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Catabolite Repression of the *lac* Operon

REPRESSION OF TRANSLATION

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1. Experiments were devised to show whether catabolite repression of β-galactosidase synthesis operates at the level of transcription or of translation. *Escherichia coli* K12 was induced for a short period in non-repressing medium (glycerol–minimal medium), and transcription of the *lac* operon was terminated by either of two methods; glucose was then added as a source of the catabolite repressor during the subsequent translation of the accumulated β-galactosidase messenger RNA. 2. When induced bacteria in glycerol medium were infected with T6 phage, which is known to halt transcription, the addition of glucose up to 3min. later diminished the yield of β-galactosidase. 3. When induced bacteria in glycerol medium were removed from the inducer and resuspended in fresh medium (a process that is also known to halt transcription), the yield of enzyme was again diminished by the presence of glucose in the resuspension medium. 4. It is concluded that repression of β-galactosidase synthesis can be brought about by the presence of glucose during the translation phase only. 5. In *E. coli* strain 300U the effect on translation was sufficient to account for almost all the catabolite repression of β-galactosidase synthesis observed during exponential growth of the organism in glucose–minimal medium. In *E. coli* strain 200P, however, much more severe repression occurred during exponential growth, and an additional effect of glucose is postulated.

The rate of synthesis of β-galactosidase by fully induced cultures of *Escherichia coli* depends to a large extent on the medium in which the cells are growing. In glucose–salts medium, for example, the differential rate of synthesis of the enzyme is much lower than in glycerol–salts medium. This repression of β-galactosidase synthesis by glucose is an example of catabolite repression (Magasanik, 1961). It is presumed that the catabolism of glucose gives rise to some substance that interferes with the synthesis of β-galactosidase, and that, in cells growing on glycerol, the concentration of this catabolic product is substantially lower.

Although the identity of the postulated catabolite repressor is not known, we can nevertheless ask 'at which point in the transfer of information from DNA to protein does the repressor act? In particular, does it act by diminishing the rate at which messenger RNA is synthesized on DNA (transcription), or by diminishing the rate at which protein is synthesized on messenger RNA (translation)?'.

The present evidence is conflicting. Nakada & Magasanik (1964) devised experiments in which transcription and translation of β-galactosidase were separated in time, and reported that catabolite repression interfered with transcription. On the other hand, Moses & Yudkin (1968) showed that catabolite repression persisted in a mutant strain of *E. coli* that carried a deletion of all of the genetic elements known to control transcription (the regulator, promoter and operator regions).

One possible reason for this apparent conflict is that Nakada & Magasanik (1964) might have been studying a phenomenon different from the catabolite repression that is defined above. In the experiments of Nakada & Magasanik (1964), bacteria were incubated in conditions that precluded growth. In these circumstances a very severe repression occurs that is probably related to the acute transient repression observed when bacteria are transferred from poor to rich media. Transient repression is known to operate at the level of transcription (Tyler, Loomis & Magasanik, 1967; Palmer & Moses, 1968), but we now believe that it is different from the permanent catabolite repression that is observed during exponential growth in glucose–minimal medium.

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We have therefore sought to answer the question 'does catabolite repression act during the stage of transcription or during the stage of translation?' by studying bacteria that are induced during exponential growth. In principle this question could be answered by separating the two stages and ensuring that the substance giving rise to catabolite repression (glucose) is present during one of them only. We used two different methods for separating translation from transcription: the first was to add T6 phage to induced cultures (Kaempfer & Magasanik, 1967), and the second was to remove the inducer by filtration (Nakada & Magasanik, 1964). From a careful study of these procedures, Kaempfer & Magasanik (1967) concluded that both of them prevent the further initiation of synthesis of *lac* messenger RNA; thereafter the bacteria complete the synthesis of the messenger RNA that has been started, and translate the accumulated messenger RNA until it has all decayed.

MATERIALS AND METHODS

Organisms. Three strains of *E. coli* K12 were used: 3000, a wild-type strain, originally from Dr W. Hayes; 300U, deficient in galactoside permease, originally from Dr J. Monod; and 200P, deficient in galactoside permease, kindly supplied by Dr B. Magasanik. They were maintained on salts medium (Davis & Mingioli, 1950), supplemented with 0·5% of glycerol and 1mg. of thiamine/l.; for strain 200P, L-threonine and L-leucine (50mg. of each/l.) were also added. Both these media are referred to as 'minimal medium'.

