On the Regulation of Gene Activity

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INTRODUCTION

According to modern concepts, the deoxynucleotide sequence which constitutes a gene participates in two distinct chemical processes. In the first, for which the term *replication* should be reserved, free deoxyribonucleotides are linearly assembled by specific base-pairings, forming an identical sequence or replica of the original sequence; the second process, which we shall call *transcription*, allows the gene to perform its physiological function, i.e., to specify the molecular structure of a certain protein or polypeptide chain. Transcription does not appear to be a direct process, since it most probably involves the formation of an intermediate as carrier of the genetic information. Two stages may then be distinguished in transcription, the first of which is presumably closely similar to replication, involving, however, ribonucleotides instead of deoxynucleotides, and resulting in an RNA "transcript" of the original DNA sequence. In the second transcription stage, the RNA transcript in turn directs the assembly of amino acids into the polypeptide (Fig. 1).

Although, as assumed in this description, a single gene may be both necessary and sufficient to specify the structure of a protein, it is well known that the synthesis of many, if not most or all, proteins, that is to say the expression of most if not all genes, is controlled by other specific agents.

In *E. coli* the formation of the enzyme tryptophan-synthetase is under the control of a structural gene, clearly identified and located on the chromosomal map (Yanofsky and Crawford, 1959). When bacteria are grown in the absence of tryptophan, the enzyme is actively synthesized. As soon as tryptophan is added to the medium, the enzyme ceases to be synthesized (Monod and Cohen-Bazire, 1953). This effect of tryptophan, which has been called *repression* of enzyme synthesis (Vogel, 1957), is extremely specific: tryptophan inhibits the synthesis of the series of enzymes involved in tryptophan synthesis (Cohen and Jacob, 1959), but not of other pathways; no other compound (except analogs of tryptophan) exerts this effect.

In *E. coli* the formation of the enzyme β-galactosidase is under the control of a structural gene, also identified and mapped on the bacterial chromosome. When bacteria are grown in the absence of a galactoside, only traces of β-galactosidase are formed by the cells. As soon as a galactoside is added, the rate of synthesis of this enzyme increases by about 10,000-fold. This effect, called *induction*, is also very specific. Galactosides increase the rate of synthesis of β-galactosidase and of certain other enzymatic components involved in lactose utilization, without affecting other systems; no compounds other than galactosides exert an inducing effect on β-galactosidase synthesis (see Monod and Cohn, 1952; Cohen and Monod, 1957; Jacob and Monod, 1961).

When *E. coli* is infected by a temperate phage, phage DNA replicates rapidly, and viral proteins are formed by the cells which eventually lyse and release viral particles. If, however, the genetic material of the phage is carried in the prophage state by the lysogenic cells, the viral DNA replicates at the pace of the bacterial chromosome and viral proteins are not formed. Furthermore, if such lysogenic cells are infected with phage particles of the same strain as the prophage, the genetic material of the virus is injected into the cell; but no viral DNA or proteins are formed after infection. This phenomenon is called *immunity*. In lysogenic cells, therefore, the viral genes of the prophage or of superinfecting particles are not expressed. Only as a result of a change in cellular conditions, either spontaneous or induced by various agents such as UV light, X rays, or various chemicals, do the viral genes express their potentialities (i.e., their capacity to specify viral proteins) and the bacteria produce phage particles (see Lwoff, 1953; Jacob, 1954, 1960).

The three systems which we have used as examples appear to be widely different physiologically. Yet, the results of genetic analysis and biochemical characterization of mutations affecting these systems are so closely similar that they point to a common basic mechanism operating in the three systems. The main conclusions drawn from this study have led to a general picture of the activity of structural genes and of the genetic control of protein synthesis in bacteria (Jacob and Monod, 1961). In the present paper we wish to discuss this general model and the main experimental findings which support it. For the sake of clarity and brevity, it will be convenient to describe the model briefly before reviewing the evidence which has led to its formulation.

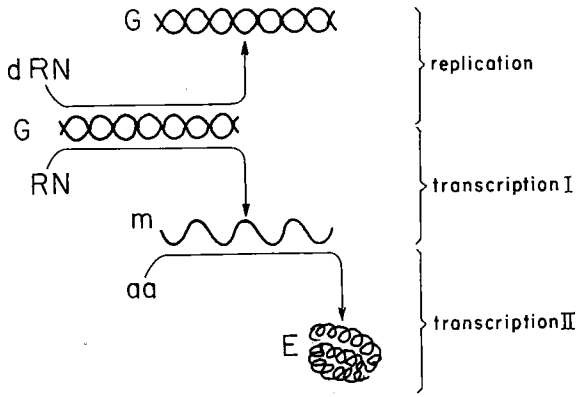

FIGURE 1. *Replication and transcription of DNA.* G: gene; dRN: deoxyribonucleotides; RN: ribonucleotides; m: messenger; aa: amino acids; E: enzyme.

structural genes. Such a group of genes, whose transcriptive activity is thus coordinated by a single operator, constitutes an *operon*. The operon may therefore be defined as the unit of primary transcription.

3. The genetic material contains determinants functionally distinct from structural genes (and operators), called regulator genes. A regulator gene produces a cytoplasmic *repressor* which may be visualized as an RNA transcript of the regulator gene. The repressor formed by a given regulator gene has an affinity toward, and tends to associate reversibly with, a specific operator (probably by homology of their base sequences). This combination blocks the initiation of transcription of the whole operon controlled by the operator and therefore prevents the synthesis of the proteins governed by the structural genes belonging to the operon.

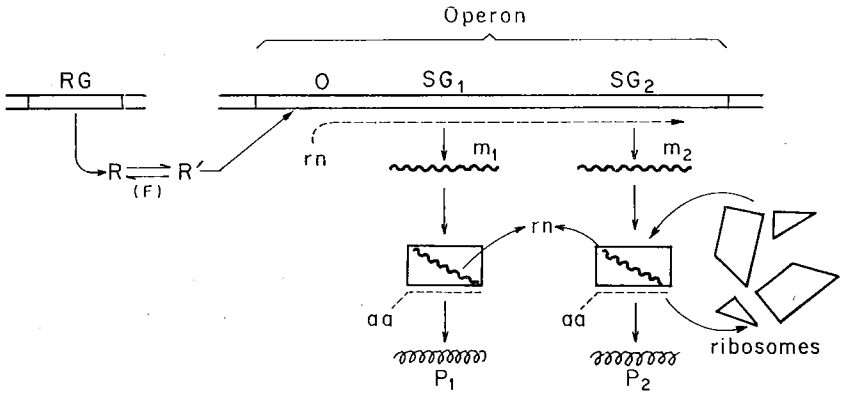

FIGURE 2. *General model of the regulation of enzyme synthesis.* RG: regulator gene; R: repressor converted to R′ in presence of effector F (inducing or repressing metabolite); O: operator; SG_1, SG_2: structural genes; rn: ribonucleotides: m_1, m_2: messengers made by SG_1 and SG_2; aa: amino acids; P_1, P_2: proteins made by ribosomes associated with m_1 and m_2.

GENERAL MODEL

The model diagrammatically represented in Fig. 2 involves the following assumptions.

1. The primary product of structural genes, or "*messenger RNA*," which brings structural information from genes to cytoplasmic protein-forming centers, is a short-lived intermediate. Once completed, it is detached from the DNA and associates in the cytoplasm with pre-existing, non-specialized ribosomal particles. The second transcription takes place on ribosomes, and the messenger is destroyed in the process. Once completed, the polypeptide folds and is detached from the ribosomal particle, which is set free for a new transcription cycle involving the same or any other specific messenger.

2. The synthesis of messenger RNA is supposed to be a sequential and oriented process which can be initiated only at certain regions, or *operators*, on the DNA strands. In many instances, a single operator may control the first transcription of several adjacent

4. The repressors have the property of reacting with certain small molecules (which we shall call effectors). The reactions are specific with respect to both the repressors (R) and the effectors (F) and may be expressed as

$$R + F \rightleftharpoons R' + F'.$$

In certain systems, called *inducible*, only the R form of the repressor can associate with the operator and block the transcription of the operon. The presence of the effector (called inducer) inactivates the repressor and therefore allows transcription to take place. In other systems (called *repressible*) only the modified repressor (R′) is active. The transcription of the operon, allowed in the absence of the effector, is therefore prevented in its presence.

This model may appear rather complex and abstract. It is, however, precise enough to imply very distinctive predictions by which its validity can be tested. In the course of the discussion that follows

we hope to show that, although some details of the mechanisms are speculative, the main outlines of this scheme are not pure assumptions and that, in its major features, it is firmly grounded on experimental evidence. Before discussing the evidence, it may be useful to state clearly these "major features."

a. The first postulate is the distinction between two classes of genes, structural and regulator, which fulfill different functions in the genetic control of protein synthesis.

b. The second is the postulate that the regulatory mechanisms, in inducible and repressible systems as well as in lysogenic systems, are primarily *negative,* i.e., operate by inhibiting, rather than provoking, specific protein synthesis.

c. The third is the postulate that *several* linked structural genes may constitute a coordinated unit of transcriptive activity.

d. The fourth is the postulate that the regulatory mechanisms operate at the genetic level, by controlling the rate of synthesis of the messenger.

e. The fifth is the postulate that the primary gene product (messenger) is a short-lived intermediate.

The experimental material which is to be discussed has already been described and analyzed in detail in other publications (Jacob and Monod, 1961; Monod, Jacob, and Gros, 1961). In the present paper, we will summarize briefly the evidence concerning points a, b, and e and discuss more extensively points c and d, i.e., the problem of the operon as the unit of genetic activity and regulation.

MESSENGER RNA AS THE PRIMARY GENE PRODUCT

It has long been believed that structural information was transferred from the genes to stable templates, such as ribosomal RNA, copied along the genes and maintaining in the cytoplasm the information necessary for protein synthesis. Every gene was supposed to determine the production of a particular type of ribosomal particles which in turn ensured the synthesis of a particular protein (see Crick, 1958). In recent years, however, this hypothesis has encountered several difficulties.

1. The diversity of base composition found in the DNA of different bacterial species is not reflected in the base composition of ribosomal RNA (Belozersky and Spirin, 1960), as would be expected if ribosomal RNA were transcript of DNA.

2. The introduction, by conjugation, of the structural gene which determines the structure of β-galactosidase in *E. coli,* into a cell hitherto lacking it, results in almost immediate synthesis of the enzyme at maximal rate. Furthermore if, after the beginning of enzyme synthesis, the gene is inactivated by ^{32}P decay, it is observed that the capacity for enzyme production does not survive beyond the integrity of the gene. (The "after effects" of ^{32}P disintegration within a cell may be quite complex as shown by the recent work of McFall [1961]. In the experiment referred to above it seems likely, however, that the effect is due to damage in the injected genetic material itself.) Such results are hardly compatible with the hypothesis of stable intermediates carrying in the cytoplasm all the information for protein synthesis (Riley, Pardee, Jacob, and Monod, 1960).

3. Finally, as will be discussed in a later section of this paper, regulation of protein synthesis appears to operate directly at the level of the genetic material. Since both the initiation and cessation of synthesis of an adaptive enzyme follows almost immediately upon addition or removal of the specific effector, it appears that the information-carrying intermediate of protein synthesis must be both rapidly formed and rapidly destroyed.

