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Catabolite Repression of the *lac* Operon

SEPARATE REPRESSION OF TWO ENZYMES

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1. Catabolite repression of β-galactosidase and of thiogalactoside transacetylase was studied in several strains of *Escherichia coli* K 12, in an attempt to show whether a single site within the structural genes of the *lac* operon co-ordinately controls translational repression for the two enzymes. In all experiments the rate of synthesis of the enzymes was compared in glycerol–minimal medium and in glucose–minimal medium. 2. In a wild-type strain, glucose repressed the synthesis of the two enzymes equally. 3. The possibility that repression was co-ordinate was investigated by studies of mutant strains that carry deletions in the genes for β-galactosidase or galactoside permease or both. In all of the strains with deletions, the repression of thiogalactoside transacetylase persisted, and it is concluded that there is no part of the structural gene for β-galactosidase that is essential for catabolite repression of thiogalactoside transacetylase. 4. Subculture of one strain through several transfers in rich medium greatly increased its susceptibility to catabolite repression by glucose. It is concluded that unknown features of the genotype can markedly affect sensitivity to catabolite repression. 5. These results make it clear that one cannot draw valid conclusions about the effect of known genotypic differences on catabolite repression from a comparison of two separate strains; to study the effect of a particular genetic change in a *lac* operon it is necessary to construct a partially diploid strain so that catabolite repression suffered by one *lac* operon can be compared with that suffered by another. 6. Four such partial diploids were constructed. In all of them catabolite repression of β-galactosidase synthesized by one operon was equal in extent to catabolite repression of thiogalactoside transacetylase synthesized by the other. 7. Taken together, these results suggest that catabolite repression of β-galactosidase and thiogalactoside transacetylase is separate but equal.

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* promotor region; *leu*$^-$, auxotrophy for leucine; *met*$^-$, auxotrophy for methionine; *pro*$^-$, auxotrophy for proline; *thr*$^-$, auxotrophy for threonine; *trp*$^-$, auxotrophy for tryptophan; *Sm*$^{S(R)}$, sensitivity (resistance) to streptomycin.

The results described in the preceding paper (Yudkin, 1969) make it clear that catabolite repression of the *lac* operon in *Escherichia coli* can persist even when the *o*, *p* and *i* regions (responsible for the control of transcription) are deleted. The most probable conclusion is that catabolite repression operates in part at the level of translation; the same conclusion was reached by Yudkin & Moses (1969) from the results of different types of experiment. The question now arises: where is the genetic determinant of sensitivity to translational repression? It seems possible that the determinant might lie within the structural genes of the operon, and that a single site might determine sensitivity to translational repression for the entire operon. If both of these proposals are correct, it should follow first that catabolite repression is co-ordinate for all of the *lac* proteins, and secondly that deletion of the relevant part of the operon would diminish catabolite repression of the remaining protein(s). (In practice it is impossible to assay galactoside permease with the necessary degree of accuracy, and the experimental work is confined to β-galactosidase and thiogalactoside transacetylase.)

The present experiments involved a comparison between the rates of synthesis of these two enzymes by bacteria growing in glycerol–minimal and

glucose–minimal medium. The results show that in wild-type bacteria β-galactosidase and thiogalactoside transacetylase are equally repressed by glucose. However, this effect cannot be attributed to coordinate repression at a single site, since the genetic deletions of *z* and *y* that have been tested have no effect on catabolite repression. The results also show that, in partially diploid strains that contain two *lac* operons, catabolite repression is equal for the separate genomes.

MATERIALS AND METHODS

Media. These were as described by Yudkin (1969). Unless otherwise specified, organisms were maintained on glycerol–minimal medium. For *E. coli* strain CA7078, L-tryptophan (50mg./l. of medium) was added. *E. coli* strain JC2637 was grown on Difco casein hydrolysate.

Organisms. The names, genetic characters and sources of the parental strains used (which are all *E. coli* K12) are listed in Table 1.

