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THE ROLE OF THE REGULATOR-GENE PRODUCT (REPRESSOR)
IN CATABOLITE REPRESSION OF β-GALACTOSIDASE SYNTHESIS
IN ESCHERICHIA COLI

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ABSTRACT

The role of the regulator-gene product (repressor) in catabolite repression of β-galactosidase synthesis in Escherichia coli. J. Palmer and V. Moses; Laboratory of Chemical Biodynamics, University of California, Berkeley, Calif., 94720, U.S.A.

July 19, 1967

The specific role of the lac repressor (i-gene product) in transient catabolite repression evoked by the introduction of glucose into the medium has been investigated in Escherichia coli using mutants of the i-gene. A temperature-sensitive mutant (iTL) is normally inducible and demonstrates transient repression when grown at 32°. At 42° it is about 20% constitutive and transient catabolite repression is abolished. A strain carrying an amber suppressor-sensitive mutation in the i-gene is phenotypically constitutive and also fails to show transient catabolite repression. Insertion of Flac i+ into this strain restores both inducibility and transient repression. It is concluded that the i-gene product interacts with the catabolite corepressor in such a way that its affinity for the operator is increased.
The Role of the Regulator-Gene Product (Repressor) in Catabolite Repression of β-Galactosidase Synthesis in Escherichia coli

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July 19, 1967

1. The specific role of the lac repressor (i-gene product) in transient catabolite repression evoked by the introduction of glucose into the medium has been investigated in Escherichia coli using mutants of the i-gene. 2. A temperature-sensitive mutant (iTL) is normally inducible and demonstrates transient repression when grown at 32°. At 42° it is about 20% constitutive and transient catabolite repression is abolished. 3. A strain carrying an amber suppressor-sensitive mutation in the i-gene is phenotypically constitutive and also fails to show transient catabolite repression. 4. Insertion of F lac i⁺ into this strain restores both inducibility and transient repression. 5. It is concluded that the i-gene product interacts with the catabolite corepressor in such a way that its affinity for the operator is increased.

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It has previously been shown (Palmer & Moses, 1967) that acute transient catabolite repression of β-galactosidase synthesis, observed when glucose is added to glycerol-grown cells of *Escherichia coli* (Moses & Prevost, 1966), requires the presence of a functional operator gene (o). The specific involvement of the i-gene product (repressor) was not then tested since a regulator gene deletion mutant (i del) was not available to us. While such a deletion mutant has still not been isolated to the best of our knowledge, the isolation of certain temperature-sensitive control mutants by Sadler & Novick (1965) and of amber suppressor-sensitive mutants by Müller-Hill (1966) has made possible an investigation of the role of the repressor in acute transient repression.

Two types of temperature-sensitive regulatory mutants have been obtained (Sadler & Novick, 1965). In one series the repressor itself appeared to be increasingly unstable as the temperature was raised, so that growth at 42° produced a cell largely constitutive in character, while the same strain cultured at 30° was typically inducible. This mutation has been designated iTL. In another series (iTSS) the synthesis of the repressor appeared to be prevented at high temperature although the repressor, once formed, was not destroyed by heat. There is some doubt, however, about the status of the repressor in the iTSS mutants. Sadler & Novick (1965) observed that such strains acquired active repressor unusually rapidly when transferred from a high growth temperature, believed to prevent repressor synthesis, to a temperature at which repressor synthesis was possible. Further, the constitutive synthesis of β-galactosidase at high temperatures is susceptible to inhibition by o-nitrophenyl-β-fucoside (J. Palmer, unpublished work), a substance regarded as an activator of the repressor rather than an antagonist of the inducer (Jayaraman, Müller-Hill & Rickenberg, 1956). It is thus probable that some repressor functions in iTSS strains are reversibly inactivated at high temperature, and it is possible that other
repressor functions might persist under these conditions. Lowering the ambient temperature of the cells from 42° to 32° might permit spontaneous re-activation of a heat-inactivated repressor, accounting for the rapid onset of inducibility reported by Sadler & Novick (1965). We have therefore confined our studies of transient repression in heat-sensitive strains to the iTL mutation. Investigation of the catabolite repression behaviour of the iTL mutant at 42° and 32°, and comparison with its wild-type parent, has allowed a more definite correlation than heretofore possible between the functional state of the repressor and the transient repression effect.