These difficulties, however, can be overcome by the single assumption that the transfer of structural information from genes to cytoplasm does not involve stable structure persisting in the cytoplasm, but rather metabolically unstable and rapidly renewed messenger molecules (Jacob and Monod, 1961). This assumption also accounts for other observations. It is known, for instance, that infection with virulent phages such as T2 or ϕII, which results in rapid, visible lysis of bacterial DNA, is attended by almost immediate inhibition of bacterial protein synthesis (Cohen, 1949; Monod and Wollman, 1947; Benzer, 1953), whereas infection with a temperate phage such as λ, which does not bring about the destruction of host nuclei, does not prevent bacterial proteins from being formed during the latent period (Jacob, 1951; Siminovitch and Jacob, 1952). The difference in effects between virulent and temperate phages is readily understood if the integrity of bacterial genes is required for continued synthesis of bacterial messengers necessary for continued synthesis of bacterial proteins.

The assumption that the transfer of genetic information from genes to protein-forming centers involves a short-lived messenger implies two predictions. The first is the existence of a molecular species, probably a polyribonucleotide, exhibiting the characteristic features of turnover, size, capacity to attach to ribosomal particles, and base composition, expected of the messenger. The second is that the same ribosomal particle (or population of particles) should prove to synthesize different protein species, depending on which instructions it receives *via* messenger.

The first of these expectations appears to be fulfilled by the discovery that the rapidly renewed RNA fraction first observed in phage infected cells (Volkin and Astrachan, 1957) also exists in growing yeasts (Yčas and Vincent, 1960) or *E. coli* (Gros *et al.*, 1961). The various properties of this RNA fraction (m-RNA) seem to satisfy the requirements for its identification

as messenger. The second expectation has been verified by the demonstration that the same population of ribosomal particles which produces bacterial proteins, when associated with bacterial m-RNA in normal cells, shall produce phage proteins once associated with viral m-RNA in phage infected cells (Brenner, Jacob, and Meselson, 1961). Since other papers in this Symposium deal with messenger RNA and ribosomal particles, we shall not discuss further the reasons for identifying m-RNA with the unstable messenger postulated to account for the genetic-biochemical properties of adaptive enzyme systems and lysogenic systems. It should be pointed out, however, that a final proof of the role of m-RNA as carrier of genetic information shall be obtained only when the synthesis of a specific protein controlled by an identified structural gene will be shown to be synthesized by a reconstructed system in which all the fractions, except messenger RNA, came from cells known to lack this gene.

STRUCTURAL AND REGULATOR GENES

The recognition and identification of "regulator genes" genetically and functionally distinct from "structural genes" is based on the genetic analysis of mutations which affect adaptive enzyme systems (both inducible and repressible) and temperate phage systems, and on detailed studies of the biochemical phenotypes of these mutants (in both the haploid and the heterozygous diploid conditions), including in particular their response to the specific agents known to provoke or inhibit expression of the wild-type properties. Since this evidence has been extensively discussed in recent papers, the following is a brief and deliberately generalized summary of the experimental observations.

A. Structural Genes

The identification of a gene as a determinant of structure rests upon the demonstration that mutations of this gene lead to alterations of the structure of protein. This type of mutation has been recognized in several adaptive enzyme systems, and has been found, as a general rule, to affect exclusively a single protein (or peptide chain). Conversely, all the mutations which affect the molecular properties of an "adaptive" protein (or peptide chain) are found to be clustered in a very small segment of the genetic map and to belong to a single cistron (see Yanofsky, this Symposium). Adaptive systems therefore obey, in this respect, the one gene-one enzyme postulate. In the lysogenic systems similar mutations have been found, but their identification as structural mutations results from indirect, if very likely, inferences from their physiological manifestations rather than on a direct study of the proteins concerned.

An important negative property of the structural mutations is that *they do not affect the regulatory system*, which continues to respond, qualitatively and quantitatively, to the same specific stimuli as in the wild type. (This negative rule suffers one exception, as we shall see in the next section.) For instance, in heterogenotes z^-_{CRM}/Fz^+, carrying both the wild allele of the structural gene for β-galactosidase (z) and a mutant allele which results in the synthesis of a protein antigenically similar to β-galactosidase but devoid of any detectable affinity for galactosides (CRM), both the enzyme and the CRM are induced to the same extent under various cultural conditions (see Table 1). In other words, a genetically altered inducible or repressible protein remains inducible or repressible to the same extent, and by the same agents, as the wild-type protein (Perrin, Jacob, and Monod, 1960).

We may already note at this point that structural genes controlling metabolically sequential enzymes tend to form clusters in bacteria (Demerec and Hartman, 1959). The significance of this remarkable correlation of genetic structure and biochemical function will be discussed later.

B. Regulator Genes

Regulator genes are identified by two types of mutations which occur in adaptive and in lysogenic systems.

1. *Constitutive-Regulator Mutations*

Mutations which result in uncontrolled synthesis of the proteins (often at a very high rate never attained by the wild type), irrespective of the presence or absence of the agent to which the wild type is sensitive, have been found in all three types of systems. Very often these mutations simultaneously affect, to the same extent, the synthesis of *several* proteins; they are invariably found to map in a gene distinct from the structural genes identified as governing the structure of these proteins, and they do not appear to modify in any way the molecular properties of the proteins.

Table 1. Induced Production of β-Galactosidase and CRM by Heterogenotes z^-_{CRM}/Fz^+

(*) Expressed as units of enzyme (or equivalent units for cross reacting material CRM) per mg of dry weight.

Bacteria $i^+z^-_{CRM}/Fi^+z^+$ were grown in minimal medium with glycerol as carbon source and induced with different concentrations of isopropyl-β-D-thiogalactoside (IPTG). Note that heterogenotes produce more enzyme than CRM. Since reverse heterozygotes $i^+z^+/Fi^+z^-_{CRM}$ produce more CRM than enzyme, this is presumably due to the presence of several F factors per chromosome (From Perrin, Jacob, and Monod, 1960.)

	per cent of maximum induction	β-galactosidase*	CRM	CRM/galactosidase
IPTG 5.10^{-5} M	21.5	1,920	670	0.35
" 10^{-4} M	47	5,280	1,900	0.36
" 10^{-3} M	100	11,200	3,900	0.35

TABLE 2. PRODUCTION OF β-GALACTOSIDASE, GALACTOSIDE-TRANSACETYLASE AND GALACTOSIDE-PERMEASE BY HAPLOID AND HETEROGENOTE, REGULATOR-CONSTITUTIVE MUTANTS

(*) The high levels observed in non-induced cultures of i^+/i^- heterogenotes are due to the presence of a small fraction of homozygous i^-/i^- constitutive recombinants.

This table summarizes the results of many experiments. The three activities are given in per cent of those obtained with fully induced haploid, wild type. Note that the activities found in heterogenotes are two to three times greater than those found in haploids. This is probably due to the presence of several F factors per chromosome. i: regulator gene (i^+: inducible; i^-: constitutive). z and y: structural genes for β-galactosidase and galactoside permease respectively. F: sex factor of *E. coli* K12. Δ_{izy} : deletion of the *Lac* region.

Genotypes	Non-induced			Induced		
	β-galactosidase	galactoside-permease	galactoside-transacetylase	β-galactosidase	galactoside-permease	galactoside-transacetylase
1. $i^+z^+y^+$	<0.1	<1	<1	100	100	100
2. $i^-z^+y^+$	120	120	120	120	120	120
3. $i^+z^-y^+/Fi^-z^+y^+$(*)	2	2	2	200	250	250
4. $i^-z^-y^+/Fi^+z^+y^-$(*)	2	2	2	250	120	120
5. $i^-z^-y^+/Fi^-z^+y^+$	250	250	250	200	250	250
6. $\Delta_{izy}/Fi^-z^+y^+$	200	200	200	200	200	200

As an example, the effects of constitutive regulator mutations which alter the inducible system of lactose utilization in *E. coli* are given in Table 2 for haploid and heterogenote bacteria. These mutations map in a regulator gene (i) distinct from the structural genes (z and y) of the system (see Fig. 3). They affect to the same extent the synthesis of all the known components of the system (Pardee, Jacob, and Monod, 1959). Similar mutants have been found for a series of inducible systems, such as penicillinase of *B. cereus* (Kogut, Pollock, and Tridgell, 1956); amylomaltase (Cohen-Bazire and Jolit, 1953); glycuronidase (Stoeber, 1961); and the enzymes of galactose utilization in *E. coli* (see Buttin, this Symposium; Kalckar, this Symposium).

Among repressible systems, mutations affecting a regulator gene, located far from the cluster of structural genes of the tryptophan pathway, result in constitutive (derepressed) synthesis of all the enzymes of the pathway in *E. coli* (Cohen and Jacob, 1959). Similar situations have been observed in the arginine pathway (see Gorini, this Symposium; Maas, this Symposium). In the case of alkaline phosphatase, two distinct regulator genes appear to govern its repression by orthophosphate (Echols, Garen, Garen, and Torriani.)

Finally, in phage the ability of a temperate phage to become a prophage, i.e., to become integrated with the chromosome of the lysogenic host, depends upon inhibition of expression of the phage structural genes. Mutations have been found which impair the capacity of the phage to lysogenize. These mutations are recessive to wild type and affect a regulator gene (C_1) (see Fig. 4) whose cytoplasmic product prevents transcription of the structural genes required for initiating the vegetative multiplication of the phage (Jacob, 1960; Jacob and Campbell, 1959).

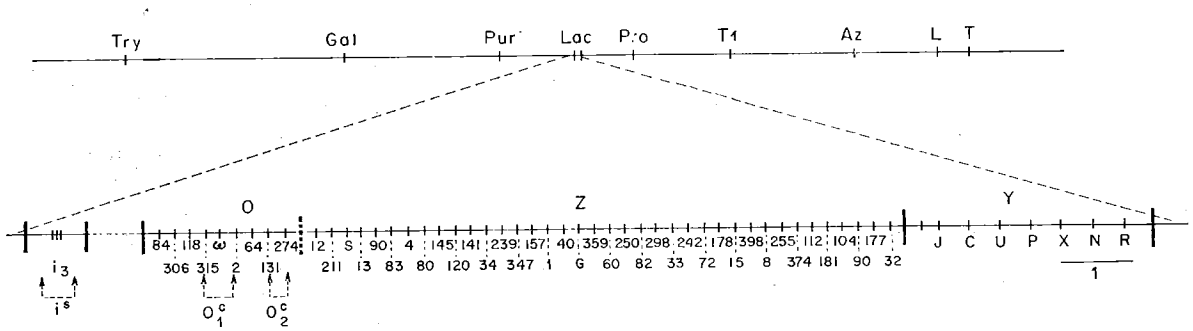

FIGURE 3. *Genetic map of the Lac region of E. coli.* The upper line represents the position of the *Lac* region among linked characters in the bacterial chromosome. The lower line represents an enlargement of the *Lac* region, with the two structural genes z and y and the regulator gene i. The operator o appears to correspond to the extremity of the z gene.

The behavior of these "regulator-constitutive" mutations in the diploid condition is of particular significance. Although their effect may be described as a positive one and to imply the acquisition of a new potentiality since they allow the mutant cells to synthesize a given protein(s) under conditions where the wild type cannot do so, the "constitutive" allele is invariably *recessive* to wild type; moreover, deletion of the gene also results in a constitutive phenotype, proving the latter to correspond to an inactive state of the gene, or gene product.

