Catabolite Repression in *Escherichia coli*

A STUDY OF TWO HYPOTHESES

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1. Two hypotheses to account for general catabolite repression of the lactose enzymes in *Escherichia coli* were tested: the dilution model of Palmer & Moses (1967), and the specific catabolite repressor model of Loomis & Magasanik (1965, 1967). 2. The dilution model predicts that in mutants lacking the *i-o* regulation system the differential rate of β -galactosidase synthesis should increase when amino acid-synthesizing enzymes are repressed by the presence of amino acids in the medium. It also predicts that with such mutants the total absence of P_1 from the medium should not result in the complete cessation of β -galactosidase synthesis that is observed with wild-type cells. 3. Neither prediction was confirmed experimentally, and it is concluded that this model cannot explain catabolite repression. 4. The specific repressor hypothesis depends on the properties of a strain of *E. coli* carrying the CR^- mutation. It requires both that cells of this genotype should be totally resistant to general catabolite repression and that this resistance should be specific for the lactose enzymes. 5. In fact the synthesis of β -galactosidase by CR^- cells, though showing resistance to catabolite repression by growth on glucose, was found to be repressed in several other circumstances. 6. Two other inducible enzymes, L-tryptophanase and D-serine deaminase, also showed resistance to repression by glucose in CR^- cells. 7. It is concluded that this model, too, does not account for general catabolite repression. 8. Strains carrying deletions at either end of the lactose operon that extend into the structural genes of the operon continue to exhibit catabolite repression. 9. These experiments appear to eliminate the possibility that catabolite repression operates at the level of DNA transcription, and suggest that repression affects instead the translation of messenger RNA into protein.

It has become clear from recent studies (Moses & Prevost, 1966; Paigen, 1966; Tyler, Loomis & Magasanik, 1967) that two types of catabolite repression may affect the expression of the *lac* operon in *Escherichia coli*. Such repression is typically observed in *E. coli* when cells are grown in glucose-minimal medium or in rich medium, and is usually much decreased in glycerol or succinate medium.

Transient repression is observed when cells growing in glycerol medium are supplied in addition with glucose, or are transferred to glucose medium. This repression, which is relatively severe, lasts for a short time only. It seems to be associated with a reorganization of cellular activity during the shift-up that follows the enrichment of the medium. Transient repression affects all the proteins of the lactose system (Tyler *et al.* 1967), and genetic studies (Palmer & Moses, 1967, 1968) have suggested

that it depends on the action of the *i*-gene product (repressor).

When glucose is added to cells induced for the lactose enzymes and growing in glycerol the severely repressive transient phase is followed by a phase of permanent repression. Here the degree of repression, though less severe than during the transient period, characteristically lowers the rate of enzyme synthesis to no more than 50% of the original rate in glycerol. Similarly, the rate of enzyme synthesis in cells grown for many generations in glucose-minimal medium is usually less than half that of cells grown in glycerol-minimal medium. There is thus a type or phase of catabolite repression different from the transient variety, and this has variously been termed 'permanent', 'general' or 'mild', or even just 'catabolite repression' as distinct from 'transient repression'. It is this type of repression that we consider in the present paper;

we use the terms 'transient' and 'general' to distinguish the two sorts of catabolite repression.

Little is known of the detailed working of general catabolite repression. Recently, however, two different suggestions have been made to account for the phenomenon, and the purpose of this study is to decide whether either of them can adequately explain the repression observed experimentally.

Palmer & Moses (1967) suggested that general catabolite repression might be a non-specific effect resulting from changes in protein composition when cells are grown in different media. When cells were shifted to a richer medium the rate of synthesis of some cellular proteins (e.g. ribosomal proteins) would increase, whereas the rate of synthesis of others (e.g. β -galactosidase) would not. By comparison with the overall rate of protein synthesis, the synthesis of β -galactosidase would then appear to be repressed. This proposal we can term the 'dilution model'.