A further test has been made using an amber suppressor-sensitive mutant of the i-gene (i-SUS), and comparing this with the behaviour of a diploid strain also carrying a wild-type i-gene (i-SUS/i+). The i-SUS mutant differs from i- strains in that it effectively carries a functional deletion of the i-gene rather than a point mutation. Thus, all functions of the repressor are eliminated simultaneously and the role of the i-gene may then be studied by introducing i+.

MATERIALS AND METHODS

Organisms. The strains of E. coli used are listed with their relevant genetic characters: E 103 (Hfr iTL o+ z+) and E 102 (i+ parent of E 103) were obtained from A. Novick, and their properties are described by Sadler & Novick (1965) and by Horiuchi, Horiuchi & Novick (1963); the iTL mutant differs from its parent only by lesions in the i-gene of the lactose operon and selection procedures during its isolation would not be expected to select other genotypic changes. Strains 112-12-A-84 (F- i-SUS o+ z+ y+ his- cys- gal- su-) and JC 2637 (thr- leu- pro- met- B+ SmR Flac i+ o+ z+ y+) were gifts from B. Müller-Hill and A. J. Clark, respectively.
Growth conditions. The cells were grown at 32°, 37°, or 42° in medium 63 (Pardee & Prestidge, 1961) containing 22 mM-glycerol and supplemented with the necessary specific nutrients. Temperatures were controlled to ± 0.5° in a water bath. Aeration was provided by shaking or by magnetic stirring. Growth was measured as described previously (Moses & Prevost, 1966); at an extinction value of 1.0 the cultures contain 225 μg. bacterial protein/ml.

Glucose was introduced to the cultures where indicated by adding 1 M-glucose solution to give a final concentration of 10 mM.

Enzyme induction and assay. β-Galactosidase synthesis was induced, where indicated, with 0.5 mM-isopropyl-thio-β-D-galactopyranoside (IPTG). Since many measurements were made of constitutive enzyme synthesis, particular care was exercised to minimize errors in sampling volume. Samples (0.05 to 0.20 ml.) of culture were added to clean weighed 10 ml. shell vials containing 0.2 ml. of chloramphenicol solution (1 mg./ml.), mixed and reweighed to correct for volume errors. Before assay, one drop (about 6 μl.) of toluene was added to each sample, and these were stoppered and shaken for 30 min. at 37°. The stoppers were then removed and the toluene allowed to evaporate for 30 min. at 37°. Enzyme activity was measured by the rate of hydrolysis of o-nitrophenyl-β-D-galactoside (ONPG) as described by Kepes (1963), using 0.8 ml. of 3.3 mM-substrate solution, and 0.8 ml. of 0.75 M-Na₂CO₃ to stop the reaction. About 100 mg. of solid BaCO₃ was added to each vial, and after mixing the vials were centrifuged for 1 min. at 1400 x g in a bench-top centrifuge to clarify the solution and remove turbidity due to the bacteria. The amount of enzyme catalyzing the hydrolysis of 1 μmole of substrate/min. at 37° is defined as 1 unit.

Mating experiments. Strain JC 2637 was used to insert i⁺ into the i⁻sus strain 112-12-A-84. The requirement of JC 2637 for proline was confirmed; colonies of this strain failed to grow for at least 7 days in the absence of
added proline. About $2 \times 10^5$ cells/ml. of the donor were incubated with $1 \times 10^8$ cells/ml. of the recipient strain for 2 hr. at 37° without agitation in glycerol-minimal medium containing all the growth requirements for both strains. The culture was then diluted with buffer and plated on glycerol-minimal plates containing histidine and cysteine to select for the recipient strain and against the donor. When visible colonies were obtained the plates were inverted for 1-2 min. over filter paper soaked with toluene and the colonies were then tested for β-galactosidase activity with sterile ONPG solution. Colonies showing no nitrophenol colour for at least 5 min. were replated and further colonies selected which showed no ONPG hydrolysis for 1 hr. These were found to be lac⁺ on eosin-methylene blue-lactose plates. One isolate was designated PM1 and was used for further studies. It showed the same growth requirements as the recipient parent and not those of the donor. PM1 is therefore i⁻sus o⁺ z⁺ y⁺/i⁺ o⁺ z⁺ y⁺.