Construction of diploid strains. (*a*) Strain RV/F′*lac*$^+$. Young exponential cultures of strain JC2637 and strain RV in casein-hydrolysate medium were mixed in the ratio 5:1 and shaken at 37° for 2hr. The mating mixture was diluted and plated on lactose–minimal medium–agar. After about 48hr. at 37° a colony was picked, grown overnight in lactose–minimal medium and plated again on lactose–minimal medium–agar. A single colony was picked.

(*b*) Strain XA8030/F′M15. Young exponential cultures of strain E7049 and strain XA8030 in glycerol–minimal medium were mixed in the ratio 5:1 and shaken at 37° for 4hr. The mating mixture was diluted and plated on lactose–minimal medium–streptomycin–agar. After about 28hr. at 37° several colonies were picked, grown overnight in glycerol–minimal medium and checked to ensure that they did not produce β-galactosidase constitutively (and therefore that they had received the i^+ gene from the F′episome). One culture was plated again on lactose–minimal medium–streptomycin–agar, and a single colony was picked.

(*c*) Strain XA8030/F′MS37. This was constructed by mating strains RV/F′MS37 and XA8030. The procedure followed that described for strain XA8030/F′M15, except that the selection and purification were done on glycerol–minimal medium–streptomycin–agar.

(*d*) Strain RM32/F′M15. Young exponential cultures of strain E7049 and strain RM32 in glycerol–minimal medium were mixed in the ratio 5:1 and shaken at 37° for 4hr. The mating mixture was diluted and plated on lactose–minimal medium–streptomycin–agar. After about 28hr. at 37° several colonies were picked and grown overnight in lactose–minimal medium. Portions of each culture were diluted into glycerol–minimal medium containing 1mM-isopropyl β-D-thiogalactoside, and after 3hr. 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 lactose–minimal medium–streptomycin agar, and a single colony was picked.

(*e*) Strain RM32/F′MS37. This was constructed by mating

Table 1. *Parental strains of* E. coli

Strain	Genetic characters	Source
3000	Hfr *lac*$^+$	W. Hayes
CA243	Hfr $i^-z^{del}_{M12}y^+a^+$	J. G. Scaife
CA7078	Hfr $trp^-i^+z^{del}_{rI}y^+a^+$	J. G. Scaife
RV	F$^-$*lac*^{del}	M. H. Malamy
RV/F′MS1054	*lac*^{del}/F′*lac* $i^+z^+y^{del}_{MS1054}a^+$	J. Roth via V. Moses
RV/F′MS37	$Sm^S lac^{del}$/F′*lac* $i^+z^-y^{del}_{MS37}a^+$	M. H. Malamy
E7049	$Sm^S i^+z^{del}_{M15}y^+a^+$/F′*lac* $i^+z^{del}_{M15}y^+a^+$	J. G. Scaife
XA8030	F$^-$$Sm^R i^-z^+y^-a$*	J. G. Scaife
RM32	F$^-$$Sm^R lac^{del}$/φ80d*lac* $i^+z^+y^+a^{del}$	A. Fowler via V. Moses
JC2637	*thr*$^-$*leu*$^-$*pro*$^-$*met*$^-$/F′*lac*$^+$	J. Clark via V. Moses

* This gene produces negligible quantities of thiogalactoside transacetylase, owing to the polar mutation in the adjacent *y* gene.

Table 2. *Constructed strains of* E. coli

Strain	Genetic characters
RV/F′*lac*$^+$	*lac*^{del}/F′*lac*$^+$
XA8030/F′M15	$i^-z^+y^-a$*/F′*lac* $i^+z^{del}_{M15}y^+a^+$
XA8030/F′MS37	$i^-z^+y^-a$*/F′*lac* $i^+z^-y^{del}_{MS37}a^+$
RM32/F′M15	*lac*^{del}/φ80d*lac* $i^+z^+y^+a^{del}$/F′*lac* $i^+z^{del}_{M15}y^+a^+$
RM32/F′MS37	*lac*^{del}/φ80d*lac* $i^+z^+y^+a^{del}$/F′*lac* $i^+z^-y^{del}_{MS37}a^+$

* This gene produces negligible quantities of thiogalactoside transacetylase owing to the polar mutation in the adjacent *y* gene.

strains RV/F'MS37 and RM32. The procedure followed that described for strain RM32/F'M15.