The second model is due to Loomis & Magasanik (1965, 1967), who isolated a mutant strain of *E. coli* (LA-12G) showing resistance to general catabolite repression of the lactose enzymes, primarily that exerted by glucose. They found that the genetic determinant of this resistance (*CR*) was far removed from the lactose operon. In partial diploids resistance was recessive to sensitivity. Loomis & Magasanik (1965, 1967) proposed the existence of a new apo-repressor molecule, coded for by the locus *CR*, the function of which was to mediate specifically the catabolite repression of the lactose enzymes by a mechanism presumably analogous to that of the *i*-gene repressor. This proposal we can term the 'specific repressor model'.

We conclude that neither model offers a satisfactory explanation of general catabolite repression.

EXPERIMENTAL

Organisms. The strains of *E. coli* used together with their relevant genetic characteristics and sources are listed in Table 1. Cultures of strain LA-12G were checked as a routine for the presence of the *CR*-character by the indicative plate technique of Loomis & Magasanik (1967).

Growth conditions and media. These were as described by Moses & Prevost (1966) with a few additions. For de-repression of alkaline phosphatase, cells were grown in medium similar to M63 except that the concentration of P_i was decreased to 2 mM and buffering was provided by 0.1 M-tris-HCl, pH 7.2. De-repression was achieved by filtering the cells on a Millipore filter (0.45 μ m pore size) and resuspending them in a similar medium totally devoid of P_i . The cultures were aerated by shaking in a reciprocal-shaker water bath rather than by magnetic stirring.

Determination of bacterial protein. This was carried out by the method of Lowry, Rosebrough, Farr & Randall (1951).

Enzyme induction and assays. These were performed as described earlier: β -galactosidase (Palmer & Moses, 1968); D-serine deaminase and L-tryptophanase (Pardee & Prestidge, 1961); thiogalactoside transacetylase (Epstein, 1967). Enzyme units are defined as those quantities giving rise to 1 μ mole of product/min. at 37°. For D-serine deaminase arbitrary units are used, which are satisfactory for the comparative studies made here.

In several experiments we compared the rate of synthesis of β -galactosidase by cells growing in different media. There are two ways of doing this; neither is entirely satisfactory. The first method involves subculturing cells into two different media, allowing them to grow for many generations so that they have reached a steady state, and then measuring the differential rate of synthesis of enzyme. This method has the disadvantage that at the time of the

Table 1. *Bacterial strains used in the present study*

Abbreviations: *i*, gene for inducibility of lactose operon; *o*, lactose operator gene; *p*, lactose promoter gene; *z*, structural gene for β -galactosidase; *y*, structural gene for galactoside permease; *ac*, structural gene for thiogalactoside transacetylase; *lac*, lactose operon; *pro*, proline biosynthesis locus; *CR*-, resistant to catabolite repression of the lactose operon by glucose; *pur E*, purine biosynthesis locus *E*; del, deletion.

Strain	Genetic characteristics	Source
O ₆₇	<i>i</i> ⁻ <i>o</i> ^c <i>z</i> ⁺ <i>y</i> ⁺	E. Steers
3000	<i>i</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ⁺	E. Steers
LA-12G	<i>i</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ⁺ <i>CR</i> ⁻	W. F. Loomis, jun.
3000-L8	<i>i</i> ⁺ <i>o</i> ⁺ <i>p</i> ⁺ <i>z</i> ⁺ <i>y</i> ⁺	J. R. Beckwith
EC6*	<i>lac</i> _{X74} <i>Sm</i> ^S /F <i>lac</i> <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺ - <i>pur E</i> -d 25 (F'- <i>ad</i> - <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺)(<i>pro</i> ⁻)/ ϕ 80 <i>lac</i> ⁺	J. R. Beckwith
EC9*	<i>lac</i> _{X74} <i>Sm</i> ^S /F <i>lac</i> <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺ - <i>pur E</i> -d 25 (F'- <i>ad</i> - <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺)(<i>pro</i> ⁻)/ ϕ 80 <i>lac</i> ⁺	J. R. Beckwith
EZ11*	<i>lac</i> _{X74} <i>Sm</i> ^S /F <i>lac</i> <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺ - <i>pur E</i> -d 25 (F'- <i>ad</i> - <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺)/ ϕ 80 <i>lac</i> <i>i</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ^{del} <i>ac</i> ^{del}	J. R. Beckwith
EZ15*	<i>lac</i> _{X74} <i>Sm</i> ^S /F <i>lac</i> <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺ - <i>pur E</i> -d 25 (F'- <i>ad</i> - <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺)/ ϕ 80 <i>lac</i> <i>i</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ⁺ <i>ac</i> ^{del}	J. R. Beckwith
EZ16*	Same as strain EZ15	
EZ16-3*	<i>lac</i> _{X74} <i>Sm</i> ^S /F <i>lac</i> <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺ - <i>pur E</i> -d 25 (F'- <i>ad</i> - <i>z</i> ⁻ <i>y</i> ⁺ <i>ac</i> ⁺)	Derived from strain EZ16 as <i>lac</i> ⁻ on eosin-methylene blue-lactose agar