These observations identify a "regulator gene" as a determinant which, in the active state, controls negatively the transcription of certain specific structural genes without itself contributing any structural infor-

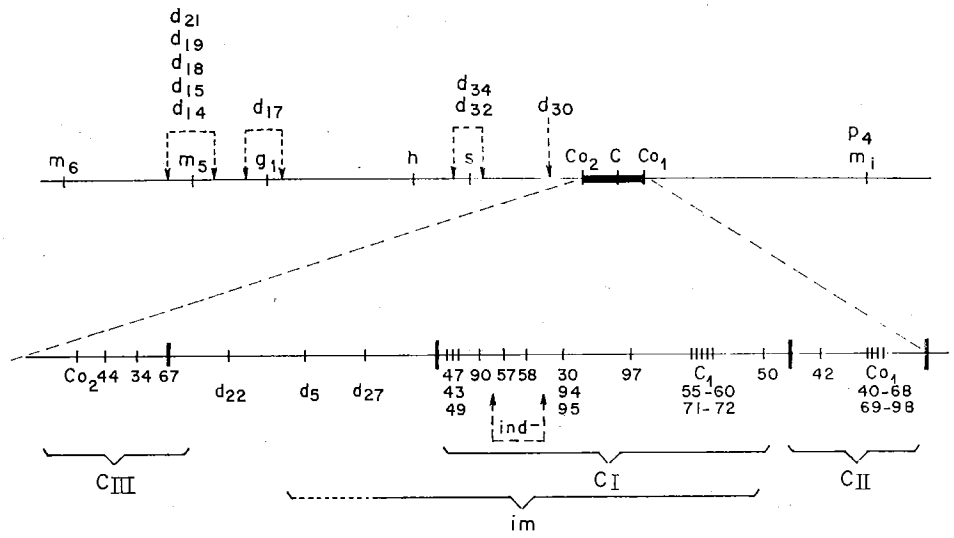

FIGURE 4. *Genetic map of the C region of the temperate bacteriophage* λ. The upper line represents the linkage-group of λ. Symbols refer to various plaque size, plaque type, and host range markers. Symbols *d* refer to various defective mutations. The C region represented by a thicker line is enlarged in the lower diagram. Figures correspond to various C mutations. C_1 corresponds to the regulator gene; ind⁻ corresponds to the "non-inducible" mutation. The segment controlling immunity is designed *im*.

TABLE 3. PRODUCTION OF β-GALACTOSIDASE, GALACTOSIDE-TRANSACETYLASE AND GALACTOSIDE-PERMEASE BY HAPLOID AND HETEROGENOTE, "SUPERREPRESSED" REGULATOR MUTANTS

(*) The levels observed in i^s mutants are always higher than in the wild type. This is due to the presence of a significant fraction of constitutive (i^- or o^c mutants) present in the cultures.

This table summarizes the results of several experiments. The three activities are given in per cent of those obtained with fully induced, haploid wild type. *i*: regulator gene (i^+: inducible; i^-: constitutive; i^s: superrepressed). *z* and *y*: structural genes for β-galactosidase and galactoside-permease respectively. *F*: sex factor of *E. coli* K12.

Genotypes	Non-induced			Induced		
	β-galactosidase	galactoside-permease	galactoside-transacetylase	β-galactosidase	galactoside-permease	galactoside-transacetylase
1. $i^+z^+y^+$	<0.1	<1	<1	100	100	100
2. $i^sz^+y^+$(*)	2	2	2	2	2	2
3. $i^sz^+y^+/Fi^+z^+y^+$(*)	2	2	2	2	2	2
4. $i^sz^+y^+/Fi^-z^+y^+$(*)	2	2	2	2	2	2

mation to the proteins. Since the active (wild-type) allele is equally effective toward the expression of structural genes placed in *cis* or in *trans* position, it is clear that the regulator gene exerts this negative control via a specific cytoplasmic product.

2. *Superrepressed Regulator Mutations*

All the available evidence suggests that the cytoplasmic product of the regulator gene acts directly as a *repressor* of structural gene expression rather than indirectly as an antagonist of yet another cytoplasmic agent required to activate the synthesis of the protein, such as, for instance, an endogenous inducer. If the latter were the case, one would expect mutations of the gene which controls the synthesis of the positive agent (the internal inducer) to occur. These mutations would be expressed as a loss of the capacity to synthesize the proteins. They would be recessive to wild type, and mutations of the regulator gene to the constitutive state would not restore the capacity to synthesize the enzymes. Mutations characterized by these properties have not been found in any system.

In contrast, both in an inducible enzyme system and in a lysogenic system, certain mutations within the regulator gene have been observed to result in a loss of the capacity to synthesize the proteins controlled by the regulator.

The properties of (haploid and heterogenote) bacteria carrying such a "superrepressed regulator" mutation affecting the system of lactose utilization in *E. coli* are reported in Table 3. Such mutations (i^s), which map in the regulator gene *i* (see Fig. 3), result in a loss of the ability to produce all the known components of the system. As shown by the behavior of the heterogenote (i^+/i^s), the mutant allele is dominant over the wild type (Jacob and Monod, 1961; Willson, Perrin, Jacob, and Monod, 1961). In the temperate

phage λ a similar mutation of the regulator gene (C_1) has been isolated. The vegetative reproduction of such a mutant prophage can no longer be induced by inducing agents such as UV light. Furthermore, when it superinfects *induced* lysogenic cells carrying a wild-type prophage, this mutant has the unique property of inhibiting phage multiplication. The study of heterogenotes, or of doubly lysogenic cells, shows that this mutation is dominant over the wild type (Jacob and Campbell, 1959).

Thus, while the constitutive regulator mutation appears as a *recessive* "gain of function," the uninducible mutation results in a *dominant* "loss of function." These relationships are immediately accounted for by the assumption that the wild type regulator gene controls the synthesis and structure of a cytoplasmic *repressor* able to inhibit the transcription of one or several structural genes, and to interact with a specific inducer, the interaction resulting in inactivation of the repressor. The constitutive mutation then corresponds to a genetic inactivation of the repressor, while the "uninducible" mutation represents loss of the capacity to react with the inducing agent (see Fig. 5).

If these assumptions are correct, one expects that the ability to synthesize the proteins would be restored to an "uninducible" mutant by a further mutation to an inactive (i.e., constitutive) state of the gene. This, in fact, is found to be the case.

It is obvious that, if the product of the regulator gene were assumed to act indirectly as an antagonist of an endogenous "activating" agent, none of these findings would be accounted for. It is also easy to see that, while the "uninducible" phenotype may occur in an inducible system and is distinct from the "constitutive" phenoytpe, the corresponding "non-repressible" phenotype of a repressible system could not operationally be distinguished from the "constitutive" phenotype.

Thus, the study of biochemical phenotypes in the haploid and the diploid conditions reveals, besides "classical" structural genes, the existence of functionally specialized determinants which appear to be concerned exclusively with the formation of specific cytoplasmic repressors.

More direct evidence of the existence of *repressors* as cytoplasmic components has been obtained in certain systems (see Pardee *et al.*, 1959; Jacob, 1960), and it has been shown that the synthesis of these agents occurred under conditions where the synthesis of proteins is blocked by inhibitors, such as chloramphenicol or 5-methyl-tryptophan (Pardee and Prestidge, 1959; Jacob and Campbell, 1959). No positive evidence has been obtained as yet concerning the chemical identity

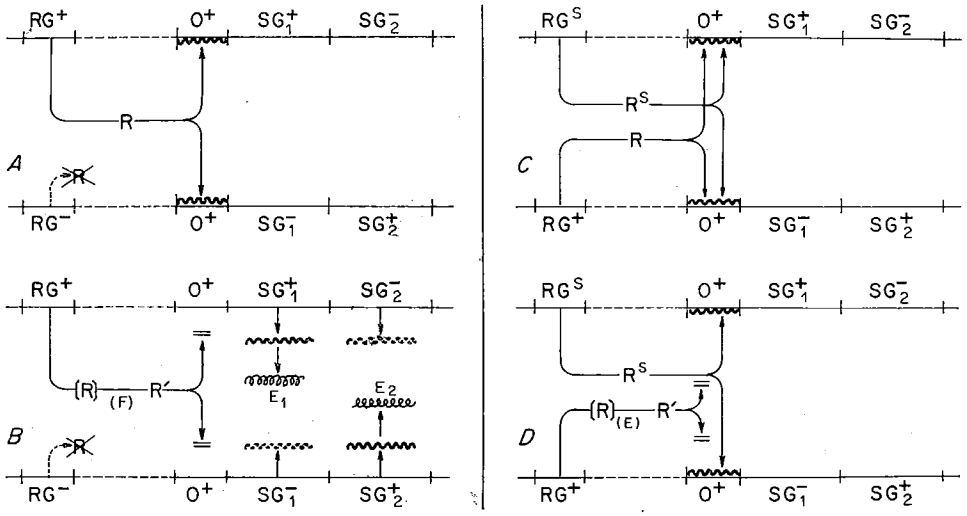

FIGURE 5. *Schematized behavior of two diploids heterozygous for the regulator and for both structural genes.*

Left. Heterozygote for a constitutive-regulator mutation (RG^-)/wild type (RG^+). A. *Non-induced.* The mutated regulator gene (RG^-) produces an inactive repressor while the normal regulator gene (RG^+) produces the repressor R which blocks both operators (*o*). No messenger and therefore no enzyme is made. B. *Induced.* In presence of inducer (F), the repressor (R) is converted to the inactive form (R′). Gene activity is allowed. SG_1^+ on one chromosome produces E_1, and SG_2^+ on the other chromosome produces E_2. The diploid, therefore, behaves in every respect like a haploid cell.

Right. Heterozygote for a "superrepressed" mutation of the regulator gene (RG^s)/wild type (RG^+). C. *Non-induced.* Both types of repressor (R: normal; R^s: altered) are simultaneously produced. They block the operators. No enzymes are made. D. *Induced.* Only the normal repressor reacts with the inducer while the altered (R^s) repressor does not and still blocks transcription in both chromosomes. No enzyme is made.

of repressors, but since they are presumably primary products of the regulators, the assumption that they are polyribonucleotides appears as the most reasonable guess. The nature of the interaction between repressor and effector (inducer or repressing metabolite) is unknown. The symmetrical reaction between the repressor R and the effector F:

$$R + F \rightleftharpoons R' + F',$$

which we assumed, is to be taken only as a convenient manner of expressing the fact that inducible and repressible systems are similar in every respect, suggesting that the primary interaction of the effector is most probably the same in both types of systems.

An important line of argument, which we will not develop here, is the following: since the regulator gene does not control the structure of the protein, and since the effectors appear to react with the product of the regulator, rather than with products of the structural gene, there need exist no steric relationships between the protein itself and the effector. Rather detailed and elaborate proof of this negative statement has been obtained (see Monod, 1956, 1959; Cohn, 1957; Yates and Pardee, 1957; Gorini and Maas, 1958; Jacob and Monod, 1961). Conversely, since the regulator is frequently, if not as a rule, pleiotropic in its action, simultaneously controlling the synthesis of several proteins, one expects the effector to show the *same* pleiotropy, i.e., to provoke or inhibit simultaneously the synthesis of precisely the *same* group of enzymes that are affected by mutations of the regulator. This has invariably proved to be the case, confirming the conclusion that the effector reacts with the product of the regulator, but not directly with a product of the structural genes.

