Catabolite Repression of the *lac* Operon

THE CONTRIBUTION OF TRANSCRIPTIONAL REPRESSION

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1. Experiments were carried out to distinguish the contributions of transcriptional and translational repression to catabolite repression of the *lac* operon. 2. In strain EZ16-3-G of *Escherichia coli* the synthesis of thiogalactoside transacetylase is directed by a gene situated on an episome, and the operator, promoter and regulator genes that lay *cis* to this gene have been deleted, so that the normal mechanism for controlling transcription is abolished. The extent of catabolite repression in this strain was much less than that in wild-type strains. 3. The same episome is responsible for the synthesis of thiogalactoside transacetylase in strain RM32/F'd25, and in this strain a second *lac* operon directs the synthesis of β -galactosidase under the control of a wild-type operator-promotor-regulator system. The extent of catabolite repression of thiogalactoside transacetylase in strain RM32/F'd25 was substantially more than in strain EZ16-3-G, but less than that of β -galactosidase in strain RM32/F'd25. 4. Since the synthesis of thiogalactoside transacetylase in these organisms is presumably subject to translational repression only, it is concluded that in strain RM32/F'd25 the synthesis of β -galactosidase is subject to both transcriptional and translational repression. It is also concluded that the extent of translational repression varies between strains.

The genetic symbols used in this paper are as follows: *lac*⁺(-), ability (inability) to ferment lactose; *z*, structural gene for β -galactosidase; *y*, structural gene for galactoside permease; *a*, structural gene for thiogalactoside transacetylase; *i*, *lac* regulator gene; *o*, *lac* operator gene; *p*, *lac* promoter region; *pur* *E*, purine biosynthesis locus *E*.

The *lac* operon of *Escherichia coli* (Fig. 1) contains three structural genes, termed *z*, *y* and *a*, which specify the amino acid sequences of the proteins β -galactosidase, galactoside permease and thiogalactoside transacetylase respectively. In wild-type strains a control system [consisting of the regulator (*i*) and operator (*o*) genes] ensures that these proteins are synthesized in substantial quantity only in the presence of a galactoside inducer (Jacob & Monod, 1961, 1965). There is abundant evidence to support the following account of the control of synthesis of these proteins (for a review see Epstein & Beckwith, 1968). The synthesis of an m-RNA* molecule corresponding to the whole operon is initiated at the promoter region (*p*). In the absence of inducer this synthesis is blocked by the attachment to the operator gene of a repressor protein synthesized under the direction of the regulator gene. Inducers are capable of interacting

with the repressor and thus of preventing its attachment to the operator, so that in the presence of an inducer the synthesis of m-RNA proceeds unimpeded. Ribosomes begin to translate at the operator end of the β -galactosidase m-RNA; but many more ribosomes complete the translation of the β -galactosidase m-RNA than of the m-RNA corresponding to the remaining genes.

The rate of synthesis of the *lac* proteins, however, depends not only on the concentration of inducer but also on the medium in which the bacteria are growing. For example, in glucose-minimal medium the differential rate of enzyme synthesis is often much less than half of that in glycerol-minimal medium. This effect of glucose is an example of catabolite repression (Magasanik, 1961).

Catabolite repression occurs in constitutive strains of *E. coli* (Mandelstam, 1962; Loomis & Magasanik, 1964). Silverstone, Magasanik, Reznikoff, Miller & Beckwith (1969) reported that a deletion that covered part of the *p* region diminished (or in some strains abolished) the sensitivity of β -galactosidase synthesis to catabolite repression; it appears to follow from these results that catabolite repression, even if it does not involve the *i* gene, operates at the level of transcription.

By contrast, Moses & Yudkin (1968) found that

* Abbreviation: m-RNA, messenger RNA.

