PRODUCTION OF GALACTOKINASE BY AN O^c-MUTANT OF THE GALACTOSE OPERON IN ESCHERICHIA COLI

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O^c-mutants are operationally defined as revertible, pleiotropic mutations, which abolish the function of all genes of an operon in cis position. Initially it was thought that O^c-mutations are located in the operator, the region of an operon, which serves as the recognition site for the gene product of the corresponding regulator gene (Jacob and Monod, 1961). Recent evidence, however, mainly obtained in the lactose operon of E.coli, places the O^c-mutations in the first structural gene of the operon, while the operator region seems to be located in a segment of the operon distinct from any structural gene (Jacob et al., 1964). The O^c-mutation is thought to manifest itself as a defect at the translation step (Beckwith, 1964). In the present communication we want to report some experiments with an O^c-mutant of the galactose operon. These experiments make it likely that the O^c-mutation is also able to change the response of the operon towards the inducer.

Materials and Methods: All experiments were carried out in the synthetic medium M9 fortified with 0,75 % casamino acids. Glucose was omitted from the medium. 10^{-3}M d-fucose was added as an inducer, when mentioned in the text. Incubations were at 37° with aeration. W8 is a galactose-positive K12 strain, strain W3109, an O^c-mutant of the galactose operon, was obtained from Dr. M. Yarmolinsky. Strain W13 is a mutant lacking both galactokinase (kinase) and galactose-1-phosphate uridyl transferase (transferase).

Strains were lysogenized with λ, the transducing λdg or both of them. Lysates were obtained from the lysogenic strains by uv-induction. O^c-homogenotes were obtained from cells carrying λdg only by isolating galactose-negative cells which still were immune to λ. These could be additionally lysogenized by λ, and lysates could be obtained from the doubly lysogenic strains.

These lysates did not transduce the original O^o-strain to galactose-fermentation, and did transduce strains carrying mutations in the galactose structural genes.

Kinase was determined according to Sherman (Sherman, 1963), and transferase was assayed according to Buttin (Buttin, 1963).

For the calculation of the number of kinase molecules, a molecular weight of 38 000 and a specific activity of the pure enzyme of 4×10^3 µmoles/h/mg protein were assumed (Wilson and Hogness, 1964). Most enzyme determinations were carried out in lysozyme-EDTA-lysates of cells. The low activities of the O^o-strain, without phage induction or infection had to be carried out with alumina extracts at a protein concentration of >10 mg protein/ml. The values obtained with these concentrated extracts are somewhat variable and may be minimum estimates, since high protein concentrations were found to be inhibitory. It was shown that the reaction was linear with time and was absolutely dependent on ATP, thus excluding unspecific binding of radioactive galactose to the exchange paper. - In the induction and infection experiments, time curves of enzyme production were measured, and the rates were calculated from the linear part of these curves.

Results: W3109 produces an amount of galactokinase (Table 1, line 3 and 4), which is small compared to wildtype (line 1 and 2) and independent of the presence of inducer.

While growth in the presence of d-fucose does not increase the rate of kinase synthesis in W3109, other conditions are effective in doing so:

a) uv-induction of a homogenote O^o_{gal} (λdgO^o_{gal}) (line 5 and 6)
b) uv-induction of the doubly lysogenic homogenote O^o_{gal} ($\lambda, \lambda dgO^o_{gal}$) (line 7 and 8). In experiments a and b, kinase synthesis was not stimulated by d-fucose.
c) Infection of O^o-cells with a lysate obtained by induction of the homogenote of experiment b (line 9).

The following conditions do not stimulate galactokinase synthesis:

d) Infection of O^o-cells lysogenic for λ with the same lysate as in c does not stimulate kinase synthesis (line 10). This seems to indicate that on the phage chromosome the galactose genes are under the control of λ-immunity, as in wildtype λdg (Buttin, 1963).
e) Infection of sensitive O^o-cells with λ does not lead to an

increase in kinase production (line 11). This excludes the possibility that the effects observed are due to the presence on λ of a suppressor gene for the O^c-mutation, active only in the vegetative state of the phage.