OPERATORS

The property of a particular repressor to inhibit specifically the synthesis of one (or several) protein(s) implies that, at some stage of the transcription process from gene to protein, it can form a steric combination with some element of the system. Once this element is combined with the repressor, the transcription is blocked. This element which can switch on and off the transcription of the system is called *operator* (Jacob and Monod, 1959, 1961).

In order to discuss in some detail the properties of the operator which control the system of lactose utilization in *E. coli*, we shall first summarize the main biochemical and genetic characteristics of this system. It involves three known components whose rate of synthesis is determined by a single regulator gene (i) and induced by β-galactosides: the two enzymes, β-galactosidase and galactoside-transacetylase (which have both been isolated in vitro in a high state of purity and by all criteria correspond to two different proteins), and the galactoside-permease identified only by in vivo tests (see Cohen and Monod, 1957; Zabin, Képès and Monod, 1959; Képès, 1960).

The structural gene (z) specifying β-galactosidase has been unambiguously identified by the study of a variety of mutations which result in alterations of the molecular properties of the enzyme. About ¼ or ⅓ of the studied mutants produce, instead of the enzyme, an enzymatically inactive, antigenically cross-reacting protein (CRM).

The structural gene (y) for galactoside-permease has been identified as adjacent to the z gene (see Fig. 3). All y^- mutants have lost the capacity to produce β-galactoside-permease. Many of them have also lost the capacity to produce β-galactoside-transacetylase. Revertants to Lac^+ recover both properties. This correlation seemed strongly to suggest the identity of permease and transacetylase. However, biochemical studies of the transacetylase have revealed that this enzyme does not exhibit the properties expected of the permease protein. Furthermore, the properties found in several mutants of the z gene (which will be described in the next section) have cast some doubt on the significance of the fact that both forward and backward mutations of the y gene simultaneously affect permease and transacetylase. The question is still open as to whether the acetylase is actually the (or a part of) permease and is controlled by the y gene or is controlled by another gene close, or adjacent, to y. This question is not essential for the following argument, which applies to both cases. The essential point is that the three known elements, β-galactosidase, transacetylase, and permease, are submitted to the same control mechanism governed by a single regulator gene, i.

The functional analysis of z^- and y^- mutants has been undertaken by means of heterogenotes obtained by sexduction (see Jacob and Wollman, 1961). As a rule, z^- mutants are complemented by y^- mutants, with a few exceptions to which we shall return in a further section. Most z^- mutants are not complemented by other z^- mutations, with the exception of certain pairs which produce partial complementation. Mixtures of extracts of such pairs also result in slightly increased enzymatic activity in vitro (Willson, unpublished results). No complementation has been observed as yet between any tested pair of y^- mutants. It therefore appears that the z mutants belong to a single functional unit (cistron) and the y mutants to another functional unit.

We may now return to the problem of the operator. Whatever the nature of the operator and the level at which it intervenes in the transcription process, its specific configuration required by the specific action of the repressor must be genetically determined. It should therefore be altered by mutations. Actually, two distinct types of mutations affecting the operator may be expected: operator-constitutive mutations, re-

sulting from a loss of affinity of the operator toward the repressor; and operator-negative mutations resulting in a permanent block of the transcription process.

The two types of mutants have been isolated in the *Lac* system of *E. coli*. The study of their properties shows that *a single operator controls the expression of the two adjacent structural genes* (*z* and *y*) *located on the same chromosome* (Jacob, Perrin, Sanchez, and Monod, 1960; Jacob and Monod, 1961).

A. Constitutive-Operator Mutations

By contrast to constitutive-regulator mutations, which are recessive, the constitutive operator mutants (o^c) are expected to be dominant, since under any hypothesis, the presence, in a diploid cell, of a repressor-sensitive operator would not affect the activity of a repressor-insensitive operator.

In the lactose system of *E. coli* such constitutive mutants have been isolated in heterogenotes carrying an *F-Lac* factor, homozygous i^+/i^+ in order to avoid selecting recessive i^- constitutive regulator mutants. The properties of such o^c constitutive-operator mutants can be summarized as follows (see Table 4).

1) The o^c mutants isolated so far are partially constitutive. Depending on the cultural conditions, they produce in the absence of inducer about 10 to 50 per cent of the galactosidase which they synthesize when grown in the presence of inducer, i.e., 100 to 500 times more than the uninduced wild type.

2) The o^c allele is dominant over the wild allele o^+, since heterogenotes o^+/o^c are constitutive.

3) The o^c mutations are pleiotropic, and affect *to the same extent* the synthesis of β-galactosidase, galactoside-transacetylase, and permease.

4) The o^c mutations result in a constitutive expression only of those structural genes which are located in position *cis*, i.e., on the same chromosome. This is shown by the behavior of cells carrying an *F-Lac* factor and heterozygous for *o* as well as for one of the structural genes. For instance, of the two heterogenotes o^+z^-/Fo^cz^+ and o^+z^+/Fo^cz^- which carry an active z^+ gene and a mutant allele, only the former synthesizes the enzyme constitutively. In the same way, of the two heterogenotes o^+y^+/Fo^cy^- and o^+y^-/Fo^cy^+ carrying an inactive y^- allele unable to form galactoside-transacetylase and permease, only the latter produces these two factors constitutively. The operator therefore has no *independent* cytoplasmic expression. It controls the activity of the chromosomal *zy* segment as an integrate unit.

5) We have seen previously that some mutations (i^s) of the regulator *i* gene result in the production of a "superrepressor" which can no longer be antagonized by the external inducer (see Table 3). Such mutations result in dominant loss of the capacity to produce the enzymes. If the o^c mutation does indeed correspond to a decreased sensitivity of the operator to the repressor, it is a definite prediction that the o^c mutant should also be insensitive to the presence of the altered repressor synthesized by the i^s allele. That this is indeed the case, as shown by the constitutive behavior of heterogenotes i^so^+/Fi^+o^c, is a very strong confirmation of the interpretation of both the i^s and o^c mutation (see Table 4).

6) The o^c mutations map at one extremity of the group of structural genes *z* and *y*, between the *z* and the *i* mutations (see Fig. 3).

Table 4. Production of β-Galactosidase, Galactoside-Transacetylase and Galactoside-Permease by Haploid and Heterogenote, Constitutive-Operator Mutants

(*) The y^- mutant used in these experiments does not produce galactoside-transacetylase.

This table summarizes the results of several experiments. Activities are expressed in per cent of those observed with fully induced haploid wild type. The levels obtained with heterogenotes are always higher than those observed with haploid bacteria. This is presumably due to the presence of several *F* factors per chromosome. The high basal level observed in strains carrying an i^s mutation is due to the presence of constitutive (i^- or o^c) mutants in the cultures. *i*: regulator gene (i^+: inducible; i^s: superrepressed). *z* and *y*: structural genes for β-galactosidase and galactoside permease respectively. *o*: operator (o^+: wild type; o^c: constitutive). *F*: sex factor of *E. coli* K12.

Genotypes	Non-induced			Induced		
	β-galactosidase	galactoside-permease	galactoside transacetylase	β-galactosidase	galactoside-permease	galactoside transacetylase
1. $o^+z^+y^+$	<0.1	<1	<1	100	100	100
2. $o^cz^+y^+$	25	25	25	100	100	110
3. $o^+z^-y^+/Fo^cz^+y^+$	75	75	75	250	300	300
4. $o^+z^-y^+/Fo^cz^+y^-$(*)	75	1	1	250	120	120
5. $o^+z^+y^-/Fo^cz^-y^+$	1	75	75	100	250	250
6. $i^so^+z^+y^+$	2	2	2	2	2	2
7. $i^so^+z^+y^+/Fi^+o^+z^+y^+$	2	2	2	2	2	2
8. $i^so^+z^+y^+/Fi^+o^cz^+y^+$	150	150	150	150	150	150

B. Operator-Negative Mutations

Among the mutants selected for their Lac^- phenotype, a series of mutants (o°) turned out to exhibit the properties expected of operator-negative mutants (see Table 5).

1) The o° mutations are pleiotropic: o° mutants are unable to synthesize both proteins, galactosidase and transacetylase, as well as permease.

2) The o° allele is recessive as shown by the study of o^+/o° heterogenotes which behave like the wild type.

TABLE 5. PRODUCTION OF β-GALACTOSIDASE, GALACTOSIDE-TRANSACETYLASE AND GALACTOSIDE-PERMEASE BY HAPLOID AND HETEROGENOTE, OPERATOR-NEGATIVE MUTANTS

This table summarizes the results of several experiments. Activities are expressed as per cent of the fully induced, haploid wild type. The levels obtained with heterogenotes are higher than those observed in haploid bacteria. This is presumably due to the presence of several *F* factors per chromosome. Levels observed in non-induced cultures of i^+/i^- heterogenotes are due to the presence of a small fraction of homozygous i^-/i^- constitutive recombinants in the cultures. *i*: regulator gene (i^+: inducible; i^-: constitutive) *z* and *y*: structural genes for β-galactosidase and galactoside permease respectively. *o*: operator (o^+: wild type; o°: operator-negative). *F*: sex factor of *E. coli* K12.

Genotypes	Non-induced			Induced		
	β-galactosidase	galactoside-transacetylase	galactoside-permease	β-galactosidase	galactoside-transacetylase	galactoside-permease
1. $i^+o^+z^+y^+$	<0.1	<1	<1	100	100	100
2. $i^+o^\circ z^+y^+$	<0.1	<1	<1	<0.1	<1	<1
3. $i^+o^\circ z^+y^+/Fi^+o^+z^+y^+$	<0.1	<1	<1	250	250	250
4. $i^+o^\circ z^+y^+/Fi^+o^+z^-y^+$	<0.1	<1	<1	<0.1	250	250
5. $i^+o^\circ z^+y^+/Fi^+o^c z^+y^+$	75	75	75	250	250	250
6. $i^+o^\circ z^+y^+/Fi^-o^+z^+y^+$	1	1	1	250	250	250

3) The o° mutants are not complemented by any z^- or y^- mutants of the structural genes.

4) The o° mutations are clustered at the extremity of the chromosomal segment bearing the two structural genes *z* and *y*, between *z* and *i* at the same place as the o^c mutants (see Fig. 3).

The properties and mapping of the o^c and o° mutations allow the recognition of an "operator locus" which in some way can switch on and off the expression of the adjacent structural genes *z* and *y* located on the same chromosome and which controls the sensitivity of the system to the action of the repressor synthesized by the regulator gene *i* (see Fig. 6).

The "operator locus" thus defined by the *o* mutation is contiguous to the *z* structural gene. The important question arises, therefore, whether the "*o* locus," thus defined, is distinct from, as opposed to part of, the *z* gene. This amounts to asking whether or not the nucleotide sequence constituting the *o* segment determines part of the molecular structure of β-galactosidase. This question may be approached by studying the molecular properties of the enzymes formed by operator-constitutive mutants or by revertants of operator-negative mutants. The β-galactosidase formed by the o^c mutants so far studied appears not detectably different, in any of its tested properties, from the wild-type enzyme. Several Lac^+ revertants of o° mutants, however, produce altered β-galactosidase (see Fig. 7) while synthesizing apparently normal permease and acetylase. By all the available criteria, the reversion appears to affect the very site, or a closely linked site, of the original o° mutation. These results suggest that the operator of the *Lac* system actually corresponds to the extremity of the structural gene which specifies the configuration of β-galactosidase.

