M12 involves a deletion extending over a major part of the *z* region.

AMES *et al.*[7] have suggested two mechanisms for restoration of operon activity in suppressed o^0 mutants for the histidine pathway. They propose that in one revertant a deletion connects the inactivated histidine operon to an operator for another system, thus restoring its activity. In the second case, the defective operon appears to have been duplicated on an extrachromosomal fragment, again possibly coming under the control of a new operator. The revertants reported on in this note do not belong to either of these classes. M12 does not appear to carry a deletion extending out of the *lac* operon. It was found that when the heterogenote, $F^-i^+z^+y^-/i^-z^-y^+$, was formed by introducing an *F-lac* factor into M12, repression of the previously constitutive permease activity resulted. The repressor-sensitive region of the operon is thus probably still intact. It does not seem plausible that the i^+ repressor could act on the new operator for the *lac* region as would have to be supposed if the model of AMES *et al.* is valid for this system. Secondly, when an Hfr carrying a deletion of the *lac* region was mated with representatives of the three external suppressors, 26, M31 and M41, it was found that melibiose$^-$ (or *lac*$^-$) recombinants appeared immediately the lac region entered the recipient. If these suppressor loci were actually translocated and reactivated operons, such recombinants should not appear until after the wild-type "suppressor loci" have entered the cell. Thus, neither extended deletions nor translocations can be invoked to account for the various suppressors described here.

With these mechanisms excluded for restoration of operon activity, it becomes difficult to interpret the many suppressors in terms of the accepted model of gene control. Either the factors involved in action at the operator site are more complex than previously supposed, or new types of suppressors which affect the gene transcription process are involved.

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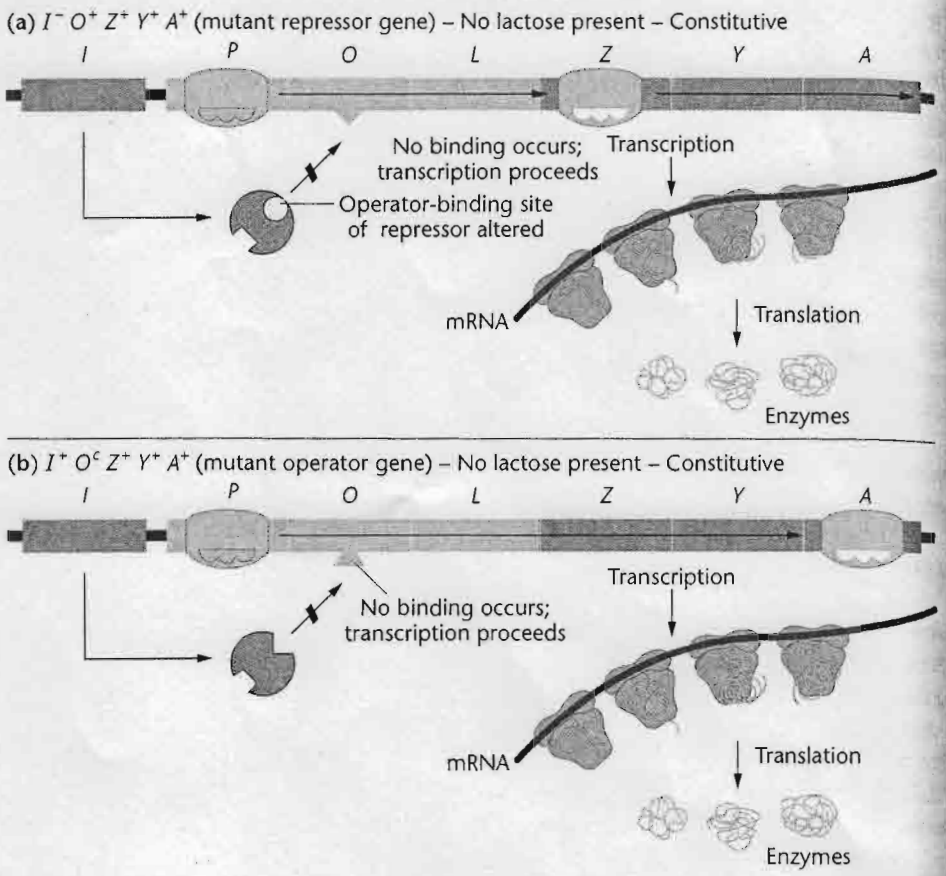

FIGURE 17–6 The response of the *lac* operon in the absence of lactose when a cell bears either the I^- or the O^C mutation.

molecule, no direct chemical evidence was immediately forthcoming. A single *E. coli* cell contains no more than 10 or so copies of the *lac* repressor; direct chemical identification of 10 molecules in a population of millions of proteins and RNAs in a single cell presented a tremendous challenge.

TABLE 17–1 A Comparison of Gene Activity (+ or −) in the Presence or Absence of Lactose for Various *E. coli* Genotypes

		Presence of β-Galactosidase Activity	
	Genotype	*Lactose Present*	*Lactose Absent*
	$I^+O^+Z^+$	+	−
A.	$I^+O^+Z^-$	−	−
	$I^-O^+Z^+$	+	+
	$I^+O^cZ^+$	+	+
B.	$I^-O^+Z^+/F'I^+$	+	−
	$I^+O^cZ^+/F'O^+$	+	+
C.	$I^+O^+Z^+/F'I^-$	+	−
	$I^+O^+Z^+/F'O^c$	+	−
D.	$I^sO^+Z^+$	−	−
	$I^sO^+Z^+/F'I^+$	−	−

Note: In parts B to D, most genotypes are partially diploid, containing an F factor plus attached genes (F′).

In 1966, Walter Gilbert and Benno Müller-Hill reported the isolation of the *lac* repressor in partially purified form. To achieve the isolation, they used a *regulator quantity* (I^q) mutant strain that contains about 10 times as much repressor as do wild-type *E. coli* cells. Also instrumental in their success was the use of the gratuitous inducer, IPTG, which binds to the repressor, and the technique of **equilibrium dialysis**. In this technique, extracts of I^q cells were placed in a dialysis bag and allowed to attain equilibrium with an external solution of radioactive IPTG, which is small enough to diffuse freely in and out of the bag. At equilibrium, the concentration of IPTG was higher inside the bag than in the external solution, indicating that an IPTG-binding material was present in the cell extract and that this material was too large to diffuse across the wall of the bag.

Ultimately, the IPTG-binding material was purified and shown to have various characteristics of a protein. In contrast, extracts of I^- constitutive cells having no *lac* repressor activity did not exhibit IPTG-binding activity, strongly suggesting that the isolated protein was the repressor molecule.

To confirm this thinking, Gilbert and Müller-Hill grew *E. coli* cells in a medium containing radioactive sulfur and then isolated the IPTG-binding protein, which was labeled in its sulfur-containing amino acids. This protein was mixed with DNA from a strain of phage lambda (λ), which carries the $lacO^+$ gene. The DNA sediments at 40*S*, while the IPTG-binding protein sediments at 7*S*. The DNA and protein were mixed and sedimented in a gradient, using ultracentrifugation. The radioactive protein sediments at the same rate as