Experiments with phage. Strain 3000 was grown exponentially in glycerol–minimal medium at 30°. When the culture reached about 35μg. of bacterial protein/ml., isopropyl β-D-thiogalactoside (final concn. 0·5mM) and L-tryptophan (10μg./ml.) were added. After 3min., T6 phage was added to give about 20 infective particles/cell. (The phage, kindly supplied by Dr M. R. Lunt, was grown in liquid cultures of *E. coli* BB, and its infective titre was measured by plating it on strain 3000.) The infected culture was divided, and glucose (final concn. 0·2%) was added to some portions at various times after infection.

Filtration experiments. Strains 300U and 200P were grown overnight at 37° in glycerol–minimal medium or in a similar medium in which glucose (0·5%) replaced the glycerol. The cultures were diluted with fresh medium and allowed at least to double at 37°. They were then induced by the addition of isopropyl β-D-thiogalactoside (final concn. 1mM). After a short time, the bacteria (about 30ml. of culture containing about 70μg. of protein/ml.) were collected on a Millipore filter (0·65μm. pore size), washed with 4×2ml. of salts medium at room temperature and resuspended in fresh warm glycerol- or glucose–minimal medium. (This whole procedure took about 1min.) E_{600} was measured to determine the recovery of bacteria from the filter. Nakada & Magasanik (1964) showed that filtration and washing (especially with strains lacking galactoside permease) lowers the concentration of inducer to a value at which no further induction can occur.

In experiments in which bacteria from a single culture were resuspended after filtration in two different media, the filter was cut in half at the end of the washing procedure; this method ensured that the bacteria from each half-filter had been treated identically up to the moment of resuspension.

In some experiments bacteria were transferred from glycerol–minimal medium to glucose–minimal medium, or vice versa, before induction. All such transfers were accomplished by filtering, washing and resuspending the bacteria, as described above.

Long-term experiments. The rate of synthesis of β-galactosidase by bacteria in glycerol- or glucose–minimal medium was determined in cultures that had been maintained in the appropriate medium for at least ten generations before induction with isopropyl β-D-thiogalactoside.

Sampling and assay of β-galactosidase. This was as described by Moses & Prevost (1966).

Bacterial protein. This was determined by measuring E_{600} and relating the extinction to a standard curve prepared for bacterial protein by the method of Lowry, Rosebrough, Farr & Randall (1951).

RESULTS

When T6 phage was added to *E. coli* strain 3000 induced to synthesize β-galactosidase in glycerol medium, the synthesis of enzyme stopped after about 15min. at 30° (Fig. 1). By plotting on a

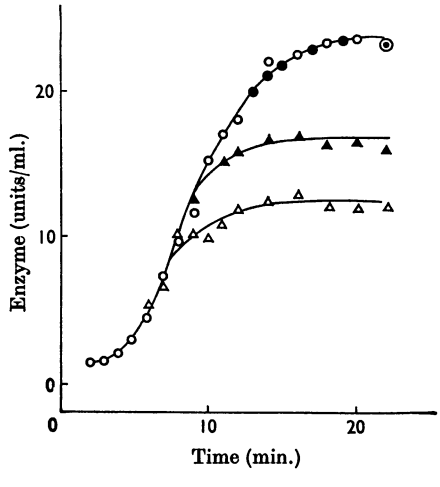

Fig. 1. Effect of glucose on β-galactosidase synthesis in T6-phage-infected cells. *E. coli* strain 3000 was grown exponentially in glycerol–minimal medium at 30° to a bacterial density of about 35μg. of protein/ml. At zero time isopropyl β-D-thiogalactoside (final concn. 0·5mM), and L-tryptophan (10μg./ml.) were added. At 3min., T6 phage was added (about 20 infective particles/cell). The culture was divided. One portion was left unsupplemented (○). The others received glucose (final concn. 0·2%) 1min. after the addition of the phage (△), 3min. after the addition of the phage (▲), or 5min. after the addition of the phage (●).

logarithmic scale the remaining capacity at any time of the T6-phage-infected cells to synthesize enzyme (cf. Kaempfer & Magasanik, 1967), we could determine the time at which the enzyme-synthesizing capacity began to decline exponentially. This corresponds to the time at which transcription of the messenger RNA for β-galactosidase is completed; in our experiments it was 3min. after the addition of the phage.

