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

ARTHUR B. PARDEE‡, FRANÇOIS JACOB AND JACQUES MONOD

Institut Pasteur, Paris and University of California, Berkeley, California, U.S.A.

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-galacto-sides.

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 (Ricken-berg, Cohen, Buttin & Monod, 1955; Cohen & Monod, 1957; Cohn, 1957).

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 (S⁸), 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 S⁸, 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 ³⁵S from ³⁵SO₄⁻ 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	ONPG = o-nitrophenyl-β-D-galactoside
IPTG = *iso*propyl-thio-β-D-galactoside	TMG = methyl-thio-β-D-galactoside

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_w^- ; z_z^- ; 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:

$$\male\ z^+y^+i^+Sm^s \times \female\ z_s^-y^+i^+Sm^r \tag{A}$$

is performed in the presence of inducer (IPTG) and of 1 mg/ml. of streptomycin. The $\female$ lack the z^+ factor; the $\male$ are inhibited by streptomycin (cf. Methods); the zygotes are not, because they inherit their cytoplasm from the $\female$ 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 cotransduction 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_5^-) 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
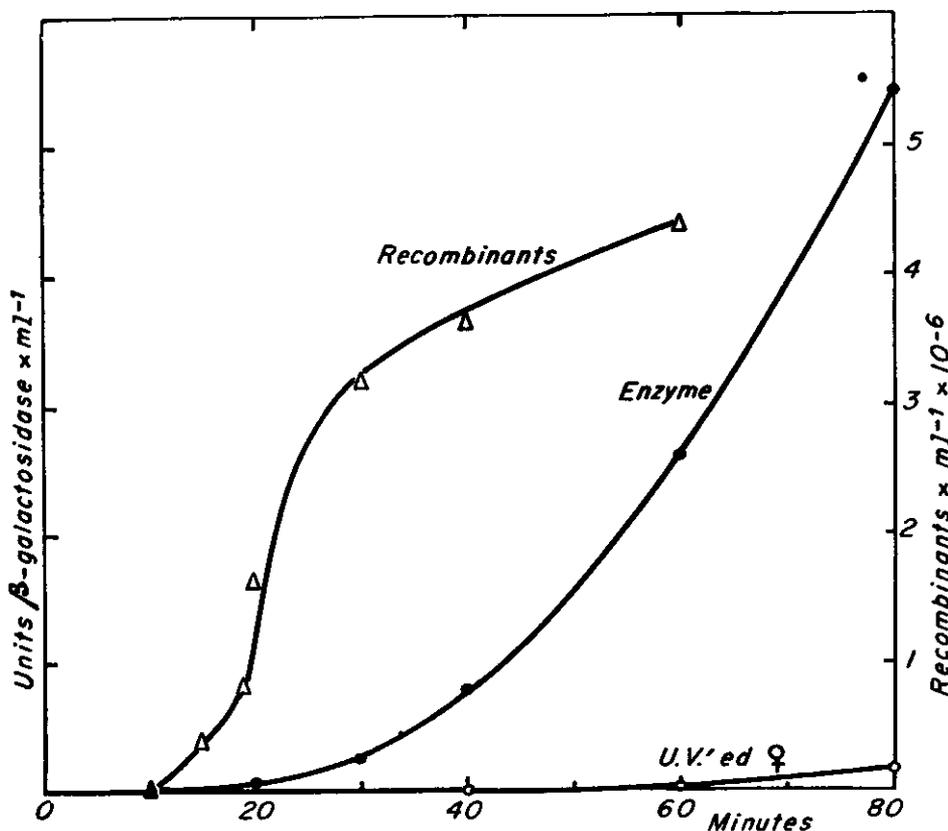