Few systems have been investigated so far as to the possible existence of operator mutants. In phage, however, mutants similar to the o^c type have been isolated. These mutants (*v*) are characterized, and can easily be selected, by their ability to multiply vegetatively in immune cells, lysogenic for the wild type: they behave, therefore, as having lost sensitivity to the repressor present in such cells. The difficulties of biochemical investigations of these systems has not yet permitted an analysis of the role of the operator and of its relation with structural genes to be carried out. Mutants in every respect similar to the o^c-mutants of the *Lac* system have recently been isolated in the "galactose" system of *E. coli*, in which the synthesis of three enzymes, determined by three closely linked structural genes, are induced by galactose or analogs of galactose. These mutations are pleiotropic, dominant, and affect exclusively the expression of structural genes located in position *cis* with respect to the mutated operator (see Buttin, this Symposium).

Mutations similar to the o° mutations of the *Lac* system also occur in the galactose segment *E. coli* (E. Lederberg, 1960; Kalckar, Kurahashi, and Jordan, 1959), and a mutation of the same type affects the sequence of enzymes involved in histidine biosynthesis by *S. typhimurium* (Ames, Garry, and Herzenberg, 1960). The latter system includes 8 or 9 clustered structural genes which presumably specify the individual structures of the enzymes of the pathway, the synthesis of the whole series of enzymes being repressed by histidine. A mutant has been isolated which results in the pleiotropic loss of the capacity to synthesize all the enzymes of the pathway. This mutant exhibits the properties expected of an "operator-negative" allele. It maps as adjacent to, if not part of, a structural gene at one *extremity* of the cluster, and is not complemented by any of the structural gene mutants.

THE OPERON AS THE POLARIZED UNIT OF TRANSCRIPTION

Besides identifying the genetic segment responsible for the structure of the operator, the properties of the *o* mutations—most specifically the absence of any *trans* effect of the *o* alleles—define a new genetic unit which has been called the *operon* (Jacob *et al.*, 1960; Jacob and Monod, 1961). This polycistronic unit involves the

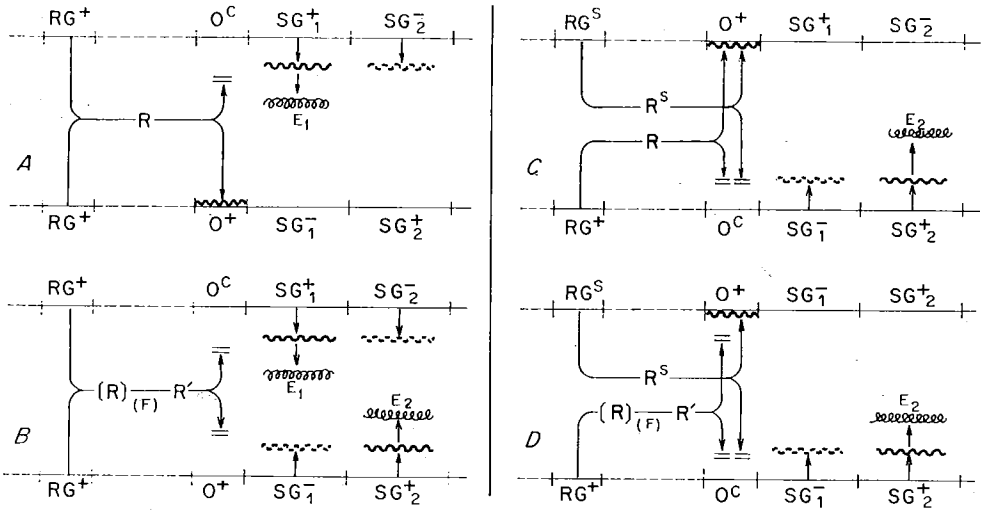

FIGURE 6. *Schematized behavior of two diploids heterozygous for the operator and for the structural genes.*

Left. Heterozygote for a constitutive operator (o^c) mutation/wild type (O^+). A. *Non-induced.* The repressor made by the regulator genes (RG) blocks gene expression in the lower chromosome which carries a normal operator (o^+). It does not act on the upper chromosome which carries a mutated operator (o^c), with a low affinity for the repressor. Only enzyme E_1 is produced constitutively since the upper chromosome carries a mutation in SG_2 (SG_2^-). B. *Induced.* In presence of inducer (F) the repressor (R) is converted to the inactive form (R′). Gene activity is allowed in both chromosomes. Both enzymes E_1 and E_2 are made.

Right. Heterozygote for a constitutive operator (o^c) mutation/wild type and for a "superrepressed" mutation (RG^S) of the regulator gene/wild type. C. *Non-induced.* Both types of repressor (R: normal, and R^S: altered) are simultaneously produced. They block gene transcription in the normal operator (o^+) of the upper chromosome, but not in the mutated operator (o^c) of the lower chromosome. Only enzyme E_2 is produced since the lower chromosome carries a mutated structural gene SG_1^-. D. *Induced.* Only the normal repressor (R) reacts with the inducer while the altered (R^S) repressor does not. It still blocks transcription in the normal operator (o^+) of the upper chromosome but not in the mutated (o^c) operator of the lower chromosome. Only enzyme E_2 is made.

cluster of linked structural cistrons whose expression is controlled by the operator. The absence of any *trans* effect shows that the primary product, if any, of the operator segment cannot recombine, in the cytoplasm, with the products of structural genes. The operon is thereby defined as a genetic unit of transcription. Whether or not the primary products of an operon also constitute an integral unit or particle in the cytoplasm, and whether the repressor acts directly at the genetic level, by combining with the operator segment, or at the cytoplasmic level, by combining with the *product* of this segment, is another problem which will be considered later. In the present section we wish to discuss some observations which further identify the operon as a *polarized* unit of *coordinated* transcription.

A. Quantitative Coordination within the Operon

The pleiotropic effect of operator mutations proves that the expression of the different genes included in an operon simultaneously behave as repressible, or inducible, or altogether suppressed, depending on the allelic state of the operator segment. Since the operator is the receiver of the controlling signals (i.e., the receptor of the repressor), we may expect the effects of inducing or repressing conditions to be *quantitatively*

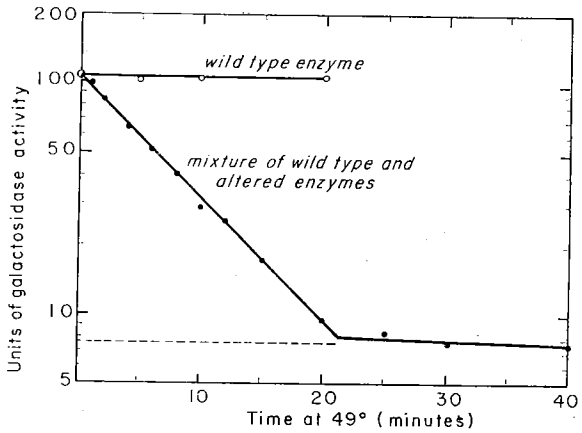

FIGURE 7. Altered β-galactosidase produced by a Lac^+ revertant of an operator-negative (0°) mutant. The revertant was obtained by plating a culture of mutant O_{84}^c on EMB agar. An extract from an induced culture of this revertant was mixed with an extract of the induced wild type (92.5% enzyme activity from the revertant, with 7.5% enzyme activity from the wild type). The mixture was incubated at 49°, and enzyme activity was assayed after various time intervals. Control extract from the induced wild type was treated in the same way. Units of β-galactosidase activity are plotted (on a log scale) versus the time of incubation at 49°. The dotted line corresponds to the expected activity of the wild-type fraction present in the mixture.

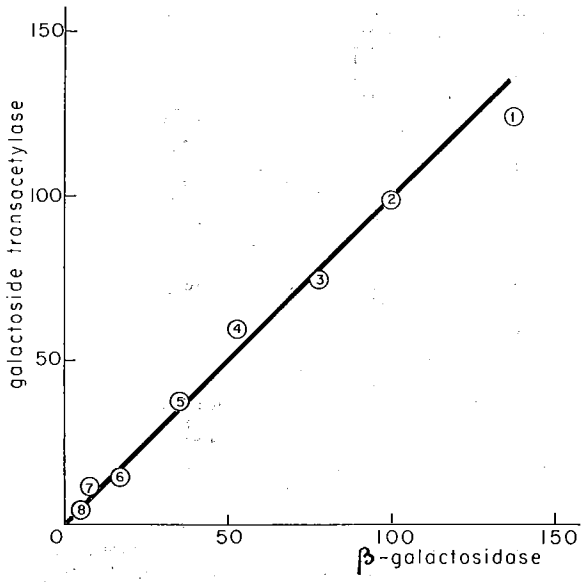

FIGURE 8. *Coinduction of β-galactosidase and galactoside-transacetylase.* The rates of synthesis of β-galactosidase and galactoside-transacetylase are expressed in arbitrary units, the rates achieved by fully induced wild-type organisms being taken as 100.

1. Uninduced constitutive mutant of the regulator (i^-).
2. Wild type induced by isopropyl-β-D-thiogalactoside 10^{-4}M.
3. Wild type induced by methyl-β-D-thiogalactoside 10^{-4}M.
4. Wild type induced by methyl-β-D-thiogalactoside 10^{-4}M + phenyl-β-D-thiogalactoside 10^{-3}M.
5. Wild type induced by melibiose 10^{-3}M.
6. Wild type induced by lactose 10^{-3}M.
7. Wild type induced by methyl-β-D-thiogalactoside 10^{-5}M.
8. Wild type induced by phenyl-ethyl-β-D-thiogalactoside 10^{-3}M.

the same for different proteins controlled by different genes belonging to the same operon.

The lactose system of *E. coli* offers an excellent test of this prediction, since many different inducers are available whose inducing activity covers a very wide range (see Monod, Cohen-Bazire and Cohn, 1951). As shown in Fig. 8, while the *absolute* rates of synthesis of β-galactosidase and galactoside-transacetylase vary greatly, depending on the nature and concentration of the inducer used, the *ratio* of the two rates is invariant. Nor is this ratio altered in different types of constitutive organisms.

In the repressible "histidine" system of *S. typhimurium*, the same situation is observed (Ames and Garry, 1959). We shall summarize it by formulating the rule that the expression of structural genes belonging to a single operon is *quantitatively coinduced* or *corepressed*.

It must be pointed out that a coordinated effect of induction or repression must be expected only for the expression of those structural genes which are *linked* and belong to the *same operon*. In the case of arginine, in contrast, the seven structural genes determining the synthesis of the seven known enzymes of the pathway are distributed among five distinct regions of the bacterial chromosome. Although the available data suggest that a single regulator gene controls the repressibility of these seven enzymes, the repressor must be expected to act on a series of distinct operators; and the regulatory effect, while qualitatively similar, need not be quantitatively the same.

B. Polarity of the Operon

A priori the role of the operator can be most easily understood at the molecular level if the transcription of the whole operon is a polarized process, the operator being the initiating point. Some experimental evidence suggests that the operon is actually a polarized unit.