When glucose was added to the culture 3min. after the phage, the final yield of enzyme was diminished by about 30% (Fig. 1); since transcription is believed to be completed by this time, the effect of glucose here is presumably due to the occurrence of repression during translation. The addition of glucose 1min. after the phage diminished the final yield of enzyme by about 50%, whereas addition of glucose 5min. after the phage had no effect on the final yield.

Filtration experiments with strain 300U. In preliminary experiments we exposed bacteria to inducer for brief periods during growth on either glycerol medium or glucose medium, filtered them and resuspended them in glycerol medium without inducer. We generally found that the final yield of enzyme was not affected by the nature of the carbon source during induction, but the results were not sufficiently reproducible for unequivocal conclusions to be drawn (see the Discussion section). We therefore studied the effect of addition of glucose during the second incubation.

When bacteria were induced in glycerol medium for 2min. at 37°, filtered and resuspended in either glycerol medium or glucose medium, we found that the yield of enzyme in glucose was about 40% less than that in glycerol (Fig. 2). A similar repression by glucose was observed in bacteria that had been exposed to inducer for only 15sec. before resuspension. At first sight these results suggest that catabolite repression acts during the translation of messenger RNA. However, since the exposure to inducer initiates the synthesis of messenger RNA that is completed after filtration and resuspension, glucose could still be exerting a repression during the transcription phase.

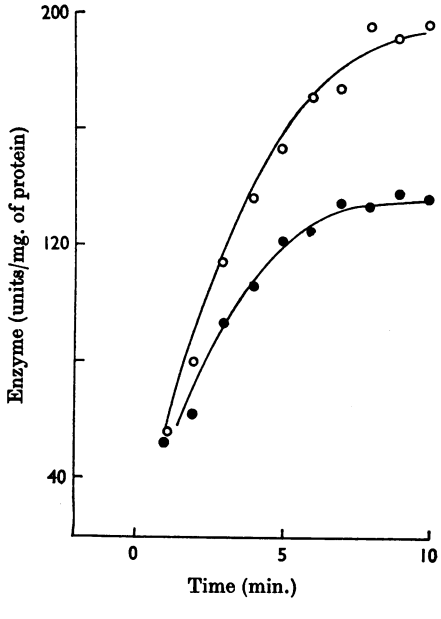

Fig. 2. Effect of carbon source on the translation of β-galactosidase messenger RNA. *E. coli* strain 300U was grown at 37° in glycerol–minimal medium. At a bacterial density of 70μg. of protein/ml., isopropyl β-D-thiogalactoside was added (final concn. 1mM). After 2min. the bacteria from 30ml. of culture were collected on a filter and washed with salts medium. The filter was cut in half and the bacteria were resuspended at zero time in glycerol–minimal medium (○) or glucose–minimal medium (●). The density of each culture was measured, and samples were taken for the determination of β-galactosidase.

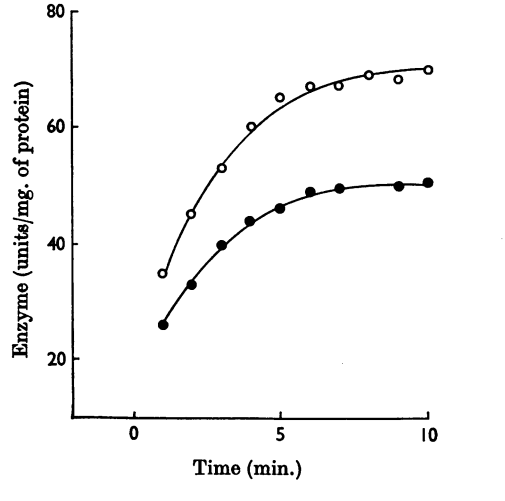

Fig. 3. Effect of carbon source on the translation of β-galactosidase messenger RNA. *E. coli* strain 300U was grown at 37° in glycerol–minimal medium. At a bacterial density of 75μg. of protein/ml., isopropyl β-D-thiogalactoside was added (final concn. 1mM). After 15sec. the bacteria from 28ml. of culture were collected on a filter, washed with salts medium and resuspended in glycerol–minimal medium at 37°. After a further 90sec., the bacteria were again collected on a filter and washed with salts medium. The filter was cut in half and the bacteria were resuspended at zero time in glycerol–minimal medium (○) or glucose–minimal medium (●). The density of each culture was measured, and samples were taken for the determination of β-galactosidase.