RESULTS

Effect of temperature on transient repression in iTL and i⁺ strains. The effect on β-galactosidase synthesis of adding glucose to cells growing on glycerol at 32° and 42° is shown for the i⁺ strain E102 (Fig. 1) and the iTL strain E103 (Fig. 2). Both strains had similar growth responses to the addition of glucose: little change in doubling time when glucose was added at 32°, but a 10-25% increase in the growth rate when glucose was introduced at 42°. For strain E102 a transient repression of enzyme synthesis was observed at both temperatures, lasting about the same time in each case. Similar experiments performed with E103 showed that transient repression, while present at 32°, was no longer evident at 42° (Fig. 2). The mutation from i⁺ to iTL thus abolished acute transient repression and simultaneously permitted a partially constitutive synthesis of β-galactosidase when the cells were grown at 42°.
Effect of glucose on i^-sus and i^-sus/i^+ strains. Addition of glucose to i^-sus cells growing on glycerol increased the growth rate by 11%. No transient repression of β-galactosidase synthesis was observed, and the steady differential rate of enzyme synthesis fell immediately to 61% of the rate in the absence of glucose (Fig. 3). When glucose was added to the i^-sus/i^+ diploid there was almost no effect on the growth rate, but transient repression was obtained (Fig. 3). Immediately after the addition of glucose the differential rate of enzyme synthesis fell by 79%; some 55 min. later (0.9 generation) it recovered to about 65% of the original rate on glycerol.

DISCUSSION

It was pointed out elsewhere (Palmer & Moses, 1967) that arguments excluding repressor from a role in transient repression are not acceptable when based on the fact that i^- constitutive mutants exhibit such repression. It is possible that i^- point mutants possess an altered repressor which, while having lost its normal capacity to bind to the operator, may be activated to do so under metabolic conditions giving rise to catabolite repression. Similarly, the observation of repression by glucose of β-galactosidase synthesis during the one-hour period of constitutive synthesis of the enzyme following transfer by conjugation of i^+ z^+ into lac-deletion recipients (Loomis & Magasanik, 1964), also does not eliminate repressor from the mechanism of catabolite repression. One might argue that repressor is effective at lower concentrations in the presence of glucose metabolites. In a recent paper Barbour & Pardoe (1966) have indeed obtained evidence that the repressor or its precursor is present in low concentration from a very early period during mating.

The observation that transient repression depends on a functional operator gene (Palmer & Moses, 1967) strongly suggests the likelihood of the repressor
also being involved. A formal test of this possibility requires a comparison of the glucose effect in related strains, one of which contains the repressor while in the other it is absent. Such a comparison cannot yet be made with a deletion mutant, but the i^TL temperature-sensitive regulator strain and the i^-sus mutant offer opportunities of testing repressor involvement.

In comparing E 103 with its parent E 102 we note that the change from i^+ to i^TL simultaneously rendered the synthesis of β-galactosidase about 20% constitutive at 42° and at that temperature eliminated the transient repression due to glucose. At 32° the i^TL strain was inducible and showed transient repression. We have attempted to insert F lac i^+ from JC 2637 into an F^- pheno-cop y (Lederberg, Cavalli & Lederberg, 1952) of E 103 but failed to isolate an i^TL/i^+ diploid. A successful isolation would have permitted a more rigorous test of these changes arising entirely as a result of mutation in the i-gene.