In a previous section we have briefly discussed the properties of the z^- mutations, which affect β-galactosidase, and of the y^- mutations, which affect galactoside-permease. We have seen that most of the z^- mutants are complemented by all the y^- mutants, a result which indicates the existence of two independent functional units. A few z^- mutants, however, exhibit no, or only partial, complementation with any of the y^- mutants. When tested for enzymatic activities, the z^- mutants are found to vary greatly in their properties. Most of them produce no β-galactosidase activity but a normal amount of transacetylase and permease. A few of the z^- mutants, however, which appear to be randomly distributed along the z gene, not only produce no β-galactosidase activity, but also produce acetylase and permease in reduced amounts. The properties of several z^- mutants are reported in Table 6. It is clear from these results that z mutations may eventually alter to various degrees (from 5 to 50 per cent of the wild type) the capacity to produce both these factors. When the productions of acetylase and of permease are impaired, however, it is always *to the same extent*. Furthermore, among the Lac^+ revertants of such z^- mutants, it is always found that the reversion restores not only the production of β-galactosidase but also those of acetylase and permease, and that the restoration affects these two factors *to the same extent*.

In contrast, among the y^- mutants, which are unable to produce the permease, none has been found in which the capacity to produce β-galactosidase was impaired. It is clear, therefore, that some polarity exists in the $o \rightarrow z \rightarrow y$ operon in such a way that some rare blocks in the z region decrease the rate of transcription in the distal part of the operon, whereas further blocks in the y region cannot alter the rate of the initial transcription in the z region.

Such findings have cast some doubt about the previously assumed identity of the protein of the galactoside-permease with the galactoside transacetylase. This

identification had been based on the finding that several y^- mutants, isolated as permeaseless, were also unable to synthesize the transacetylase, whereas reverse mutants were found to have recovered both activities. However, investigation of a greater number of y^- permeaseless mutants has shown that only in a fraction of these mutants the ability to synthesize the transacetylase is impaired, these mutants being randomly distributed along the y gene among other y^- mutants still able to manufacture the transacetylase in a normal amount. If the transcription of the operon is polarized, these findings could be equally explained by the hypothesis that the transacetylase is controlled by another, unknown structural gene (x) of the *Lac* operon, the order being $o \rightarrow z \rightarrow y \rightarrow x$, so that some mutations in z might block, or decrease, the rate of further transcription in y and x, while some mutations in y might block further transcription in x, but not in z.

Several hypotheses might account for the decrease in the rate of transcription observed in the distal part of the operon as a result of mutations in the z gene. Since they have not yet received any experimental support, they need not be discussed here.

The concept of operon, as the unit of primary transcription which may contain more than one gene, provides an interpretation for the observed fact that, at least in bacteria, genes controlling the successive steps of a single biochemical pathway remain linked (see Demerec and Hartman, 1959). It is clear indeed that, if as a result of chromosomal rearrangement, some of the genes of an operon would be removed from the control of the original operator, displaced and submitted to the control of another operator, such a rearrangement would result in a much less specific and therefore less efficient control. Wild-type bacteria would therefore exhibit a selective advantage over such chromosomal mutants. Hence a tendency for the genes of an operon to remain linked.

The case of those structural genes which although unlinked are under the control of a single system of regulation, such as the genes of the arginine pathway, has also some interesting implications. Since a single repressor appears to be active on the whole system, each group of genes must be controlled by a separate operator; and all these operators must possess a common structure. If, as in the lactose system, each operator corresponds to the extremity of a structural gene, one may expect the different enzymes of the pathway to possess, despite their difference in activity, a common primary structure, perhaps in one extremity of their peptide chains. This prediction should become subject to experimental test soon. It has some evolutionary implications which will be discussed in another paper of this Symposium (see Monod and Jacob, this Symposium).

TABLE 6. POLARIZED EFFECTS OF MUTATIONS AT DIFFERENT LOCI ON THE LACTOSE OPERON OF *Escherichia coli*

(1) The figures refer to specific activity of β-galactosidase, permease, and galactoside-transacetylase induced in bacteria under standard conditions. The activities are expressed in per cent of that found in wild type.

(2) *Lac*$^+$ revertants of mutant z_S ; revertant 3 produces apparently normal galactosidase; revertant 8 produces altered enzyme showing increased heat sensitivity and lower specific activity. When tested immunologically, revertant 8 produces close to 100 per cent of galactosidase antigen.

	β-galactosidase (1)	galactoside-permease (1)	galactoside-transacetylase (1)
Wild type	100	100	100
Operator mutants:			
o_2	<0.1	<1	<1
o_ω	<0.1	<1	<1
o_{84}	<0.1	<1	<1
o_{118}	<0.1	<1	<1
z mutants:			
z_{40}	<0.1	100	100
z_1	<0.1	100	100
z_{250}	<0.1	50	46
z_{73}	<0.1	19	14
z_{211}	<0.1	18	11
z_S	<0.1	5	5
z_S Rev 3 (2)	50	50	50
z_S Rev 8 (2)	50	100	100
y mutants:			
y_J	100	<1	100
y_X	100	<1	100
y_V	100	<1	60
y_R	100	<1	47
y_U	100	<1	5
y_1	100	<1	<1
y_{65}	100	<1	<1

REGULATION AT THE GENETIC LEVEL

The question we wish to discuss now is: at which level does the repressor act in the transcription process? It may either block the synthesis of the primary gene product, messenger RNA, or the synthesis of the protein itself. The results of genetic analysis do not, by themselves, allow a direct distinction between the two alternatives. They only show that regulation operates at a level where the information derived from several adjacent structural genes is still contained in a single, continuous, functionally integrated structure. The assumption that this structure is the operon itself is indeed a very tempting one. Taking into account the kinetics of induction and repression (cf. Monod, 1956; Cohn, 1957), it necessarily implies, however, that the

structural intermediate is a very unstable molecule. This, in fact, was one of the main arguments which led to postulating the existence of unstable messengers as information carriers from gene to protein-forming centers. Although the discovery of an RNA fraction which appears to qualify nicely as an unstable messenger certainly brings strong support to the hypothesis that regulation operates at the genetic level, by switching on and off the synthesis of messenger RNA, it does not constitute direct proof of the validity of this hypothesis. The alternative hypothesis, namely that the repressor acts at the cytoplasmic level, i.e., controls the activity of already made messengers, would imply the existence of messenger molecules able to specify the structure of all the proteins belonging to a single operon (up to 8 or 9 enzymes in the case of histidine biosynthesis). Assuming a coding ratio of 3, and an average (monomeric) molecular weight of 60,000 for the proteins, such a messenger RNA should have a minimum molecular weight of 5×10^6, which would correspond to molecules much larger than appear to be present in any of the RNA fractions so far analyzed. Given the present state of our knowledge concerning the molecular weight and heterogeneity of messenger RNA, however, this type of argument cannot eliminate the hypothesis that the repressor acts at the level of protein synthesis.

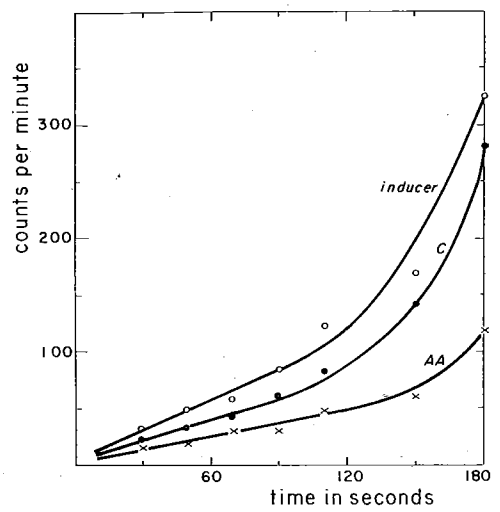

FIGURE 9. *Rates of incorporation of radioactive adenine into nucleic acids under various conditions.* Bacteria carrying an *F-Lac*$^+$ factor were grown in minimal medium at 19°, and the exponentially growing culture was divided into three samples. One sample used as control received only C_{14}-adenine (curve C); another sample received casamino acids (casein hydrolysate, final concentration 1.25%) one minute before the addition of adenine (curve AA). The third fraction received a β-galactoside (isopropyl-β-D-thiogalactoside, 10^{-3}M final) as inducer ten minutes before the addition of adenine (curve: inducer). In every sample, aliquots were taken at various time intervals after addition of C_{14}-adenine, and the radioactivity incorporated in the TCA-insoluble fraction was determined. The TCA-insoluble radioactivity is plotted as a function of the time after addition of radioactive adenine. Centrifugation in a sucrose gradient indicates that, under these conditions, only the messenger-RNA is labeled during the first 80–90 seconds.

Direct evidence in support of the assumption that regulation operates at the level of m-RNA synthesis may be obtained experimentally. In a culture exponentially growing at 37°, the incorporation of radioactive bases, both in messenger and ribosomal RNA, is too fast to allow a distinction between the two fractions by kinetic measurements of incorporation. At 19°, instead, the kinetics of incorporation of adenine or uracil clearly exhibits two distinct phases (Fig. 9). During the first 80 to 90 seconds the radioactivity incorporated increases almost linearly. After the 90th to 100th second incorporation proceeds at a much faster rate. Examination of the labeled extracts in sucrose gradients indicates that during the first 80–90 seconds only the messenger RNA is labeled, while after the 100th second the RNA of 30S ribosomes also begins to be labeled. The determination of incorporation during the first phase provides, therefore, an estimate of the rate of messenger RNA synthesis. By changing the specific cultural conditions in such a way as to induce or repress a significant protein fraction, one may hope to provoke a detectable change in the synthesis of the messenger RNA.

A strain carrying an *F-Lac* factor was used for these experiments because, when induced with a galactoside, cultures of such a strain produce up to 20 per cent of the total proteins as β-galactosidase and an additional (unknown) amount of protein as transacetylase (and permease). Bacteria were grown at 19° in minimal medium containing glycerol as carbon source. In order to repress the synthesis of many enzymatic systems, a mixture of amino acids was dumped into the culture. This addition was found to result within a minute in a decrease of about 30 to 40 per cent in the rate of messenger RNA, as compared with a control culture (Fig. 9). In order to induce the enzymes of the lactose system, a gratuitous, non-metabolized β-thiogalactoside was added to another sample of the cultures. Under these conditions the formation of β-galactosidase starts only after an induction lag of about 10 minutes. During this lag no change in the synthesis of messenger RNA can be detected, but after the tenth minute an increase of about 20 per cent is observed (Fig. 9). This increase is not a consequence of an increase in *total* protein synthesis, which is found to proceed at constant rate during the same period of time. A homozygous strain, carrying an operator-negative (o^c) mutation in the chromosome and in the *F-Lac* factor, was used as a control. Under the same conditions, the rate of incorporation of radioactive adenine is depressed by amino acids but is not stimulated by a galactoside inducer (Hiatt, Gros, and Jacob, unpublished experiments).

It therefore appears that changes in cultural condi-

tions which are known either to repress, or to induce, the synthesis of an important fraction of the total proteins, results in detectable decrease, or increase, respectively, in the rate of synthesis of messenger RNA. Inasmuch as m-RNA itself is a primary gene product (a more than likely assumption), this experiment strongly suggests that the regulation operates directly at the genetic level and controls the rate of synthesis of this gene product. The specificity of the additional messenger, however, remains to be demonstrated.