Fig. 1. The *lac* operon of *E. coli*.

catabolite repression persists in a strain in which the whole of the *o*, *p* and *i* regions have been deleted, and they tentatively concluded that repression might operate by blocking the translation of *lac* m-RNA rather than its synthesis. This conclusion has gained support from the demonstration that the yield of β -galactosidase is diminished by adding glucose to cultures of bacteria that can be assumed to have already completed the synthesis of β -galactosidase m-RNA in glycerol medium (Yudkin & Moses, 1969); the effect of glucose in such experiments is most easily explained by assuming that it represses the translation of accumulated β -galactosidase m-RNA. Yudkin & Moses (1969) found, however, that the extent of translational repression (as thus defined) varied between different strains of *E. coli*, and in some strains there appeared to be a contribution from transcriptional control to the total repression observed. It seems probable that these variations can be ascribed to minor metabolic differences between strains that result in different concentrations of the relevant catabolites.

It is possible to account for the results both of Silverstone *et al.* (1969) and of Yudkin & Moses (1969) by assuming that both transcriptional and translational repression vary in extent among different strains. The effect of this assumption can be illustrated by considering a *lac* operon carrying a deletion that covers the *o*, *p* and *i* regions. This deletion would abolish transcriptional control, and, if the operon happened to be in a strain that also suffered only slight translational repression, little catabolite repression of any kind would be observed. But the same operon would suffer substantial catabolite repression when transferred to a strain in which translational repression was comparatively severe.

Such an operon is carried on the episome F'd25 in strain EZ16-3-G, the chromosomal *lac* operon of which is deleted. This episome contains an intact *a* gene, so that the extent of catabolite repression in strain EZ16-3-G can be determined by studying the synthesis of thiogalactoside transacetylase. It is also possible to transfer the episome to another strain that possesses a chromosomal *lac* operon, and thus to construct a partially diploid strain with two

lac operons. By transferring the episome to strain RM32 (which synthesizes β -galactosidase but no thiogalactoside transacetylase), one can construct a partial diploid, strain RM32/F'd25, in which β -galactosidase synthesis is under the control of an intact *o-p-i* system but synthesis of thiogalactoside transacetylase is not. If catabolite repression is dependent in part on the integrity of the *o-p-i* system, one would expect that in strain RM32/F'd25 the synthesis of β -galactosidase would be more severely repressed than that of thiogalactoside transacetylase. [For a fuller discussion of the use of such partial diploids see Yudkin (1969).]

The present results suggest that control of transcription does contribute to catabolite repression, and that the extent of translational repression varies substantially between strains.

MATERIALS AND METHODS

Media. Minimal medium (Davis & Mingioli, 1950) was supplemented with thiamin (1mg./l.). The required carbon source (glycerol, glucose or lactose) was added to a final concn. of 1% (w/v). Solid media contained 1.5% (w/v) of agar. When streptomycin was used as a selective agent, 100mg. of its sulphate was added/l. Adenine, when used, was added to give 100mg./l.

Organisms. (a) Strain EZ16-3-G is a variety of strain EZ16-3 (Moses & Yudkin, 1968) that has been maintained since its original isolation on glycerol-minimal medium. It is incapable of growth on lactose and is sensitive to streptomycin. It contains an F' episome with the *d25* deletion (see Fig. 1) described by Jacob, Ullman & Monod (1965), which abolishes the whole of the *o*, *p* and *i* regions together with part of the *z* gene, and brings the synthesis of thiogalactoside transacetylase under the control of the nearby *pur E* locus. Synthesis of the enzyme is repressed by adenine, but induced in the absence of purines.

(b) Strain RM32 grows on lactose as sole source of carbon and is resistant to streptomycin. It has the genetic constitution F'*lac*^{del}/φ80*dlac* *i*⁺*p*⁺*o*⁺*z*⁺*y*⁺*a*^{del}. Thus it synthesizes no thiogalactoside transacetylase; but its transcriptional control for the *lac* operon is wild-type and it synthesizes β -galactosidase in response to galactoside inducers.

(c) Strain RM32/F'd25, which has the genetic constitution *lac*^{del}/φ80*dlac* *i*⁺*p*⁺*o*⁺*z*⁺*y*⁺*a*^{del}/F'*lac* *z*⁻*y*⁺*a*⁺-*pur E*-*d25*, was constructed by F'-duction. Young exponential cultures of strains EZ16-3-G and RM32 in glycerol-minimal medium were mixed in the ratio 5:1 and shaken at 37° for 4 hr. The mating mixture was diluted and plated on lactose-minimal

medium-streptomycin-agar. After about 28 hr. at 37°, several colonies were picked and grown overnight in lactose-minimal medium. Portions of each culture were diluted into glycerol-minimal medium, and after 3 hr. growth at 37° the cultures were tested for the presence of thiogalactoside transacetylase. The overnight clone corresponding to one culture that produced the enzyme was plated again on

which was then reweighed before the addition of the assay mixture. The final value for the enzyme present was corrected accordingly.