The absence of transient repression in the i^-sus strain supports the idea of a role for the repressor in this phenomenon. The repressor in this strain has presumably lost affinity for the operator, giving rise to the constitutive phenotype. Gilbert & Müller-Hill (1966) have shown that this repressor has also lost affinity for the inducer. This argues strongly for a large functional deletion in the i-gene and is thus consistent with transient catabolite repression being mediated by the i-gene product. The simultaneous restoration of both inducibility and transient repression by the insertion of i^+ into i^-sus confirms this conclusion.

The observed relation of transient catabolite repression to various mutations in the i-gene may be explained by a model in which the repressor is a trivalent molecule, rather than the bivalent entity originally suggested by Jacob & Monod (1961). This concept has previously been briefly discussed by Clarke & Gammar (1964), and was also considered by Loomis & Magasanik (1964) but was rejected by them for reasons which were subsequently criticized (Clarke...
& Grammer, 1964; Palmer & Moses, 1967). The three postulated sites on the repressor would be an inducer-interacting site (I), an operator-interacting site (O), and a catabolite corepressor-interacting site (CR). According to this model, the O-site interacts with the operator in the absence of inducer and prevents transcription. In the presence of inducer the I-site binds to the latter and the O-site is allosterically modified in such a way that it no longer has affinity for the operator and transcription proceeds (Monod, Changeux & Jacob, 1963). The CR-site interacts with the catabolite corepressor and allosterically increases the affinity of the O-site for the operator. Thus the CR- and I-sites have opposite effects on the degree of binding of the repressor to the operator and can be viewed as being competitive. Evidence has been published indicating that induction and catabolite repression may be competitive phenomena (Clark & Marr, 1964).

Each of the proposed three repressor sites may be conceived as existing in several allelic forms giving rise to the following genotypes:

- **I⁺**: repressor is capable of interacting reversibly with inducer and thus losing affinity for the operator;
- **I⁻**: repressor is incapable of interacting with inducer;
- **I⁺⁺**: repressor binds to inducer more tightly than normal;
- **CR⁺**: repressor is capable of interacting reversibly with the catabolite corepressor and thus increasing affinity for the operator;
- **CR⁻**: repressor is incapable of interacting with the catabolite corepressor;
- **CR⁺⁺**: repressor binds to the catabolite corepressor more tightly than normal;
- **O⁺**: repressor is capable of interacting reversibly with the operator;
- **O⁻**: repressor is incapable of interacting with the operator;
- **O⁺⁺**: repressor binds to the operator more tightly than usual.

In order to account for the presence of transient catabolite repression in i⁻ constitutive mutants, it is also necessary to postulate a further allele of the O-site:
CR: repressor interacts with the operator only when the CR-site is occupied by the catabolite corepressor.

By combining the appropriate alleles for the three sites into one gene all the known phenotypic variations of transient catabolite repression behaviour can be accounted for by this model.

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REFERENCES


Fig. 1. Repression by glucose of β-galactosidase synthesis in strain E 102 at 32° and 42°. IPTG (0.5 mM) was added at † and glucose (10 mM) at †. Mass doubling times (min.) before and after glucose addition and length of transient repression, respectively: at 32°, 78, 77, 25; at 42°, 70, 66, 29.

Fig. 2. Repression by glucose of β-galactosidase synthesis in strain E 103 at 32° and 42°. IPTG (0.5 mM) was added at † and glucose (10 mM) at †. Mass doubling times (min.) before and after glucose addition and length of transient repression, respectively: at 32°, 70, 72, 23; at 42°, 118, 65, no transient.

Fig. 3. Repression by glucose of β-galactosidase synthesis in strains 112-12-A-84 (i-Sus) and PM 1 (i-Sus/i+) at 37°. IPTG (0.5 mM) added at †; 10 mM-glucose added at †. Mass doubling times (min.) before and after glucose addition and length of transient repression, respectively: 112-12-A-84, 59, 53, 0; PM 1, 60, 60, 55. A, i-Sus; B, i-Sus/i+.
Fig. 1

β-Galactosidase (units/ml.)

Bacterial protein (μg./ml.)

32°

42°
Fig. 3

Bacterial protein (µg./ml.)

P-galactosidase (units/ml.)

A

B