All of these constructed strains synthesized both β-galactosidase and thiogalactoside transacetylase inducibly. All were sensitive to the male-specific phage MS2. Strain XA8030/F'MS37 was *lac*⁻ on Eosin–Methylene Blue–lactose–agar; the others were all *lac*⁺. The recipient strains were recovered in every case by treatment of the partial diploids with Acridine Orange (Hirota, 1960). The names and genetic characters of the constructed strains are given in Table 2.

Growth and induction. Comparisons were made between the rates of synthesis of β-galactosidase and thiogalactoside transacetylase in cultures growing exponentially in either glycerol– or glucose–minimal medium. The organisms were grown from a small inoculum overnight at 37° with the appropriate carbon source. In the morning they were diluted with fresh warm medium containing the same carbon source, to a density of about 30 μg. of protein/ml. They were allowed at least to double at 37°, and then (except for the constitutive strain CA243) isopropyl β-D-thiogalactoside was added to a final concentration of 1 mM.

Sampling, and assay of protein and of enzymes, and expression of results. These were as described by Yudkin (1969).

RESULTS

If a single genetic site determines the sensitivity of the entire *lac* operon to translational repression, one might expect that conditions giving rise to catabolite repression would affect the rates of synthesis of β-galactosidase and thiogalactoside transacetylase co-ordinately. In experiments to test whether the two enzymes were in fact co-ordinately repressed, the wild-type strain 3000 was used. It is possible to show that in this strain all of the catabolite repression suffered by β-galactosidase during exponential growth occurs at the level of translation (for filtration method used see Yudkin & Moses, 1969).

In several experiments, the rates of synthesis of β-galactosidase and thiogalactoside transacetylase were compared in cultures of strain 3000 growing in glycerol–minimal and glucose–minimal medium. In three experiments the percentage repression by glucose was 34–54% for β-galactosidase and 33–51% for thiogalactoside transacetylase; on each occasion the extent of repression of the two enzymes was almost equal.

Effect of deletions in z *and* y. These results suggested that there is a single site in the *lac* operon that determines sensitivity to catabolite repression at the level of translation. If this is so, one should be able to diminish catabolite repression by deleting this site. It might be thought that such a deletion would totally abolish catabolite repression; but that would probably not be the case, since in some strains of *E. coli* control of transcription contributes substantially to catabolite repression (Yudkin & Moses, 1969; Yudkin, 1969). Despite this difficulty, one can make a survey of organisms carrying different deletions, in the expectation that a strain in which a site for sensitivity to translational repression is deleted would show comparatively little catabolite repression.

Previous work has shown that the deletion of the whole of *a* and an adjacent portion of *y* has no effect on catabolite repression of β-galactosidase (Moses & Yudkin, 1968); in the present experiments I have therefore concentrated on *z* and the proximal part of *y*. In any case the fact that the direction of translation by ribosomes is from *z* to *a* makes it likely that a controlling site would be in or near *z*.

Strains CA243 and CA7078 both carry extensive deletions (M12 and rI) in *z* (Fig. 1). In both of these the repression of thiogalactoside transacetylase by glucose was 80–85%. In strain RV/F'MS1054, in which part of *y* is deleted, the percentage repression of both β-galactosidase and transacetylase was about 75%. Thus none of these deletions appears to alleviate catabolite repression to any substantial extent.