CONCLUSIONS

During the past few years, the one gene-one enzyme hypothesis has been greatly strengthened, and made more precise. A large part of the hereditary message contained in the genetic material appears to specify the molecular structure of individual proteins. Genetic analysis of the regulation systems in bacteria, however, shows that another part of the hereditary message has the task, not of specifying protein configuration, but of determining the rate of transcription of structural genes. The functioning of these regulatory systems, which insure coordinated growth, is of absolute survival value for the bacterial cell. According to the results obtained in the study of bacterial conjugation, the bacterial chromosome contains about 2,000 recombination units (see Jacob and Wollman, 1961). Individual structural genes have been estimated of the order of 0.25 and 0.75 recombination unit in the cases of alkaline phosphatase (Levinthal, 1959) and β-galactosidase (Jacob and Monod, 1961), respectively. Although there is not yet any way of determining the magnitude of the structural fraction of genetic information in bacteria, it appears a rather safe estimate to assume that a bacterial cell is able to manufacture at least 1,000 or 2,000 different protein species. Since a "constitutive regulator" mutation such as those which affect lactose utilization in *E. coli* results in a production of β-galactosidase which amounts to 5 or 7 per cent of the total protein synthesis, it is clear that a bacterium could not survive the breakdown of several systems of regulation.

In the bacterial cell the hereditary message is written in a single, linear structure, the bacterial chromosome, which determines the macromolecular pattern of the bacterium. The transcription of the structural message appears to involve a continuous flow of information from the bacterial chromosome to the cytoplasm, *via* metabolically unstable messenger RNA molecules which bring to the protein-forming centers the instructions for determining specific protein configurations. The fact that the primary gene products, messenger RNA, do not apparently accumulate in the cytoplasm has the important consequence that the only errors of copy which have a persistent effect are those which occur in the replication process and affect the production of the genetic material itself. Those errors which occur during the transcription process and affect messenger RNA only result in the production of one (or a few) bad samples of a given gene product.

The role of the regulatory part of the genetic message appears to be to select those of the structural potentialities which are transcribed and expressed. In the various systems studied so far a like basic mechanism appears to operate. It involves a system of transmitters (regulator genes) and receivers (operators) of specific cytoplasmic signals in the form of repressor molecules, which have the double property of recognizing a particular metabolite and a particular operator. Depending on the system, and through a mechanism still unknown, the metabolite can either activate or inactivate the repressor. The active repressor tends to combine, probably by virtue of possessing a particular base sequence, specifically and reversibly with a particular operator. This combination blocks the initiation of cytoplasmic transcription and, therefore, the formation of the messenger by the structural genes of the whole operon. The inactivation of the repressor allows the transcription of the whole operon and, therefore, the production of the group of coordinated proteins. At any moment, and depending upon intracellular and environmental conditions, the bacterial cell is able to recognize which part of the structural information needs to be expressed; and consequently it is able to produce the proper messengers which bring the necessary instruction to protein-forming centers. It is an important prediction of the operon model that certain chromosomal rearrangements might result in a structural gene being removed from its normal control system and becoming submitted to another, non-physiological one, an effect which would alter the specific conditions of activity of this gene (position effect).

Evidence for the existence of genetic systems of control has already been reported in maize (see McClintock, 1956; Brink, 1960). These systems appear to be composed, basically, of two elements. One is closely associated with the structural gene and controls its activity. The other element may be located near the first, or it may be independently located in another part of the chromosomal set. It determines the conditions to which the gene-associated element specifically responds, and therefore the change in the activity of the gene. These systems are quite specific, each gene-associated element responding only to a particular second element. As pointed out by McClintock (1961), such dual systems of control in maize may, in some respect, be compared with the regulator gene-operator system of bacteria: it is conceivable that the maize system also operates through the intermediacy of specific, cytoplasmic molecules acting as signals like bacterial repressors.

An important difference between the two systems is that, whereas in bacteria both regulators and operators appear as permanent, non-dispensable constituents of

the genome, located at precise, constant loci of the genetic map (the operator corresponding even to a part of a structural gene), the controlling elements of maize, at least some of them, seem to be dispensable and able to move from one chromosomal location to another. In this respect, the maize controlling elements behave like certain particular types of genetic elements in bacteria, called *episomes* (see Jacob and Wollman, 1961). That such episomic elements may interfere with some regulation system of the bacterial cell is clear in the case of prophage in lysogenic bacteria. The presence of the prophage in the lysogenic cell superimposes an additional system of regulation, the immunity system, which prevents the phage structural genes from being transcribed. When bacterial genes are incorporated in a phage genetic material, as happens with the galactose genes in transducing phage λ, they may, under certain conditions, become submitted to the phage system of control. Furthermore, in lysogenic bacteria carrying a prophage λ, induction of phage multiplication by UV light or chemical treatment releases repression of gene activity, not only in the prophage structural genes which are concerned with vegetative reproduction of phage, but also in those structural genes of the bacterial chromosome which control the production of the enzymes of the galactose system and which are known to be located close to the site of prophage attachment in the bacterial chromosome (see Buttin, this Symposium).

It is evident that physiological coordination of chemical activity, i.e., of synthesis and activity of macromolecules, is a fundamental requirement for the existence and survival of the cell as well as of complex multicellular organisms. The "structural" concept of gene action accounts for the multiplicity and for the phylogenetic stability of macromolecular structure. It does not account for biochemical coordination and ignores the problem of the emergence and functioning of differentiated cellular populations. The discovery of units of coordinated genetic activity and of regulator genes which control the activity of structural genes, *via* cytoplasmic repressors, able in turn to interact electively with exogenous or endogenous chemical agents, appears to offer precisely the type of elements needed to build the complex and precise chemical networks of information transfer upon which the development and physiological functioning of organisms must rest.

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References

Ames, B. N., and B. Garry. 1959. Coordinate repression of the synthesis of four histidine biosynthetic enzymes by histidine. Proc. Nat. Acad. Sci., *45:* 1453–1461.

Ames, B. N., B. Garry, and L. A. Herzenberg. 1960. The genetic control of the enzymes of histidine biosynthesis in *Salmonella typhimurium*. J. Gen. Microbiol., *22:* 369–378.

Belozersky, A. N., and A. S. Spirin. 1960. *The Nucleic Acids*, ed. E. Chargaff and J. N. Davidson, Vol. III, p. 147. New York: Academic Press.

Benzer, S. 1953. Induced synthesis of enzymes in bacteria analyzed at the cellular level. Biochim. Biophys. Acta, *11:* 383–395.

Brenner, S., F. Jacob, and M. Meselson. 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature, *190:* 576–581.

Brink, R. A. 1960. Paramutation and chromosome organization. Quart. Rev. Biol., *35:* 120–137.

Cohen, G. N., and F. Jacob. 1959. Sur la répression de la synthèse des enzymes intervenant dans la formation du tryptophane chez *E. coli*. C. R. Acad. Sci., *248:* 3490–3492.

Cohen, G. N., and J. Monod. 1957. Bacterial permease. Bact. Rev., *21:* 169–194.

Cohen, S. S. 1949. Growth requirements of bacterial viruses. Bact. Rev., *13:* 1–24.

Cohen-Bazire, G., and M. Jolit. 1953. Isolement par sélection de mutants d'*E. coli* synthétisant spontanément l'amylomaltase et la β-galactosidase. Ann. Inst. Pasteur, *84:* 1–9.

Cohn, M. 1957. Contributions of studies on the β-galactosidase of *E. coli* to our understanding of enzyme synthesis. Bact. Rev., *21:* 140–168.

Crick, F. H. C. 1958. On protein synthesis. Symp. Soc. Exp. Biol., *12:* 138–163.

Demerec, M., and P. E. Hartman. 1959. Complex loci in micro-organisms. Ann. Rev. Microbiol., *13:* 377–406.

Echols, H., A. Garen, S. Garen, and A. Torriani. 1961. Genetic control of repression of alkaline phosphatase in *E. coli*. J. Mol. Biol., *3:* 425–438.

Gorini, L., and W. Maas. 1958. Feed-back control of the formation of biosynthetic enzymes. pp. 469–478. *The Chemical Basis of Development*, ed. W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Press.

Gros, F., W. Gilbert, H. Hiatt, C. G. Kurland, R. W. Risebrough, and J. D. Watson. 1961. Unstable ribonucleic acid revealed by pulse labelling of *Escherichia coli*. Nature, *190:* 581–585.

Jacob, F. 1951. Adaptation enzymatique pendant le développement du bactériophage chez *Pseudomonas pyocyanea*. C. R. Acad. Sci., *232:* 1780–1782.

——. 1954. *Les Bactéries Lysogènes et la Notion de Provirus*. Monographies de l'Institut Pasteur. Paris: Masson ed.

——. 1960. Genetic control of viral functions. The Harvey Lectures, series *54* (1958–1959): 1–39.

Jacob, F., and A. Campbell. 1959. Sur le système de répression assurant l'immunité chez les bactéries lysogènes. C. R. Acad. Sci., *248:* 3219–3221.

Jacob, F., and J. Monod. 1959. Gènes de structure et gènes de régulation dans la biosynthèse des protéines. C. R. Acad. Sci., *249:* 1282–1284.

——, ——. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol., *3:* 318–356.

Jacob, F., D. Perrin, C. Sanchez, and J. Monod. 1960. L'opéron: groupe de gènes à expression coordonnée par un opérateur. C. R. Acad. Sci., *250:* 1727–1729.

Jacob, F., and E. L. Wollman. 1961. *Sexuality and the Genetic of Bacteria*. New York: Academic Press.

Kalckar, H. M., K. Kurahashi, and E. Jordan. 1959. Hereditary defects in galactose metabolism in *Escherichia coli* mutants. I. Determination of enzyme activities. Proc. Nat. Acad. Sci., *45:* 1776–1786.

KÉPÈS, A. 1960. Etudes cinétiques sur la galactoside-perméase d'*Escherichia coli*. Biochim. Biophys. Acta, *40:* 70–84.

KOGUT, M., M. POLLOCK, and E. J. TRIDGELL. 1956. Purification of penicillin-induced penicillinase of *Bacillus cereus* NRRL 569. A comparison of its properties with those of a similarly purified penicillinase produced spontaneously by a constitutive mutant strain. Biochem. J., *62:* 391–401.

LEDERBERG, E. M. 1960. Genetic and functional aspects of galactose metabolism in *Escherichia coli* K12. pp. 115–131. *Microbial Genetics.* Xth Symp. Soc. Gen. Microb., London. Cambridge Univ. Press.

LEVINTHAL, C. 1959. Genetic and chemical studies with alkaline phosphatase of *E. coli*. pp. 76–85. *Structure and Function of Genetic Elements.* Brookhaven Symp. in Biol.

LWOFF, A. 1953. Lysogeny. Bact. Rev., *17:* 269–337.

MCCLINTOCK, B. 1956. Controlling elements and the gene. pp. 197–216. Cold Spring Harb. Symp. on Quant. Biol., Vol. 21.

——. 1961. Some parallels between gene control systems in maize and in bacteria. In press.

MCFALL, E. 1961. Effects of ^{32}P decay on enzyme synthesis. J. Mol. Biol., *3:* 219–224.

MONOD, J. 1956. Remarks on the mechanism of enzyme induction. pp. 7–28. *Units of Biological Structure and Function.* New York: Academic Press.

——. 1959. Biosynthese eines enzyms. Angew. Chem., *71:* 685–691.

MONOD, J., and G. COHEN-BAZIRE. 1953. L'effet d'inhibition spécifique dans la biosynthèse de la tryptophane-desmase chez *Aerobacter aerogenes*. C. R. Acad. Sci., *236:* 530–532.

