THE EFFECT OF MUTATION IN A STRUCTURAL GENE ON THE INDUCIBILITY OF THE ENZYMES CONTROLLED BY OTHER GENES OF THE SAME OPERON

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It is now widely believed that, in some bacteria, protein synthesis is controlled by two kinds of genes; "structural genes" which determine the structure of proteins, and "regulatory genes" which control the rate of their synthesis through the elaboration of cytoplasmic "repressor". Furthermore, it was recently suggested by Jacob et al. (1960) that structural genes controlling a sequence of metabolic reactions are so organized as to form an "operon", together with another gene "operator" occupying a position adjacent to the structural genes. Operator is sensitive to the action of cytoplasmic repressor and regulates the expression of all the structural genes of the operon, but its effect is restricted to the genes on the same chromosome (or in cis position). When cytoplasmic repressor is present, it inhibits, through the intermediacy of operator, the synthesis of all enzymes controlled by the operon. Mutations in the operator gene called o^o result in a simultaneous loss of all enzymes of the sequence, and mutations called o^c make the synthesis of all these enzymes constitutive.

E. M. Lederberg (1960) and Kalckar et al. (1959) showed that some mutants of Escherichia coli K-12 can synthesize only traces of all three enzymes involved in galactose metabolism --- galactokinase (kinase), galactose-1-phosphate uridyl

transferase (transferase), and uridine diphosphogalactose-4-epimerase (epimerase) --- as a result of a single point mutation. These mutants can complement with none of the known mutants lacking singly kinase or transferase, and were regarded as examples of typical o^o mutation by Jacob et al. (1960).

According to the methods described by Nikaido (1961), we have recently determined the levels of these three enzymes in some galactose-negative mutants of Salmonella typhimurium LT-7, originally isolated by Z. Hartman (1956) and also by ourselves. One of them, gal-5, had only traces of all three enzymes (Table I). This mutant had been isolated by a single step selection, and reverts at a frequency of 10^{-7} to 10^{-8} to wild type galactose-positives, and gives rise to wild type recombinants in transductional crosses with any of the other

Table I

Levels of the enzymes on the Leloir pathway in various mutants[1]

		Specific Activity (micromoles/mg protein/hr.)								
		Galactokinase			Gal-1-P uridyl transferase			UDPGal-4-epimerase		
Strain	Genotype[2]	non-induced	induced	i/n[3]	non-induced	induced	i/n	non-induced	induced	i/n
LT-7	$o^+k^+t^+e^+$	0.5	3.7	7.4	0.78	12.6	16.2	4.3	62	14.4
gal-5	$o^ok^+t^+e^+$	-	<0.2	-	-	0.2	-	-	0.05	-
gal-9		-	<0.3	-	10.4	10.1	1.0	46	31	0.7
gal-13	$o^+k^-t^+e^+$	-	<0.2	-	4.8	13.4	2.8	36	43	1.2
gal-21		-	<0.2	-	5.1	11.2	2.2	46	50	1.1
gal-24		-	<0.2	-	3.0	10.2	3.4	18	29	1.6
gal-9R	$o^+k^+t^+e^+$	<0.3	4.4	>14	1.7	15.1	8.9	14	70	5.0
7M1	$o^+k^+t^+e^-$	0.3	2.5	8.3	0.50	5.3	10.6	-	<0.01	-

1. Enzymes were determined on extracts of the cells grown in broth with vigorous shaking. Induced cells were harvested after 30 minutes growth in the presence of 0.1% D-galactose.
2. o, operator; k, kinase; t, transferase; e, epimerase.
3. the ratio specific activity of the extract of induced cells / specific activity of the extract of non-induced cells.

mutants tested. Therefore, it can not be either deletion mutant or multiple step mutant; it is most probably the result of a point mutation o^0 in the galactose operator gene. These results, as well as our recent finding that a close linkage was demonstrated between this operator, kinase and epimerase genes in transduction experiments with phage P22, have led us to postulate that the genes controlling the three enzymes of galactose metabolism form an operon also in S. typhimurium.

In wild type E. coli and S. typhimurium, the synthesis of these three enzymes follows the typically inducible pattern. Kalckar et al. (1959), however, previously reported that two kinase-less mutants of E. coli K-12 exhibited constitutive synthesis of transferase, although the synthesis of epimerase still remained inducible. This result seemed rather unexpected, because the biosynthesis of all three enzymes in an operon should be simultaneously controlled. We examined, therefore, with kinase-less mutants of S. typhimurium, the levels of transferase and epimerase in the non-induced and induced states. As shown in Table I, all of the four genetically different kinase-less mutants tested showed completely constitutive synthesis of epimerase, and more or less constitutive synthesis of transferase also, which is in agreement with the operon concept.* These enzymes were fully inducible in a kinase-positive revertant (gal-9R) derived from one of these strains. An epimerase-less mutant (7M1) had usual inducible pattern of kinase and transferase.

These results demonstrate that mutations arisen in a

* After this work was completed, we were informed by Dr. K. Kurahashi that Dr. H. M. Kalckar (personal communication to K. Kurahashi) has independently obtained similar results in kinase-less strains of E. coli.

specific structural gene --- galactokinase locus --- of galactose operon have apparently a pleiotropic effect to make the synthesis of two other enzymes (controlled by the other structural genes of the same operon) fully or partially constitutive. This constitutivity might be due to either (1) the absence of specific repressor, or (2) the insensitivity of operator gene to the repressor. In the former case mutations at kinase gene may simulate the mutations at regulatory gene (i^-) which is expressed only through the mediation of cytoplasmic repressor. In the latter case they may simulate the mutations at operator gene (o^c) which affect structural genes in the *cis* position only. We can probably determine between these possibilities by several means. One of these, test of constitutivity of transferase in heterogenotes with phage lambda, was pursued in the very recent report of Yarmolinsky and Wiesmeyer (1960). Their results point to the conclusion that mutations at kinase gene lead to the loss of specific repressor, but it can still be argued that their material does not seem to be ideally suited for the study of nucleocytoplasmic interaction, since Buttin *et al.* (1960) presented an evidence that the expression of galactose operon tends to be affected by its interaction with phage lambda. On the other hand, our transductional analysis with P22 demonstrated that galactose operator gene is very closely linked to kinase gene, and gal-5 seems to belong to almost the same transductional group as gal-9, 13, 21, and 24, in agreement with the report of Z. Hartman (1956). In view of these findings, the possibility could not yet be excluded that mutations at kinase gene have an effect simulating those of the operator gene; experiments to clarify these points are now under way.

Finally it should be mentioned that kinase-less mutants have been found in nature which do not show constitutive transferase or epimerase synthesis. A mutant of yeast studied by Robichon-Szulmajster (1958) had typically inducible transferase and epimerase, and a kinase-less mutant (W-3142) of E. coli K-12 studied by Kalckar et al. (1959) also showed inducible transferase synthesis. It is interesting that in the latter mutant, the site of mutation seems to be located somewhat apart from the galactose gene cluster or operon, since E. M. Lederberg (1960) reported that this mutant could not be transformed to galactose-positives by transduction with phage lambda.

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