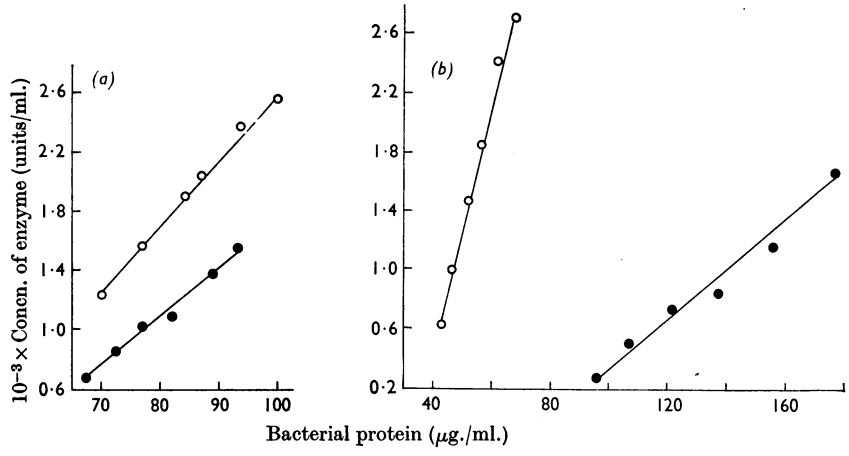

Fig. 4. Catabolite repression in exponentially growing cultures. Isopropyl β-D-thiogalactoside (final concn. 1 mM) was added to bacteria that had been growing for more than ten generations at 37° in glycerol–minimal medium (○) or glucose–minimal medium (●). Samples were taken for the measurement of bacterial density and for the determination of β-galactosidase. (*a*) *E. coli* strain 300U; (*b*) *E. coli* strain 200P. In (*b*), the severe repression caused by glucose made it inconvenient to work with glycerol- and glucose-grown cultures at the same density, but it has repeatedly been found that the differential rate of enzyme synthesis is constant during exponential growth.

We therefore added inducer to bacteria growing in glycerol medium, separated the bacteria after 15sec. and washed them, incubated them in glycerol medium without inducer for a further 90sec., filtered once more, and finally resuspended half the culture in glycerol medium and half in glucose medium. The resuspension with glycerol or glucose occurred at least 3min. after the removal of inducer, so that we could assume that transcription was completed by this time. Nonetheless we found once more that glucose repressed the synthesis of enzyme by 35–40% (Fig. 3). To show that the low yield of enzyme from the bacteria resuspended in glucose medium was not due to their not being adapted to growth on glucose, we repeated these experiments with bacteria that had been pre-grown on glucose and then transferred to glycerol medium only one generation before the induction began. The results were identical.

Comparison of strain 300U with strain 200P. The above results suggest that glucose, when added to cells of strain 300U while the translation of β-galactosidase messenger RNA is proceeding, diminishes the final yield of enzyme by 35–40%. In experiments that were designed to show how much repression glucose caused in the long term, we compared cultures of this strain growing exponentially in glycerol medium and in glucose medium; we found that the rate of synthesis of β-galactosidase in glucose was about 45% less than that in glycerol (Fig. 4*a*). We may therefore conclude that most of the catabolite repression that strain 300U suffers when growing exponentially in glucose can be accounted for by an effect on translation.

Many strains of *E. coli*, however, suffer much more repression than this. For example, in strain 200P the rate of synthesis of β-galactosidase is 80–90% less during growth in glucose medium than during growth in glycerol medium (Fig. 4*b*). But when we performed with strain 200P filtration experiments similar to those that we had done with strain 300U, we found that the results were exactly similar to those shown in Fig. 3; once again the presence of glucose during the translation phase repressed the synthesis of β-galactosidase by only 35–40%.