FIG. 2. Enzyme formation and appearance of recombinants in mating A.
Mating in presence of streptomycin (1 mg/ml.) and IPTG (10^{-3}M). A control with u.v.-treated ♀ cells (0·01 % survival) is shown. Recombinants (z^+Sm^r) selected by plating on Sm-lactose agar after blending (separate experiment with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

$$♂ \; z^+Sm^s \; maltose^+ \; \times \; ♀ \; z^-Sm^r \; maltose^-$$

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:

$$\male\ z^+i^+ \times \female\ z^-_2i^-_3 \tag{B}$$

was performed *in absence of inducer*. The $\male$ cannot synthesize enzyme, because they are i^+. The $\female$ 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).
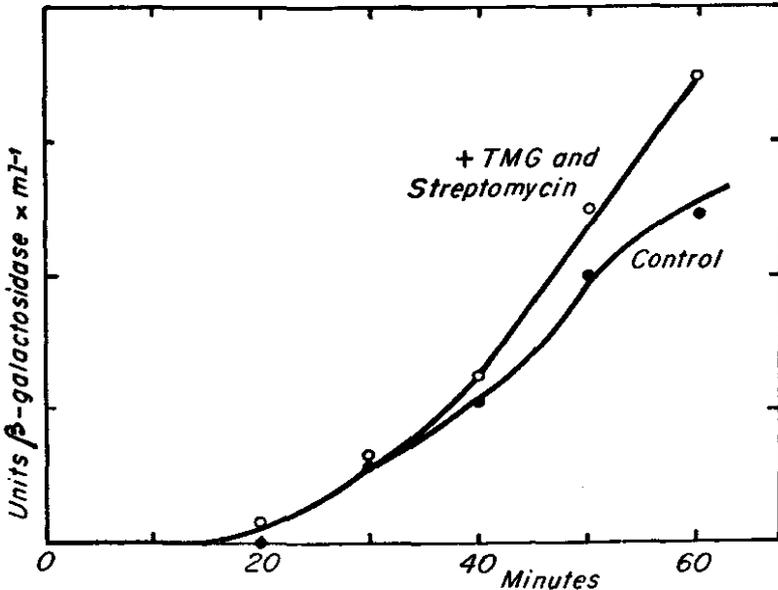

Fig. 3. Enzyme formation during first hour in mating B.
Mating under usual conditions. To an aliquot streptomycin (0·8 mg/ml.) was added at 20 minutes, and TMG at 25 minutes, to allow comparison of synthesis with and without inducer.

Such a mating therefore allows immediate and complete interaction of the z^+ from the $\male$ with the i^- from the $\female$. 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:

$$\male\ z_2^- i_2^- \times\ \female\ z^+ i^+ \tag{C}$$

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 $\female$ cell, with no significant contribution from the $\male$. Therefore the $i^- \to z^+$ interaction must be considered to take place through the cytoplasm.
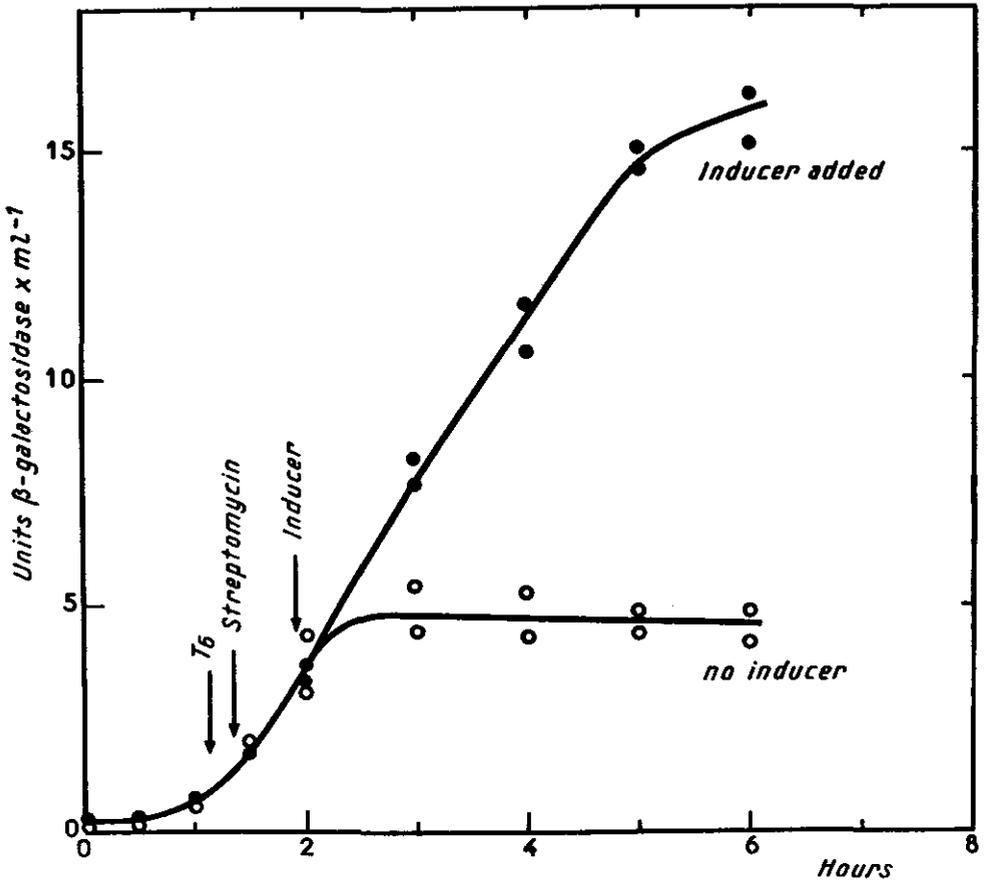