*These strains all carry the *pur-lac* fusion (Jacob, Ullman & Monod, 1965).

measurement the cultures are separated from their common parent by many generations and are therefore not entirely comparable. The second method is to transfer cells from one medium to another, and to measure the differential rate of synthesis of enzymes both before and after the transfer. This method has the disadvantage that the change of medium frequently evokes a transient repression, and this may be sometimes quite prolonged.

RESULTS

Dilution model

According to this model (see the introduction) the differential rate of synthesis of β -galactosidase in a particular medium is governed by the rate of synthesis of all the other cellular proteins. If casein hydrolysate is added to cells synthesizing enzyme in minimal medium the rate of synthesis of ribosomal proteins will rise; but the difference will amount to only a few percent of the total protein of the cell. On the other hand the cells will be relieved of the necessity of making many enzymes previously essential for their growth. If the dilution model were correct, the net effect would probably be a small rise in the differential rate of synthesis of β -galactosidase. In fact, when we added casein hydrolysate to cells making β -galactosidase we observed a marked repression of enzyme synthesis, amounting to 20% for cells growing on glycerol and as much as 60% for those growing on glucose (Fig. 1), whereas the growth rate in both cultures increased by 46%. [To avoid complications

due to transient repression, we used for this experiment the constitutive strain O_{87}^+ , in which transient repression is abolished (Palmer & Moses, 1967).] The dilution model cannot account for these results.

A further test of the dilution hypothesis involved repression of the synthesis of β -galactosidase by starvation for P_1 . It is known that in wild-type strains severe repression follows the withdrawal of an essential nutrient other than carbon (McFall & Magasanik, 1962), but it has not been clear whether this repression is of the transient or of the general type. We therefore used, once again, strain O_{87}^+ , which lacks the transient response, for this experiment.

Strain O_{87}^+ was cultured in medium containing 2 mM- P_1 , harvested by filtration and resuspended in P_1 -free medium. Samples were taken for measurements of β -galactosidase and alkaline phosphatase activities, and growth was followed turbidimetrically. Fig. 2 shows that the differential rate of β -galactosidase synthesis began to fall about 55 min. after P_1 was removed. At the same time de-repression of alkaline phosphatase occurred, so that we can say that P_1 was then exhausted. After about 85 min. the differential rate of β -galactosidase synthesis fell to zero. To explain this cessation of β -galactosidase synthesis on the dilution model we would have to say that the rate of synthesis of other cellular proteins had increased infinitely.

Neither of these experiments provides any support for the dilution model. Instead, the results suggest that general repression of β -galactosidase synthesis is due to the formation of a repressing metabolite. The formation of this metabolite is apparently stimulated by amino acids, and the absence of P_1 presumably causes it to accumulate by preventing its further metabolism.

Specific repressor model

Repression behaviour of strain LA-12G. Cells of strain LA-12G and of the wild-type strain 3000 were grown for many generations in four different media and were then induced with isopropyl β -D-thiogalactoside. Table 2 shows that the synthesis of β -galactosidase in strain LA-12G is much more resistant than the wild-type to repression by glucose, but that the two strains are equally sensitive to repression by broth. The same conclusion follows from experiments in which broth, or broth plus glucose, was added to induced cells growing in glycerol-minimal medium. After the addition of broth alone the rate of synthesis of β -galactosidase in both strains returned at the end of the transient repression to about 55% of the original rate, but broth plus glucose repressed synthesis in strain 3000 by 80% and in strain

Fig. 1. Effect of casein hydrolysate on the synthesis of β -galactosidase by strain O_{87}^+ . Cells in glycerol- or glucose-minimal medium received Difco casein hydrolysate (casamino acids) (200 μ g./ml.) as indicated by the arrows. Doubling times before and after adding casein hydrolysate were: glycerol-minimal medium, 63 and 43 min.; glucose-minimal medium, 48 and 33 min. ○, Glycerol-minimal medium; ●, glucose-minimal medium.

