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

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

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

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

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

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

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

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

Units of permease [*cf.* (5)] in percentage of the amount found in induced bacteria.

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

Genotype		Non-induced Bacteria			Induced Bacteria		
Chromosome	*F-Lac*	*Galactosidase*	*Protein Cz*	*Permease*	*Galactosidase*	*Protein Cz*	*Permease*
$i^+o^+z^+y^+$		<1	—	nd	100	—	100
$i^-_3o^+z^-_4y^+$	$/Fi^+o^+z^+y^+$	<1	nd	nd	320	100	100
$i^-_3o^+z^-_4y^+$	$/Fi^+o^cz^+y^+$	36	nd	33	270	100	100
$i^+o^+z^-_1y^+$	$/Fi^+o^cz^+y^+$	110	nd	50	330	100	100
$i^+o^+z^+y^-_R$	$/Fi^+o^cz^-_1y^+$	<1	30	—	100	400	—
$i^+o^+z^-_1y^+$	$/Fi^+o^cz^+y^-_R$	60	—	nd	300	—	100

Starting with a diploid $i^+z^-/F^-i^+z^+$, constitutive mutants (o^c) have been isolated. By appropriate recombinations and transfers, the different diploid genotypes given in the table have been obtained. It will be noted the alleles z^-_1 and z^-_4 which were used permit the synthesis of inactive proteins (Cz_1, Cz_4) which can be measured in the presence of β-galactosidase by immunochemical methods [3]. The table shows that in bacteria heterozygous for o and for z, the permease as well as the galactosidase or the Cz protein are partially constitutive, but that only the allele of z or of y which is *cis* with respect to o^c is constitutively expressed, the *trans* allele remaining strictly inducible as in the genotype o^+/o^+. The constitutive mutation o^c is thus pleiotrophic and dominant, and its effect is only manifested in the *cis* position.

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

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

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

(1) F. Jacob and J. Monod, *Comptes rendus, 249*, 1959, p. 1282.

(2) F. Jacob and E. A. Adelberg, *Comptes rendus, 249*, 1959, p. 189.

(3) D. Perrin, A. Bussard and J. Monod, *Comptes rendus, 249*, 1959, p. 778.

(4) H. M. Kalckar, K. Kurahashi and E. Jordan, *Proc. Nat. Acad. Sc., 45*, 1959, p. 1776.

(5) H. W. Rickenberg, G. N. Cohen, G. Buttin and J. Monod, *Ann. Inst. Pasteur, 91*, 1956, p. 829.

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