DISCUSSION

We have used two methods to isolate the translation phase of β-galactosidase synthesis. The addition of T6 phage or the removal of inducer is thought to halt initiation of transcription of the *lac* operon (Kaempfer & Magasanik, 1967), and within 2–3min. existing transcription is completed. Thereafter we may study the translation of β-galactosidase messenger RNA unaccompanied by further transcription.

At no time, by contrast, is it possible to study transcription unaccompanied by translation, since there is reason to believe that transcription is normally coupled to translation (Stent, 1964; Das,

Goldstein & Lowney, 1967; Mehdi & Yudkin, 1967). We had nonetheless hoped to isolate a phase of induction during which transcription predominated and to study the effect of different carbon sources added during this phase. However, we were unable to get reproducible results from experiments in which we filtered one lot of cells from glucose medium and another lot from glycerol medium, and resuspended each lot in glycerol medium. First, we found that cells taken from glucose did not invariably grow on glycerol without a lag, even when they had been pre-grown in glycerol medium only one generation before. Secondly, we did not succeed in standardizing the filtration procedure, and it therefore proved impossible to compare two lots of cells that had been separately filtered and resuspended.

Neither of these difficulties obtained when we induced cells in glycerol medium, filtered them and resuspended them in either glycerol or glucose medium. In every case the cultures grew without lag; and we were able to compare directly the two resuspended cultures, since the procedure involved filtering the bacteria from glycerol medium and washing on a single filter, which was then cut in half.

The results of the filtration experiments and the experiments with T6 phage all show that glucose, when present during the translation phase of β-galactosidase synthesis, diminishes the final yield of enzyme. We must now discuss whether this effect is really a manifestation of the catabolite repression that is observed during exponential growth in glucose medium. It has been suggested (Palmer & Moses, 1968) that the diminution by glucose of the rate of β-galactosidase synthesis is a reflection only of the cells' enhanced rate of growth on glucose, i.e. that the 'step-up' caused by a change of carbon source diverts the protein-synthesizing capacity of the organism towards the production of ribosomes etc., and away from the production of β-galactosidase. The present results, however, support our previous conclusion (Moses & Yudkin, 1968) that catabolite repression cannot be explained in this way. Fig. 1 shows that glucose represses β-galactosidase synthesis in cells that have been infected with virulent phage, and it seems plain that a 'step-up' to a higher growth rate would not occur under these conditions. Nor can the effect of glucose be regarded as an example of the 'transient repression' that is observed when induced cultures of *E. coli*, growing exponentially in glycerol medium, are transferred to glucose medium, since that repression operates at the level of transcription (Tyler *et al.* 1967; Palmer & Moses, 1968). We therefore consider that the repression by glucose observed in the present experiments is true catabolite repression.

Nakada & Magasanik (1964) concluded, on the basis of filtration experiments with strain 200P, that catabolite repression acts by inhibiting transcription rather than translation. However, in those experiments the cells were harvested from a complete medium, and then incubated with inducer in the presence of a carbon source but starved of an essential amino acid. These conditions are exactly those that would be expected to produce a severe transient repression, from which the organism, deprived of an amino acid, would be unable to escape. Thus the experiments of Nakada & Magasanik (1964) further support the view that transient repression acts by preventing transcription, but they may not be relevant to the mechanism of permanent catabolite repression.

In strain 300U, nearly all of the (comparatively mild) repression that occurs during exponential growth in glucose medium can be accounted for by a repression of translation (see Figs. 2, 3 and 4). In strain 200P, as in strain 300U, the presence of glucose during translation repressed enzyme synthesis by about 40%, but in exponential cultures of strain 200P in glucose medium synthesis was repressed by at least 80%. Thus strain 200P appears to suffer an additional repression during exponential growth in glucose medium. The fact that the addition of glucose during the translation phase does not cause the organism to manifest its full repression might suggest that this additional effect operates, like transient repression, during the transcription phase. Alternatively, the additional effect might depend on the organisms' attaining a higher content of catabolites, and perhaps this content is reached only after several minutes' growth in glucose medium.

In any case these experiments show that catabolite repression acts, at least in part, on the translation of β-galactosidase messenger RNA. This conclusion allows us to suggest that the genetic determinant(s) of sensitivity to catabolite repression may lie within the structural genes of the *lac* operon, rather than in the controlling elements *i*, *p* and *o* or in some unlinked gene.

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