Fig. 2. Effect of depletion of P_1 on growth and enzyme synthesis in strain O_{67} . Cells were grown in glycerol-minimal medium containing 2 mM- P_1 . They were later harvested and resuspended in P_1 -free medium. (a) Enzyme formation: $\blacksquare$, β -galactosidase synthesis before removal of P_1 ; $\circ$, β -galactosidase synthesis after removal of P_1 ; $\bullet$, alkaline phosphatase synthesis after removal of P_1 . (b) $\circ$, Growth after removal of P_1 ; the arrow indicates time at which alkaline phosphatase synthesis started and β -galactosidase synthesis began to slow down.

Table 2. Comparison of catabolite repression of β -galactosidase

Cells were grown for several generations in minimal medium supplemented with glycerol, glucose or glycerol plus glucose, and in nutrient broth. During exponential growth 0.5 mM-isopropyl β -D-thiogalactoside was added to each culture and the differential rate of β -galactosidase synthesis was measured during the next 60 min. Rates of enzyme synthesis reported in absolute terms and also as percentages of rate in glycerol-minimal medium.

Medium	Enzyme in strain LA-12G		Enzyme in strain 3000	
	(Units/mg. of bacterial protein)	(%)	(Units/mg. of bacterial protein)	(%)
Glycerol-minimal	28400	100	48400	100
Glucose-minimal	23600	83	15100	31
Glycerol + glucose-minimal	21800	77	10700	22
Nutrient broth	8270	29	15600	32

LA-12G by only 50%. Similarly, when glucose alone was added to induced cultures of strain LA-12G growing exponentially in glycerol medium the rate of synthesis of β -galactosidase after the

transient period was often (but not always) as high as that in glycerol (cf. Loomis & Magasanik, 1965).

Repression of enzyme synthesis by carbohydrates is very intense in non-growing cells (Mandelstam, 1961; Nakada & Magasanik, 1964). Loomis & Magasanik (1965) studied the effect of adding sources of carbon to cells of strain LA-12G that had been deprived of nitrogen and reported that glucose caused no repression of the synthesis of β -galactosidase (although considerable repression was observed when lactate was added). However, there is some doubt whether in that experiment the bacteria were in fact completely starved of nitrogen, since [14 C]leucine was present throughout the incubation and we have shown that total nitrogen starvation cannot be established under these conditions (V. Moses & M. D. Yudkin, unpublished work).

We therefore reinvestigated the response of non-growing bacteria to the addition of carbon sources (Fig. 3). When glycerol was added to nitrogen-starved bacteria repression of β -galactosidase synthesis was less severe in strain LA-12G than in the wild-type strain. However, the addition of glucose caused severe repression in both strains. We believe that nitrogen starvation was effective in this experiment, since the addition of ammonium

Fig. 3. Repression of β -galactosidase synthesis in cells starved of carbon and nitrogen. Cells of strains LA-12G (a) and 3000 (b) growing exponentially in glycerol-minimal medium were harvested by filtration on a membrane filter, washed and suspended in the same medium without $(\text{NH}_4)_2\text{SO}_4$. After incubation for 15 min. at 37° to ensure depletion of nitrogen, the cells were again harvested. They were washed and resuspended in medium devoid of both glycerol and nitrogen, and incubated for a further 30 min. at 37° to permit utilization of the remaining traces of carbohydrate in the medium. Isopropyl β -D-thiogalactoside (0.5 mM) was then added and samples were taken for measurements of enzyme activity. After 30 min. each culture was split into four equal portions and supplemented as follows: ●, no additions; ▲, 20 mM-glycerol; ■, 10 mM-glucose; ○, 1.5 mM- $(\text{NH}_4)_2\text{SO}_4$. Sampling was continued for a further 30 min.

sulphate without a carbon source stimulated enzyme synthesis in both strains (Fig. 3).