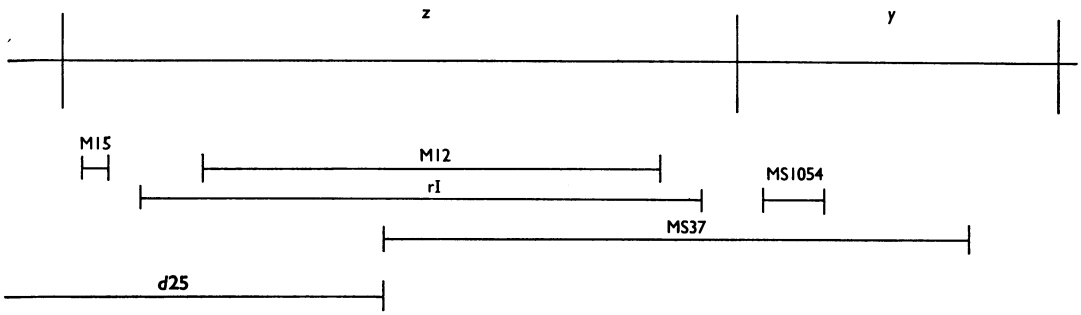

Fig. 1. Diagram showing deletions in *z* and *y* genes in *E. coli*. The information is taken from Grodzicker & Zipser (1968), Jacob, Ullman & Monod (1965), Malamy (1966) and Newton, Beckwith, Zipser & Brenner (1965). Deletion *d*25 has not been mapped with respect to deletion MS37.

In all of these strains, however, the junction between *z* and *y* remains intact, and a site determining sensitivity to catabolite repression might be located at or near this junction. In the F'MS37 episome described by Malamy (1966) a deletion extending from *z* into *y* crosses the junction (Fig. 1). This episome is carried in strain RV, the chromosomal *lac* operon of which is deleted; and by inserting a wild-type F'*lac*$^+$ episome into strain RV one can construct a strain which would be expected to have the same genetic background as strain RV/F'MS37. If deletion of the *z–y* junction alleviates catabolite repression, the extent of repression of thiogalactoside transacetylase should be less in strain RV/F'MS37 than in strain RV/F'*lac*$^+$.

In several experiments the repression of thiogalactoside transacetylase was only 20–25% in strain RV/F'MS37 but 55–60% in strain RV/F'*lac*$^+$.

At first sight this result suggests that the MS37 deletion specifically alleviates catabolite repression. But in fact a simple comparison of two strains is not sufficient to establish the effect of a particular deletion, for it has repeatedly been found that the recent history of a strain (no less than its known genotype) can greatly affect its sensitivity to catabolite repression. For example, the percentage repression by glucose of β-galactosidase synthesis in strain 300U maintained on glycerol–minimal medium was about 40% (Yudkin & Moses, 1969), but after three transfers in glucose–casein hydrolysate medium the repression by glucose increased to 70% (M. Yudkin, unpublished work; cf. Cohn & Horibata, 1959).

Catabolite repression in partial diploids. Thus, to prove that a given deletion in *lac* alleviates catabolite repression, it is necessary to include an internal control in each experiment. The control can be provided by inserting into the strain that carries the partially deleted *lac* operon a second *lac* operon. If the two operons can be distinguished (i.e. if they are mutated so that they synthesize different enzymes), one can compare the extent of catabolite repression that each of them suffers within the same experiment. For instance, by inserting the F'MS37 episome (which synthesizes thiogalactoside transacetylase but no β-galactosidase) into a strain whose chromosome synthesizes β-galactosidase but no thiogalactoside transacetylase, it should be possible to see whether the MS37 deletion diminishes the extent of catabolite repression.

The F'MS37 episome was therefore inserted into strain XA8030; in this strain the chromosome carries a highly polar mutation in *y*, the effect of which is that the rate of synthesis of thiogalactoside transacetylase is less than 5% of that in wild-type strains (and can therefore be neglected).

But before the results of experiments with strain XA8030/F'MS37 could be interpreted, it was necessary to know whether or not the episomal location of the functional *a* gene affects its susceptibility to catabolite repression. To answer this question, another partial diploid, strain XA8030/F'M15, was constructed. This strain has the same chromosome as strain XA8030/F'MS37, and its episome also synthesizes thiogalactoside transacetylase but no β-galactosidase, but in this case the lesion in the episomal *z* gene is a very small deletion (M15) near *o* (Fig. 1). (The M15 deletion does not itself alleviate catabolite repression, as is proved by the results shown in Fig. 2.)

Fig. 3 shows the results of a typical experiment with strain XA8030/F'M15. In this experiment both β-galactosidase and thiogalactoside transacetylase were repressed by about 60% by glucose. In other experiments the repression varied between 55% and 80%, although in every case the two enzymes were repressed to the same extent.