MONOD, J., G. COHEN-BAZIRE, and M. COHN. 1951. Sur la biosynthèse de la β-galactosidase (lactase) chez *E. coli*. La spécificité de l'induction. Biochim. Biophys. Acta, *7:* 585–599.

MONOD, J., and M. COHN. 1952. La biosynthèse induite des enzymes (adaptation enzymatique). Adv. Enzymol., *13:* 67–119.

MONOD, J., F. JACOB, and F. GROS. 1961. Structural and rate determining factors in the biosynthesis of adaptive enzymes. Lth Anniversary of the Biochem. Soc., London, in press.

MONOD, J., and E. L. WOLLMAN. 1947. L'inhibition de la croissance et de l'adaptation enzymatique chez les bactéries infectées par le bactériophage. Ann. Inst. Pasteur, *73:* 937–957.

PARDEE, A. B., F. JACOB, and J. Monod. 1959. The genetic control and cytoplasmic expression of "inducibility" in the synthesis of β-galactosidase by *E. coli*. J. Mol. Biol., *1:* 165–178.

PARDEE, A. B., and L. S. PRESTIDGE. 1959. On the nature of the repressor of β-galactosidase synthesis in *E. coli*. Biochim. Biophys. Acta, *36:* 545–547.

PERRIN, D., F. JACOB, and J. MONOD. 1960. Biosynthèse induite d'une protéine génétiquement modifiée, ne présentant pas d'affinité pour l'inducteur. C. R. Acad. Sci., *251:* 155–157.

RILEY, M., A. B. PARDEE, F. JACOB, and J. MONOD. 1960. On the expression of a structural gene. J. Mol. Biol., *2:* 216–225.

SIMINOVITCH, L., AND F. JACOB. 1952. Biosynthèse induite d'un enzyme pendant le développement des bactériophages chez *E. coli* K12. Ann. Inst. Pasteur, *83:* 745–754.

STOEBER, F. 1961. Etudes des propriétés et de la biosynthèse de la glucuronidase et de la glucuronide-perméase chez *E. coli*. Thèse de doctorat ès-Sciences naturelles, Paris.

VOGEL, H. J. 1957. Repression and induction as control mechanisms of enzyme biogenesis: the "adaptive" formation of acetylornithinase. pp. 276–289. *The Chemical Basis of Heredity,* ed. W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Press.

VOLKIN, E., and L. ASTRACHAN. 1957. RNA metabolism in T2-infected *Escherichia coli*. pp. 686–694. *The Chemical Basis of Heredity,* ed. W. D. McElroy and B. Glass. Baltimore: Johns Hopkins Press.

WILLSON, C., D. PERRIN, F. JACOB, and J. MONOD. 1961. In preparation.

YANOFSKY, C., and I. P. CRAWFORD. 1959. The effects of deletions, point mutations, reversions and suppressor mutations on the two components of the tryptophan synthetase of *E. coli*. Proc. Nat. Acad. Sci., *45:* 1016–1026.

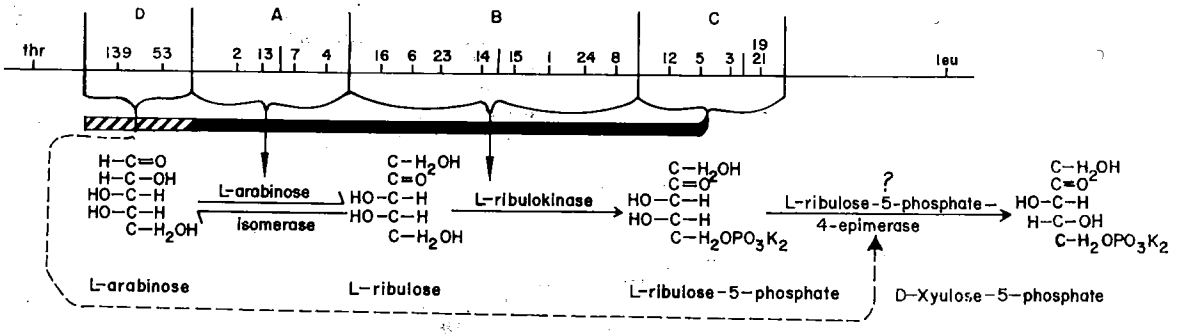
YATES, R. A., and A. B. PARDEE. 1957. Control by uracil of formation of enzymes required for orotate synthesis. J. Biol. Chem., *227:* 677–692.

YČAS, M., and W. S. VINCENT. 1960. A ribonucleic acid fraction from yeast related in composition of desoxyribonucleic acid. Proc. Nat. Acad. Sci., *46:* 804–810.

ZABIN, I., A. KÉPÈS, and J. MONOD. 1959. On the enzyme acetylation of isopropyl β-D-thiogalactoside and its association with galactoside permease. Biochem. Biophys. Res. Comm., *1:* 289–292.

DISCUSSION

ENGLESBERG: It has been observed that mutation in the structural gene for β-galactosidase, besides causing a deficiency in this enzyme, has a secondary effect leading to a decrease in β-galactoside permease activity, while mutation in the structural gene for the permease has no effect on β-galactosidase activity. Dr. Jacob has postulated that these observed effects are due to the proximity of the operator to the β-galactosidase gene and this causes the observed polarity of "dual" effects. I would like to describe some results in our laboratory with L-arabinose negative mutants of *E. coli* B/r which appear contrary to these observations. L-arabinose negative mutant sites of *E. coli* B/r have been ordered by three factor crosses between the markers thr and leu. (u) By enzymatic analysis (in the case of the D locus by accumulation studies only) it has been shown that these mutants fall into four functional groups as indicated in the diagram which follows on Pg. 210. (Gross and Englesberg, 1959 Virology, *9:* 314–331; Englesberg, 1961, J. Bacteriol., *81:* 996–1006). The C gene probably corresponds to the operator gene described by Jacob, and the entire four genes may correspond to an operon. Analysis of the L-arabinose isomerase activity and L-ribulokinase activity of mutants in the A and B locus (shown recently by antigenic analysis by Mrs. N. Lee to be the structural genes for L-arabinose isomerase and L-ribulokinase activity, respectively) revealed a striking "dual" function. Mutants in the B locus are all deficient in kinase activity but have different inducible levels of L-arabinose isomerase. For example, ara-23, ara-14, ara-15, ara-1, have 4.1, 0.17, 3.8, 0.54 times the isomerase activity



of the wild type, respectively. Mutants in the A gene are all deficient in L-arabinose isomerase activity, but each has a distinctive increase in L-ribulokinase activity, ranging from 4.7 to 2.1 times the activity of the wild type. There is no change in the basal uninduced level of either enzyme. Antigen analysis and enzyme studies by Mrs. N. Lee have shown that these changes in activity are not due to the production of modified enzymes but represent increases in amount of enzyme. Analysis of mutants in the D gene should enable us to determine just how extensive is this sort of interaction.

LURIA: I wish to report some results obtained by Dr. Helen R. Revel and myself as part of a study that was initiated some time ago with Drs. Monod and Jacob. The defective transducing phage P1 *dl*, a derivative of phage P1 that carries the *lac*$^+$ region of *E. coli* (Luria *et al.*, 1960, Virology, *12:* 348), is used to infect *lac*$^-$ bacteria; and the production of β-galactosidase is measured. Following a brief initial phase of accelerated enzyme synthesis, there is a phase of linear synthesis lasting several hours. In this period there is no integration of the *lac* genes in most of the infected bacteria (Revel and Luria, 1961, Federation Proc., *20:* 256).

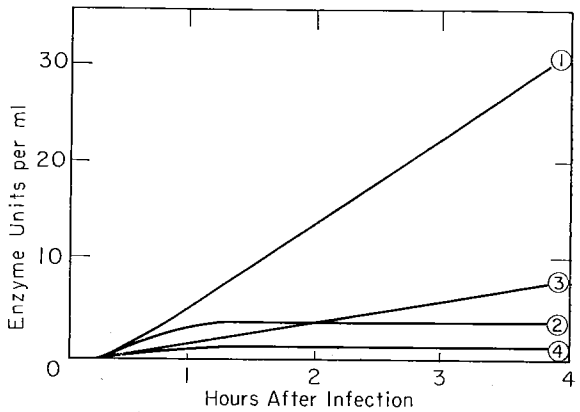
The relevant experiments include infection of bacteria i^+z^- or i^-z^- with phage carrying i^+z^+ or i^-z^+. In the presence of inducer (5×10^{-4} M TMG) enzyme synthesis occurs at comparable rates in all cases. The results of infection *in the absence of inducer* are shown in Fig. 1. Cases 1 and 2 give the expected results. The situation in case 2 is similar to that described by Pardee *et al.* (1959, J. Mol. Biol., *1:* 165) for an analogous cross. In cases 3 and 4, however, although the recipient cells are i^+ and presumably contain repressor, there is a constitutive production of enzyme in amounts about one-fourth those found with constitutive recipients. This partial escape of the z^+ gene entering as part of a phage from the repression by a chromosomal i^+ gene might be attributed: a) to an altered sensitivity of phage-carried *lac* genes to the specific repressor of the *lac* operon; b) to an early production of several copies of the *lac* genes by replication of the phage P1 *dl*; c) to the topographic location of the *lac*$^+$ genes in non-replicating phage elements reducing their accessibility to the *lac* repressor.

Against hypothesis (a) we note, in cases 2 and 4, that the z^+ gene is sensitive to repression by the i^+ gene when both are in the phage. Against hypothesis (b) we note that in case 3 the enzyme production lasts for several hours, during which time the number of z^+ copies per cell ought to be reduced by dilution, thus restoring repression.

In favor of hypothesis (c) are the following observations:

In lysogenic heterogenotes i^+z^- (P1 *dl* i^-z^+), the z^+ gene is variously repressed. Most strains produce very little enzyme constitutively, but some i^+z^- strains carrying P1 *dl* i^-z^+ produce as much as 2–3% as much enzyme constitutively as when fully induced.

In addition, when inducible heterogenotes, either carrying P1 *dl* i^-z^+ in an i^+z^- host or carrying P1 *dl* i^+z^+, are exposed to small doses of UV light, there occurs after 90–120 minutes a constitutive synthesis of β-galactosidase (experiments by H. R. Revel and N. Young), analogous to the synthesis of galactokinase and transferase in irradiated bacteria carrying λ *dg*



Case	1	Recipient	i^-z^-	Phage	i^-z^+
"	2	"	i^-z^-	"	i^+z^+
"	3	"	i^+z^-	"	i^-z^+
"	4	"	i^+z^-	"	i^+z^+

FIGURE 1

(Buttin *et al.*, 1960, C. R. Acad. Sci., *250:* 2471; Yarmolinsky and Wiesmeyer, 1960, Proc. Nat. Acad. Sci., *46:* 626). The constitutive synthesis is high when the phage carries i^-z^+, much lower when it carries i^+z^+. Thus, the z^+ gene in the phage can escape repression by the chromosomal i^+ gene more readily than when the i^+ gene is in the phage itself.

It should be mentioned that in i^+z^+ bacteria lysogenic for phage P1, whose chromosomal location is probably not near the *lac* region, there is no derepression of the chromosomal z^+ gene following UV irradiation. This is at variance with the finding with gal^+ bacteria carrying λ, which upon UV irradiation produce galactose-utilizing enzymes constitutively.