We may therefore conclude from all these experiments that under some conditions the possession of the CR^- genotype confers considerable resistance to general catabolite repression. However, this effect is very variable, and it is possible to stress the cells sufficiently so that general repression is observed in spite of the CR^- character.

Specificity of the CR locus for general catabolite repression of the lac operon. The experiments reported above suggested the possibility that the CR^- mutation, rather than altering the properties of a specific apo-repressor (Loomis & Magasanik, 1965), might affect instead the metabolism of carbohydrates. One would then expect that in strain LA-12G, where the synthesis of β -galactosidase is rather resistant to repression by glucose, other enzymes normally subject to catabolite repression might also show some resistance.

Exponential-phase cultures of strains LA-12G and 3000, growing on glucose- or glycerol-minimal medium, were simultaneously induced with isopropyl, β -D-thiogalactoside, L-tryptophan and

D-serine. Glycine was also added to prevent temporary inhibition of growth by D-serine (Davis & Maas, 1949). Samples were taken at intervals for measurement of the activities of β -galactosidase, L-tryptophanase and D-serine deaminase. Table 3 shows that in strain LA-12G all three enzymes failed to show general repression in the presence of glucose whereas in the wild-type strain all three were, to differing extents, sensitive. The CR locus is therefore not specific for the *lac* operon.

Effect on general repression of deletions at the ends of the lac operon. Although our experiments with strain LA-12G provide no support for the specific catabolite repressor model involving the CR locus, it is conceivable that another locus exists that does indeed code for such a specific apo-repressor. Unless catabolite repression acts at the level of translation, one would require for all such models an operator site at which the postulated repressor would exert its action and prevent transcription of the *lac* genes. By analogy with the *i*-gene repressor and its operator as proposed by Jacob & Monod (1961), one would expect the operator for the catabolite repressor to be located

Table 3. Glucose repression of three enzymes induced simultaneously

Cells of strains LA-12G and 3000, growing exponentially in glycerol- or glucose-minimal medium, received 0.5 mM-isopropyl β -D-thiogalactoside, 0.61 mM-L-tryptophan, 0.95 mM-D-serine and 1.33 mM-glycine simultaneously. Samples were taken during the next 2 hr. for measurements of the activities of β -galactosidase, L-tryptophanase and D-serine deaminase. Growth was followed turbidimetrically, and specific rates of enzyme synthesis are reported as enzyme units/mg. of bacterial protein.

Strain	Medium	β -Galactosidase	L-Tryptophanase	D-Serine deaminase
LA-12G	Glycerol-minimal	10040	5.6	39.4
	Glucose-minimal	10040	5.1	43.4
3000	Glycerol-minimal	15100	12.9	30.4
	Glucose-minimal	8270	0.62	23.4

Fig. 4. Diagram of relevant portions of deleted genomes used in the present study. (a) Deletions in the *lac* operons carried by ϕ 80 *lac* phages in strains EZ11, EZ15 and EZ16. These strains also carry the partially deleted F episome shown in (b). The information is taken from Fox *et al.* (1966). (b) *purE-lac* fusion strain. The parent from which this was derived is a partial diploid as shown in the upper part of this section. The lower part shows the genetic structure of strain EZ16-3 in which the chromosomal structure is unaltered, but part of the episome is deleted. The diagram is redrawn from Jacob *et al.* (1965). Abbreviations for genetic markers: *purE*, purine biosynthesis locus *E*; α and β , two cistrons at *purE* locus; *T6s*, receptor site for bacteriophage T6; *Ph*, alkaline phosphatase; *Pro*₂, one of the proline biosynthetic loci; *lac*, lactose operon; *i*, lactose regulator gene; *o*, lactose operator gene; *z*, β -galactosidase structural gene; *y*, galactoside permease structural gene; *ac*, thiogalactoside transacetylase structural gene. Deletions are indicated by broken arrows. The jagged line in the deleted episome designates the presumed site of fusion.