One unit of enzyme activity is that which liberates, under the conditions of the assay, 1 nmole of CoA/min.

Expression of results. The 'percentage repression' by glucose is defined as:

$$100 \left(1 - \frac{\text{differential rate of enzyme synthesis in glucose-minimal medium}}{\text{differential rate of enzyme synthesis in glycerol-minimal medium}} \right)$$

lactose-minimal medium-streptomycin-agar, and a single colony was picked. This organism was named RM32/F'd25, and it had the properties expected from its presumed diploid character: it produced β -galactosidase in response to isopropyl β -D-thiogalactoside, but the rate of synthesis of thiogalactoside transacetylase was unaffected by this inducer; instead the enzyme was synthesized in minimal media but repressed by adenine. Strain RM32 was recovered by treatment of the diploid strain with Acridine Orange (Hirota, 1960).

Growth, induction and sampling. The bacteria were grown at 37° from a small inoculum overnight in glycerol- or glucose-minimal medium with adenine. In the morning the cultures were diluted with fresh warm medium, containing adenine and the same carbon source, to a density of about 30 μ g. of protein/ml. They were allowed at least to double, and the bacteria were then collected on a Millipore filter (0.8 μ m. pore size), washed with warm minimal medium and resuspended at about 60–80 μ g. of protein/ml. in warm minimal medium without adenine but containing the same carbon source as that in which they had been grown. E_{600} was followed for about 20 min. to ensure that the bacteria were still growing exponentially. Isopropyl β -D-thiogalactoside (final concn. 1 mM) was then added to strain RM32/F'd25 to induce β -galactosidase. At intervals a measured volume was removed, added to one-tenth its volume of a solution of chloramphenicol (1 mg./ml.) and agitated for a few seconds with a Whirlimixer. Samples of this bacterial suspension were taken for the assay of β -galactosidase and thiogalactoside transacetylase, and the density (E_{600}) of the remaining portion of the suspension was measured.

Bacterial protein. This was estimated from the E_{600} by use of a standard curve prepared by the method of Lowry, Rosebrough, Farr & Randall (1951).

β -Galactosidase assay. A sample (usually 0.1 ml.) of the chloramphenicol-treated culture was shaken for 30 min. at 37° with 0.01 ml. of toluene. Then 1 ml. of water was added and the mixture was brought to 37°. Finally 1 ml. of warm assay mixture was added: this contained 2 g. of *o*-nitrophenyl β -D-galactoside and 3 ml. of mercaptoethanol/l. of 0.2 M-potassium phosphate buffer, pH 6.8. After a convenient time (5–30 min.) at 37°, 1 ml. of M-Na₂CO₃ was added, and E_{420} was measured.

One unit of enzyme activity is that which produces, under the conditions described above, 1 nmole of *o*-nitrophenol/min.

Thiogalactoside transacetylase assay. This was performed by the method of Epstein (1967), modified by the use of 10 μ l. of bacterial lysate in place of 5 μ l. It proved impossible to measure 10 μ l. with the necessary accuracy, and therefore the nominal 10 μ l. sample was pipetted into a weighed vial,

where the differential rate of enzyme synthesis is the gradient of a graph in which enzyme activity (units/ml.) is plotted against bacterial protein (μ g./ml.).

RESULTS AND DISCUSSION

E. coli strain EZ16-3-G synthesizes no β -galactosidase. The synthesis of thiogalactoside transacetylase in this strain is directed by a structural gene situated on an episome, and a large deletion (see *d25* in Fig. 1) has removed the *o-p-i* elements that lay *cis* to this gene. The remaining portion of the *lac* operon is fused to a nearby purine locus, and the synthesis of thiogalactoside transacetylase is induced by removing purines from the medium (Jacob *et al.* 1965).