FIG. 4. Enzyme formation in mating D.
Mating performed under usual conditions in quadruplicate in absence of inducer. At times indicated, a suspension of phage T6 (20ϕ/B final concentration) and streptomycin (1 mg/ml.) were added to all of the cultures and TMG (2×10^{-3}M) was added to two of them (black 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.
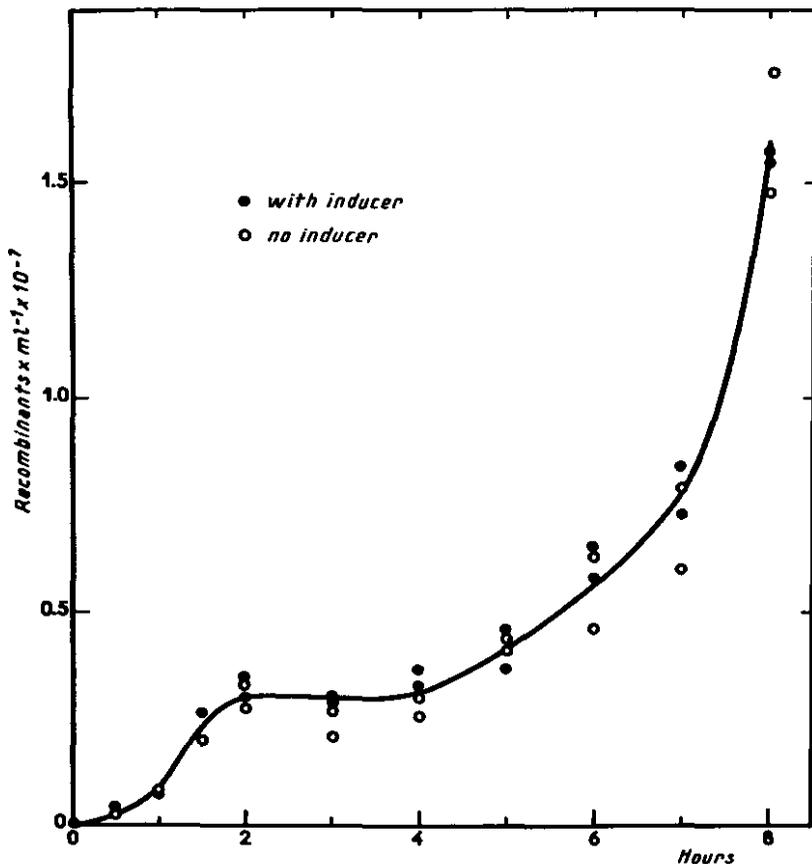

Fig. 5. Recombinant appearance in mating D.
Formation of z^+Sm^r recombinants tested by plating aliquots of the four cultures used in the experiment above (Fig. 4) on lactose-Sm agar. Portions of the culture were diluted 1000-fold and shaken vigorously at 100 minutes to prevent further mating. The increase 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:

$$\text{♂ } z^+i^+Sm^s \ T6^s \ \times \ \text{♀ } z^-_3i^-_3Sm^r \ T6^r \qquad \qquad \text{(D)}$$
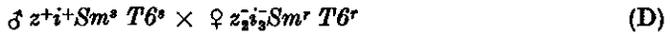
and the synthesis of enzyme, in the absence and in the presence of inducer, was followed over several hours (in order to block induction of the ♂ and remating, a mixture of streptomycin and T6 phage was used). Figure 4 shows that, in the absence of inducer, enzyme synthesis stops about 90 min (or earlier) 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 and 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*) (1953a) or with tryptophan-synthetase (of *A. aerogenes*) (1953b), and by Wijesundera & Woods (1953), and by 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

327

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)?