Fig. 5. Glucose repression of β -galactosidase synthesis in strain EZ15. Cells growing exponentially in glycerol-minimal medium $\pm$ adenine (100 μ g./ml.) were induced with 0.5 mM-isopropyl β -D-thiogalactoside; 10 mM-glucose was added at the arrows. Generation times: before glucose, 71 min.; after glucose, 52 min. O, No adenine; ●, with adenine.

at one end or the other of the lactose operon. We therefore investigated the phenomenon of general repression in a number of mutant strains bearing deletions extending from within the *lac* operon to points beyond its limits.

A series of mutants has been described by Fox,

Beckwith, Epstein & Signer (1966) in which partially deleted portions of *lac* attached to the phage ϕ 80 have been inserted into a strain of *E. coli* (Jacob *et al.* 1965) the chromosome of which is deleted for *lac*, while an F *lac* episome carries a deletion extending from within the *z* gene of *lac* to the β -cistron of the *purE* locus, and including all of the *i*, *o* and *p* sites. In this strain the F *lac* episomal genes for galactoside permease (*y*) and thiogalactoside transacetylase (*ac*) are under the control of the *purE* locus and are repressed by the

Fig. 6. Catabolite repression of acetylase synthesis in strain EZ16-3. Cells growing exponentially in minimal medium containing adenine (100 μg./ml.) with either glycerol or glucose as source of carbon were filtered, washed and resuspended in the same medium lacking adenine. Samples were taken and assayed for thiogalactoside transacetylase. ○, Glycerol-minimal medium; ●, glucose-minimal medium.

presence of adenine in the medium. The *lac* operon attached to $\phi 80$ is complete at the proximal end: thus the *i*, *o* and *p* genes are all present, and the expression of the *z* gene resulting in the synthesis of β -galactosidase is under the normal control mechanism requiring the presence of an inducer such as isopropyl β -D-thiogalactoside. However, in the strains EZ15 and EZ16 a deletion of the $\phi 80$ *lac* operon extends from within *ac* to beyond the distal end of the operon, whereas in strain EZ11 a deletion extends from within *y* to beyond the distal end of the operon, and thus *ac* is deleted as well (see Fig. 4).

Experiments in which glucose was added to induced cells of strains EZ11, EZ15 and EZ16 growing in glycerol-minimal medium (Fig. 5) showed that both transient and general catabolite repression of β -galactosidase in all three of these strains are identical with the wild-type response. The presence of adenine in the medium does not affect the response. These results show that there can be no operator locus at the distal end of the lactose operon that could be the site of action of a specific catabolite apo-repressor.

By selecting a *lac*⁻ segregant from strain EZ16 on eosin-methylene blue-lactose agar, we obtained a strain (EZ16-3) that had lost $\phi 80$ *lac* and retained only the *y* and *ac* genes of *lac* linked to *purE* on the F *lac* episome. Strain EZ16-3 formed no β -galactosidase when grown in the presence of isopropyl β -D-thiogalactoside and was therefore *lac*⁻ not by virtue of a mutation to *y*⁻ but because of the loss of $\phi 80$ *lac*. Parallel cultures of this strain were grown in glycerol- and glucose-minimal medium contain-

ing adenine (100 μg./ml.). The synthesis of thiogalactoside transacetylase was initiated by the removal of adenine and the differential rates of enzyme synthesis were measured during the subsequent growth. Fig. 6 shows that general catabolite repression continued to operate in these cells deleted for the proximal part of *lac*.

These experiments with deletion mutants have failed to locate any operator for general repression at either end of the *lac* operon. If a specific catabolite apo-repressor does exist, it cannot act at the level of the DNA. It should be noted, however, that the existence of general repression when the expression of *ac* depends on the de-repression of *purE*, as in strain EZ16-3, leads to the possibility of a model regulated at the translation stage of protein synthesis, and this is discussed below.

Effects of displacing the *lac* operon. Several strains have been constructed by Beckwith, Signer & Epstein (1966) in which *lac* attached to $\phi 80$ has been inserted at various places on the bacterial chromosome in either a forward or a reverse orientation. We examined the transient and general catabolite resistance in two such strains, EC6 and EC9, and found them similar to wild-type.