As these results showed that the synthesis of β-galactosidase from the chromosome and that of thiogalactoside transacetylase from an episome were subject to an equally severe catabolite repression by glucose, it was now possible to test strain XA8030/F'MS37. The results were very similar to those with strain XA8030/F'M15. In three experiments the percentage repression was 59–74% for β-galactosidase and 52–70% for thiogalactoside transacetylase; on each occasion

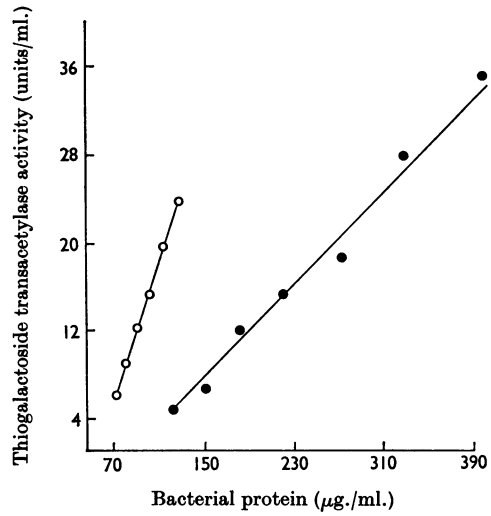

Fig. 2. Catabolite repression in *E. coli* strain E7049. The bacteria were grown for many generations in glycerol– or glucose–minimal medium, and induced with isopropyl β-D-thiogalactoside. Samples were taken for the measurement of bacterial density (E_{600}) and of thiogalactoside transacetylase. ○, Glycerol medium; ●, glucose medium.

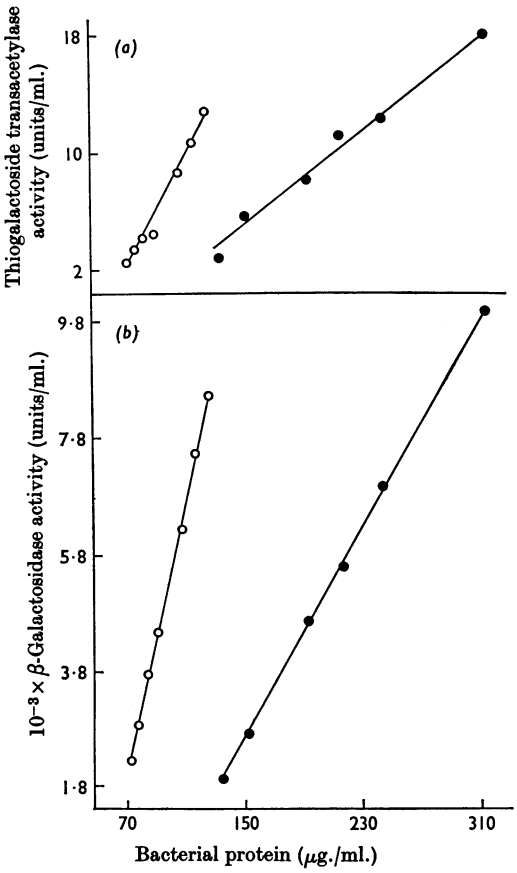

Fig. 3. Catabolite repression in *E. coli* strain XA8030/F'M15. The bacteria were grown for many generations in glycerol– or glucose–minimal medium, and induced with isopropyl β-D-thiogalactoside. Samples were taken for the measurement of bacterial density (E_{600}), of thiogalactoside transacetylase (*a*) and of β-galactosidase (*b*). ○, Glycerol medium; ●, glucose medium.