Table 1

Bacterial strains	experimental conditions	number of molecules of galactokinase synthezised per cell per minute
1) gal^+	no d-fucose	11
2) gal^+	with d-fucose	150
3) $O^c gal$	no d-fucose	0,3
4) $O^c gal$	with 10^{-3}M fucose	0,39
5) $O^c gal$ ($\lambda dg O^c gal$)	uv'ed cells, no d-fucose	2,5
6) $O^c gal$ ($\lambda dg O^c gal$)	uv'ed cells, with d-fucose	2,0
7) $O^c gal$ ($\lambda, \lambda dg O^c gal$)	uv'ed cells, no d-fucose	1,42
8) $O^c gal$ ($\lambda, \lambda dg O^c gal$)	uv'ed cells, with d-fucose	1,49
9) $O^c gal$	cells infected with $\lambda + \lambda dg O^c gal$ no d-fucose	3,47
10) $O^c gal$ (λ)	cells infected with $\lambda + \lambda dg O^c gal$ no d-fucose	0,16
11) $O^c gal$	infected by no d-fucose	0,41

Beckwith has suggested, as explanation for this finding an instability of the m-RNA carrying the O^c-mutation, which depresses even its induced level to a low and by present techniques unmeasurable value (Beckwith, 1964). This is not compatible with our observations, since on this assumption an increase in residual protein synthesis is expected in cells induced by d-fucose.

In experiments b and c, a significant synthesis of transferase was also observed. A similar observation has also been made by Yarmolinsky (Yarmolinsky et al., 1961).

Discussion: It has recently been discussed whether O^c-mutations belong to the same class of mutations as the "polar" mutants described in the histidine operon (Ames and Hartman, 1963). Since, in the O^c-mutants investigated so far, no attention was given to residual enzyme levels, this assumption could be made. The O^c-mutant described here, however, must be distinguished from the "polar" mutants, since its rate of enzyme synthesis does not respond to external inducer. In the "polar" mutants the residual synthesis is subject to repression.

For an explanation of this lack of response to inducer, two possibilities have to be considered:

A) There exist two ways to transcribe the galactose operon into m-RNA, only one of which is sensitive to repressor and affected by the O^c-mutation. On this assumption, one would additionally have to explain why the uninduced rate of enzyme synthesis in the wildtype is much higher than the residual rate in the mutant.

B) Repression and the effect of the O^c-mutation are not independent of each other. It has been reported that even in O^c-mutants which, according to their behaviour towards known suppressor genes, act at the level of protein synthesis, no increase in the amount of specific m-RNA is found in the presence of inducer (Attardi et al., 1963, Hill and Echols, 1965).

Ames and Hartman suggested that m-RNA synthesis is inhibited, if the m-RNA cannot be translated into protein, because it carries an O^c-mutation. This model is conceivable, if DNA, m-RNA and ribosomes with attached growing peptide chains form a single, complex structure (Ames and Hartman, 1963). This could also explain the dominance of O^+ over O^c. If, as has been suggested, the synthesis of a new m-RNA molecule is coupled to the successful reading of the previously synthesized molecule of m-RNA, then in the O^c-mutant reading may become limiting rather than the state of repression of the operator. Thus the maximum rate of enzyme synthesis in the mutant may be much smaller than that permitted

in the wildtype even in the fully repressed state. Removal of repressor by addition of inducer cannot, under this condition, produce an increase in enzyme production. The same could be true, if both repression and the O^c-mutation were primarily effective at the level of protein synthesis.

It is not known why genetic effects, as the translocation of the galactose operon from the bacterial chromosome to the chromosome of phage λ, change the expression of the O^c-mutant. This may either suggest a primary expression of this O^c-mutation at the DNA-level, or provide a further example of an interdependence of RNA- and protein synthesis. Studies on this problem are in progress in this laboratory.

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References

Ames, B.N., and P.E. Hartman (1963) Cold Spring Harbor Symp. Quant. Biol. XXVIII, 349

Attardi, G., S. Naono, J. Rouvière, F. Jacob, and F. Gros (1963) Cold Spring Harbor Symp. Quant. Biol. XXVIII, 363

Beckwith, J.R. (1964) Struktur und Funktion des genetischen Materials, ed. H. Stubbe, Akademie Verlag Berlin, p. 119

Buttin, G. (1963) J. Mol. Biol. 7, 164, 610

Hill, C.W., and H. Echols (1965) Biophys. J. 5, Abstracts of the Ninth Annual Meeting of the Biophysical Society, p. 91

Jacob, F. and J. Monod (1961) Cold Spring Harbor Symp. Quant. Biol. XXVI, 193

Jacob, F.A. Ullman, and J. Monod (1964) C.R. Acad. Sci. Paris 258, 3125

Sherman, J.F. (1963) Anal. Biochem. 5, 548

Wilson, D.B.and D.S. Hogness (1964) J. Biol. Chem. 239, 2469

Yarmolinsky, M., E. Jordan, and W. Wiesmeyer (1961) Cold Spring Harbor Symp. Quant. Biol. XXVI, 217