♂ $z^+i^+Sm^s\ T6^s$ × ♀ $z^-_2i^-_3Sm^r\ T6^r$ (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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