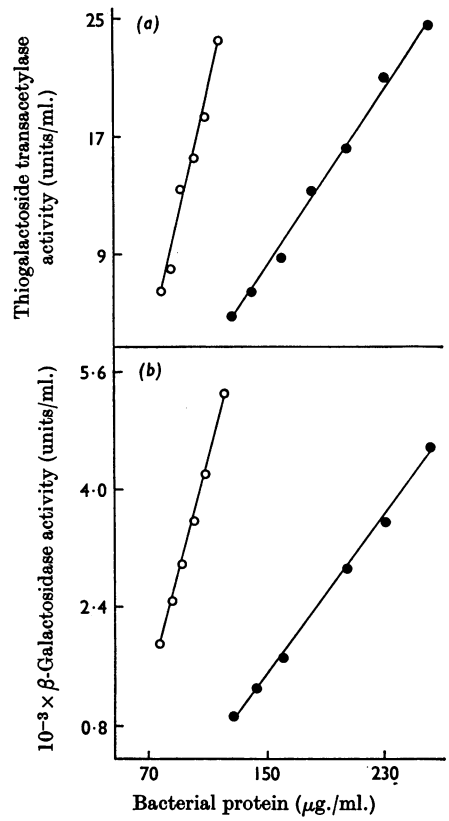

Fig. 4. Catabolite repression in *E. coli* strain RM32/F'MS37. The bacteria were grown for many generations in glycerol– or glucose–minimal medium, and induced with isopropyl β-D-thiogalactoside. Samples were taken for the measurement of bacterial density (E_{600}), of thiogalactoside transacetylase (*a*) and of β-galactosidase (*b*). ○, Glycerol medium; ●, glucose medium.

the extents of repression were almost the same for the two enzymes. One must conclude that the MS37 deletion does not alleviate catabolite repression.

This conclusion was supported by the results of experiments with strain RM32/F'MS37. In this strain the episome carrying the MS37 deletion is once again responsible for the synthesis of thiogalactoside transacetylase. But the normal *lac* operon of the chromosome has been entirely deleted, and the synthesis of β-galactosidase is under the control of a transposed *z* gene carried on φ80d*lac* from which *a* has been deleted. [Moses & Yudkin (1968) showed that the transposition does not itself affect susceptibility to catabolite repression.] In strain RM32/F'MS37, as in strain XA8030/F'MS37, β-galactosidase and thiogalactoside transacetylase were repressed to an exactly equal extent by glucose (Fig. 4). As expected, the same was true of strain RM32/F'M15.

Effect of subculturing strain RV/F'MS37 in rich medium. The results shown in Fig. 4 make it clear that the MS37 deletion has no effect on catabolite repression. Yet the original comparison between strain RV/F'*lac*$^+$ and strain RV/F'MS37 showed that the extent of repression in the latter was unusually small. If the comparative immunity to catabolite repression exhibited by strain RV/F'MS37 is, in fact, due to repeated subculture in minimal medium (see Cohn & Horibata, 1959), subculture in rich medium might be expected to increase its sensitivity to repression.

Accordingly strain RV/F'MS37 was transferred four times in medium containing 1% of glucose and 1% of Difco peptone, and the rate of synthesis of

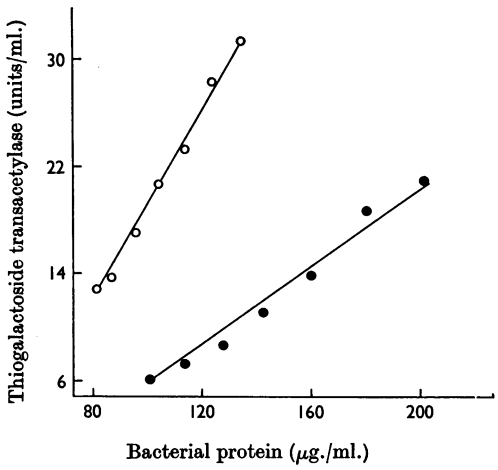

Fig. 5. Effect of subculturing in rich medium on catabolite repression in *E. coli* strain RV/F'MS37. The bacteria were transferred four times in glucose–peptone medium, and they were then grown for many generations in glycerol–minimal medium or glucose–minimal medium and induced with isopropyl β-D-thiogalactoside. Samples were taken for the measurement of bacterial density (E_{600}) and of thiogalactoside transacetylase. ○, Glycerol medium; ●, glucose medium.

thiogalactoside transacetylase was then compared again in glycerol–minimal medium and glucose–minimal medium. As a result of these subcultures, the repression by glucose had increased from about 20% to about 65% (Fig. 5).