In strain EZ16-3-G the synthesis of thiogalactoside transacetylase was repressed only about 10% by glucose (Fig. 2). Since in strains that possess an intact *o-p-i* system the percentage repression of this enzyme by glucose is usually 40–80% (see Yudkin, 1969), we may tentatively conclude that deletion of the *o-p-i* system (and thus the abolition of the normal transcriptional control of the *lac* operon) has alleviated catabolite repression.

Strain RM32/F'd25 contains the same episome as strain EZ16-3-G; it also contains a second *lac* operon, which synthesizes β -galactosidase under the control of an intact *o-p-i* system. In several experiments the synthesis of β -galactosidase in strain RM32/F'd25 was repressed by glucose by 65–80%, but that of thiogalactoside transacetylase by only 30–40% (Fig. 3). This difference cannot be explained by the fact that one of the relevant structural genes is chromosomal and the other episomal (see the results described by Yudkin, 1969). A possible explanation is that the rate of synthesis of thiogalactoside transacetylase in these strains is determined by the intracellular concentration of purines rather than catabolites; but it would then be necessary to suppose that growth on glucose gave rise to a much higher concentration of purines than growth on glycerol in strain RM32/F'd25 but not in strain EZ16-3-G. These suppositions seem quite implausible.

The most probable interpretation of the difference in sensitivity of the two enzymes is that the deletion

Fig. 2. Catabolite repression in *E. coli* strain EZ16-3-G. The bacteria were grown for many generations in glycerol- or glucose-minimal medium, with adenine. They were separated by filtration through Millipore membranes, washed and resuspended in medium containing the same carbon source, but without adenine. Samples were taken for the measurement of E_{600} and of thiogalactoside transacetylase. ○, Glycerol medium; ●, glucose medium.

Fig. 3. Catabolite repression in *E. coli* strain RM32/F'd25. The bacteria were grown for many generations in glycerol- or glucose-minimal medium, with adenine. They were separated by filtration through Millipore membranes, washed and resuspended in medium containing the same carbon source but without adenine, and after 20 min. isopropyl β -D-thiogalactoside was added. Samples were taken for the measurement of E_{600} , of thiogalactoside transacetylase (a) and of β -galactosidase (b). ○, Glycerol medium; ●, glucose medium.

of the *o*, *p* and *i* regions has abolished all means by which repression of transcription of the *a* gene can be effected. This interpretation suggests that transcriptional repression contributes substantially to the catabolite repression of β -galactosidase in this partial diploid, and, more generally, that transcriptional repression is a normal component of catabolite repression. Since the extensive deletion *d25* has removed all of the *o*, *p* and *i* regions, one cannot infer from the results which of these regions is specifically involved in catabolite repression; but the results are consistent with the suggestion of Silverstone *et al.* (1969) that it is the *p* region that determines sensitivity to catabolite repression.

In strain EZ16-3-G, deletion of the elements that control transcription abolishes all but a small fraction of the catabolite repression that normally affects the *lac* operon; a similar effect was observed in many of the strains studied by Silverstone *et al.* (1969). However, in strain RM32/F'd25 the same deletion leaves the synthesis of thiogalactoside transacetylase subject to considerable repression. One may suggest that this residual repression is mediated at the level of translation, and that by contrast translational repression operates to only a small extent in strain EZ16-3-G.

On the basis of experiments in which transcription of the gene for β -galactosidase was separated in time from translation of the corresponding m-RNA, Yudkin & Moses (1969) suggested that the extent of transcriptional repression varied between different strains of *E. coli*. The present results suggest that the extent of translational repression also varies. If these two effects vary independently, it is not surprising that in some strains of *E. coli* β -galactosidase synthesis may be repressed by glucose to the extent of 95%, whereas in others the repression may be as low as 20%. The present results, together with those of Yudkin & Moses (1969), make it clear that both transcriptional and translational repression must be operating to achieve the percentage repression of 60–90% that is observed in some strains of *E. coli*.

One can make use of these facts to study translational repression of the *lac* operon, and in particular to try to locate the genetic determinant of translational repression. The accompanying paper (Yudkin, 1969) is concerned with that question.

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