Effect of mutation at the promoter locus. Strain 3000-L8 carries a lesion mapping in the promoter region (Scaife & Beckwith, 1966; Ippen, Miller, Scaife & Beckwith, 1968), resulting in a low rate of fully induced enzyme synthesis but no apparent change in the requirement for an inducer to obtain enzyme formation. Catabolite repression responses of this strain are of the wild-type.

DISCUSSION

Investigations of the models proposed by Loomis & Magasanik (1965, 1967) and by Palmer & Moses (1967) have shown them both to be inadequate to account for general catabolite repression. The non-specific dilution model (Palmer & Moses, 1967) supposes that very considerable differences in the quantitative protein profile of the cell would result from growth in different media. That some changes do occur is beyond question: the proportion of the total cellular protein found in the ribosomes is known to increase with increasing growth rate (for review see Maaløe & Kjeldgaard, 1966). The model envisages that in media causing catabolite repression a greater degree of de-repression would prevail for many proteins concerned with growth processes, and that these would compete among themselves, and with other proteins that might be irrelevant in the particular growth conditions, for biosynthetic machinery. We attempted to test this postulate by repressing specifically some of the biosynthetic enzymes, and by setting up conditions which in wild-type cells would result in total cessation of

β -galactosidase synthesis by extreme catabolite repression. In both cases (Figs. 1 and 2) the results were not consistent with the predictions of the model, but favoured the concept of a specific repression mechanism, even for the O_{67}^c system in which both known regulator genes are non-functional.

The specific catabolite repressor model arose from results obtained with strain LA-12G, which was isolated by Loomis & Magasanik (1965) on the basis of increased resistance to glucose repression of β -galactosidase synthesis. On re-examination of the properties of this strain we found that it does not show widespread resistance to all forms of catabolite repression, nor is resistance restricted to *lac*. Enhanced resistance is most evident with glucose as the repressing substance, though even here it is often incomplete. In their original isolation of the CR^- mutant, Loomis & Magasanik (1965) first isolated a strain (LA-12) that showed enhanced resistance to catabolite repression but grew poorly on glucose. From strain LA-12 was subsequently isolated strain LA-12G, which retains the CR^- character but grows well on glucose. Strain LA-12G might then be a double mutant, and CR^- might be a mutation that leads to an impaired formation of repressing catabolites, particularly from glucose.

We also considered the possible locations of an operator region, which, at our present stage of understanding of repressor action, is required for any model involving a catabolite apo-repressor. By using mutant strains carrying deletions at either end of *lac* we sought such an operator but were unable to find any evidence for it. Unless we are prepared to jettison the concept of a polycistronic messenger RNA for the three enzymes of the lactose system, and postulate instead separately regulated transcription for each cistron, we are now left without a site at which transcription may be regulated by catabolite repression. Since general catabolite repression undoubtedly occurs in all of these deletion mutants we must consider the possibility that it acts at the level of translation.

This idea is supported by the results of our work with the mutant EZ16-3, in which a deletion stretching from the *purE* locus to the *lac* locus has brought the expression of the permease and transacetylase genes under the control of adenine (cf. Jacob *et al.* 1965). When this organism was induced to make transacetylase by depriving it of adenine, we found that the rate of enzyme synthesis when glucose was the source of carbon was only 40% of that when glycerol was the source of carbon. We presume that in this strain a hybrid polycistronic message is made corresponding to part of the *purE* and part of the *lac* loci. The fact that the synthesis of transacetylase is nevertheless repressed by growth on glucose implies either that catabolite

repression can work at the level of translation, or that transcription of the fused *pur-lac* region is subject to catabolite repression. This latter suggestion in turn implies that the *purE* region can normally itself suffer catabolite repression. We have not yet succeeded in excluding this idea experimentally, but we know of no evidence that biosynthetic sequences such as this are ever subject to catabolite repression (Maas & McFall, 1964).

It therefore seems to us likely that catabolite repression, at least of the synthesis of thiogalactoside transacetylase, can occur at the stage when messenger RNA is translated into protein.

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