DISCUSSION

The fact that the growth of strain 3000 in glucose resulted in an equal repression of β-galactosidase and thiogalactoside transacetylase suggested that catabolite repression might act co-ordinately for the whole *lac* operon. Co-ordinate repression is normally characteristic of systems that are controlled at the level of transcription and involve polycistronic m-RNA.* With strain 3000, however, it has been shown that catabolite repression can be entirely attributed to events occurring during translation (cf. strain 300U; Yudkin & Moses, 1969). Thus the equal repression of the two enzymes suggested the possibility of co-ordinate repression of translation.

Co-ordinate behaviour of this sort requires a site within the operon at which, in the presence of the repressing catabolite, ribosomes engaged in translating *lac* m-RNA are held up. Since β-galactosidase is the first *lac* protein to be synthesized, one would expect to find such a site in or near *z*. But in fact deletions (rI and MS37) that cover nearly the whole of *z* and much of *y* failed to alleviate the catabolite repression of thiogalactoside transacetylase. [The only portion of *z* not fully studied in the present experiments is a small section near *o*, although part of this section also is covered by the M15 deletion. However, the results reported by Yudkin (1969) show that catabolite repression of thiogalactoside transacetylase persists, at least in part, when the proximal part of *z* (see *d*25 in Fig. 1) is deleted.] Thus catabolite repression of thiogalactoside transacetylase appears not to depend on the integrity of any part of *z*, just as catabolite repression of β-galactosidase is independent of the integrity of any part of *a* (Moses & Yudkin, 1968).

These results could suggest that catabolite repression of the *lac* proteins is a non-specific effect that results from the diversion of the cell's protein-synthesizing machinery into other channels. However, Moses & Yudkin (1968) presented evidence that catabolite repression of the *lac* proteins cannot be accounted for in this way. One must therefore conclude that the catabolite repressions for β-galactosidase and thiogalactoside transacetylase are mediated separately, and that the fact that their extents are equal for the two is fortuitous.

This conclusion, although unattractive, is perfectly consistent with known facts about the synthesis of the *lac* proteins. In bacteria growing at 37° in non-repressing medium about 30 copies of β-galactosidase are made for only one copy of thiogalactoside transacetylase (Zabin, 1963). This fact probably indicates that, of 30 ribosomes that complete the translation of β-galactosidase m-RNA in unit time and discharge the completed enzyme, only one is permitted to translate the m-RNA corresponding to thiogalactoside transacetylase. (Possibly this rate of translation saturates the mechanism that admits ribosomes to the thiogalactoside transacetylase m-RNA.) If now the organisms are subjected to conditions that repress synthesis by even as much as 80%, six ribosomes will still complete the translation of β-galactosidase in unit time. Thus, even though there is evidence that ribosomes start the translation only at the beginning of the β-galactosidase m-RNA (Malamy, 1966), catabolite repression will not be rate-limiting in the synthesis of thiogalactoside transacetylase. (The neglect of galactoside permease in this discussion does not alter the principle.)

Cohn & Horibata (1959) showed that repeated subculture of a strain in medium that supported a low rate of growth diminished the organism's sensitivity to catabolite repression. The present

* Abbreviation: m-RNA, messenger RNA.

results with strain RV/F'MS37 provide evidence that the converse also holds: subculture through only four transfers in rich medium greatly increased sensitivity to catabolite repression. Although this phenomenon has not been investigated in any systematic way, it appears that subculture in a particular medium selects a variety whose genotype differs slightly in unknown respects from that of the original strain. The practical effect of these findings is that one cannot draw conclusions about the effect of known changes in genotype on catabolite repression by merely comparing two separate strains. To show whether a genotypic change in the *lac* operon affects susceptibility to catabolite repression, it is essential to construct a partially diploid strain that includes that operon together with another operon that functions as a control. The results of the present experiments with partially diploid strains validate this procedure, inasmuch as they show that catabolite repression, even if mediated separately for the different enzymes, leads to an equal diminution in the rate of synthesis of each.

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