REFERENCES

Anderson, T. F. & Maze, R. (1957). *Ann. Inst. Pasteur*, **93**, 194.
Benzer, S. (1957). "The elementary units of heredity", in *The Chemical Basis of Heredity*, ed. by W. McElroy & B. Glass, p. 70. Baltimore: Johns Hopkins Press.
Cohen, G. N. & Monod, J. (1957). *Bact. Rev.* **21**, 169.
Cohen-Bazire, G. & Jolit, M. (1953). *Ann. Inst. Pasteur*, **84**, 937.
Cohn, M. (1957). *Bact. Rev.* **21**, 140.
Cohn, M., Cohen, G. N. & Monod, J. (1953). *C.R. Acad. Sci., Paris*, **236**, 746.
Cohn, M. & Monod, J. (1953). In *Adaptation in Microorganisms*, p. 132. Cambridge: University Press.
Dienert, F. (1900). *Ann. Inst. Pasteur*, **14**, 139.
Fisher, K. W. (1957). *J. Gen. Microbiol.* **16**, 120.

Gorini, L. & Maas, W. K. (1957). *Biochim. biophys. Acta*, **25**, 208.

Hayes, W. (1953). *Cold Spr. Harb. Symp. Quant. Biol.* **18**, 75.

Jacob, F. (1955). *Virology*, **1**, 207.

Jacob, F. (1958–59). Harvey Lectures, Series **54**, in the press.

Jacob, F. & Wollman, E (1956). *Ann. Inst. Pasteur*, **91**, 486.

Jacob, F. & Wollman, E. (1957). *C.R. Acad. Sci., Paris*, **244**, 1840.

Jacob, F. & Wollman, E. (1958). *Symp. Soc. Exp. Biol.* **12**, 75. Cambridge: University Press.

Kogut, M., Pollock, M. R. & Tridgell, E. J. (1956). *Biochem. J.* **62**, 391.

Lederberg, J. (1947). *Genetics*, **32**, 505.

Lederberg, J., Lederberg, E. M., Zinder, N. D. & Lively, E. R. (1951). *Cold Spr. Harb. Symp. Quant. Biol.* **16**, 413.

Monod, J. (1942). *Recherches sur la croissance des cultures bactériennes*. Paris: Herman Edit.

Monod, J. (1955). *Exp. Ann. Biochim. Méd., série* **17**, 195. Paris: Masson & Cie Edit.

Monod, J. & Cohen-Bazire, G. (1953a). *C.R. Acad. Sci., Paris*, **236**, 417.

Monod, J. & Cohen-Bazire, G. (1953b). *C.R. Acad. Sci., Paris*, **236**, 530.

Neidhardt, F. C. & Magasanik, B. (1957). *J. Bact.* **73**, 253.

Pardee, A. B., Jacob, F. & Monod, J. (1958). *C.R. Acad. Sci., Paris*, **246**, 3125.

Rickenberg, H. V., Cohen, G. N., Buttin, G. & Monod, J. (1956). *Ann. Inst. Pasteur*, **91**, 829.

Stephenson, M. & Yudkin, J. (1936). *Biochem. J.* **30**, 506.

Vogel, H. J. (1957). In *The Chemical Basis of Heredity*, ed. by W. D. McElroy & B. Glass, p. 276. Baltimore: Johns Hopkins Press.

Wijesundera, S. & Woods, D. D. (1953). *Biochem. J.* **55**, viii.

Wollman, E. & Jacob, F. (1955). *C.R. Acad. Sci., Paris*, **240**, 2449.

Wollman, E., Jacob, F. & Hayes, W. (1956). *Cold Spr. Harb. Symp. Quant. Biol.* **21**, 141.

Yates, R. A. & Pardee, A. B. (1957). *J. Biol. Chem.* **227**, 677.