Catabolite Repression of the *lac* Operon

EFFECT OF MUTATIONS IN THE *lac* PROMOTER

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1. Several *lac* diploid strains of *Escherichia coli* were constructed and tested to discover whether mutations in the *lac* promoter alleviate catabolite repression. 2. In each of these diploids the chromosome carries one of the promoter mutations, L8, L29 or L1; so that the rate of synthesis of the enzymes of the *lac* operon is only 2-6% of the fully induced wild-type. Each diploid harbours the episome F'*lac*M15 that specifies the synthesis of thiogalactoside transacetylase under the control of intact regulator, promoter and operator regions, but has a deletion in the structural gene for β -galactosidase. In each diploid more than 90% of the thiogalactoside transacetylase is synthesized from the episome, and 100% of the β -galactosidase is synthesized from the chromosome, and comparison of the extent of catabolite repression that the two enzymes suffered indicated whether the chromosomal promoter mutation relieves catabolite repression. 3. In the strains in which the promoter carries either of the point mutations L8 or L29 the enzymes were equally repressed, suggesting that neither L8 nor L29 affects catabolite repression. 4. In a diploid strain harbouring the same episome but carrying deletion L1 on the chromosome, synthesis of β -galactosidase suffered much less repression than that of thiogalactoside transacetylase. 5. In a diploid strain in which the chromosome carries L1 and also a second mutation that increases the rate of expression of *lac* to that permitted by L8 or L29, the synthesis of β -galactosidase again suffered much less repression than the synthesis of thiogalactoside transacetylase. 6. The effect of L1 (which deletes the boundary between the *i* gene and the *lac* promoter) is ascribed to its bringing the expression of *lac* under the control of the promoter of the *i* gene. 7. Even in strains carrying L1, some catabolite repression persists; this is not due to a *trans* effect from the episome since it occurs equally in a haploid strain with L1.

The structural genes of the *lac** operon of *Escherichia coli* specify the sequences of three proteins, β -galactosidase, galactoside permease and thiogalactoside transacetylase. Wild-type strains of *E. coli*, when growing in glycerol-minimal medium, synthesize these proteins at a rapid rate in response to galactoside inducers. When growing in glucose-minimal medium the same strains synthesize the proteins much more slowly. This effect of glucose is an example of catabolite repression. It is not overcome by an increase in the concentration of

inducer, and it occurs even in mutant strains that synthesize the *lac* proteins constitutively.

Several attempts have been made to locate the genetic determinant of the sensitivity of the *lac* operon to catabolite repression; but two characteristics of the system have seriously complicated the interpretation of experiments. First, different strains of *E. coli* differ very much in their sensitivity to catabolite repression, quite apart from any specific change in *lac*. For example strain LA-12G, isolated by Loomis & Magasanik (1965), was reported to have a mutation that specifically relieves the catabolite repression of the *lac* operon, but on further examination the strain proved to show general metabolic changes that affected its sensitivity to catabolite repression of other systems also (Rickenberg, Hsie & Janacek, 1968; Moses & Yudkin, 1968). Even changes in genotype that were not selected specifically for any effect on *lac* can alter a strain's response to catabolite repression:

* Abbreviations and genetic symbols: *lac*, lactose locus; Lac⁻, inability to ferment lactose; acetylase, thiogalactoside transacetylase; *pur*E, purine biosynthesis locus E; *trp*, tryptophan biosynthesis locus; *z*, structural gene for β -galactosidase; *y*, structural gene for galactoside permease; *a*, structural gene for thiogalactoside transacetylase; *i*, *lac* regulator gene; *p*, *lac* promoter region; *o*, *lac* operator gene; *str*⁺(-), gene conferring sensitivity (resistance) to streptomycin.

a few subcultures of a single strain in rich medium are sufficient to increase the percentage repression of the *lac* operon from about 20% to about 65% (Yudkin, 1969a). If by chance a strain carrying a known mutation in *lac* also has unrecognized mutations that alleviate catabolite repression, the effect on catabolite repression may be erroneously ascribed to the known mutation.

A second difficulty is that mutations in *lac* may alleviate catabolite repression without abolishing it entirely (Yudkin, 1969b). If a strain carrying such a mutation is tested for the presence or absence of catabolite repression, the fact that repression can be detected may erroneously be thought to show that the mutation has no relevant effect.

I have suggested that both of these difficulties in interpretation can be overcome by including an internal control in each experiment (Yudkin, 1969a). This internal control takes the form of a second *lac* operon in the same cell. It is possible to construct diploid strains that have one mutation in the chromosomal gene for acetylase and another in the episomal gene for β -galactosidase; one can then compare the extent of catabolite repression suffered by β -galactosidase synthesized from the chromosome with that suffered by acetylase synthesized from the episome. I have shown that if both operons are under the control of wild-type *i-p-o* genes the extent of catabolite repression in any one experiment is equal for the two enzymes (Yudkin, 1969a). Thus, provided that one of the operons in such a diploid has an intact *i-p-o* region, the extent of catabolite repression that it suffers can be used as an internal standard. If the other operon shows in comparison diminished sensitivity to catabolite repression, then this diminution can probably be ascribed to a specific change in the *lac* genes.

By means of this technique, I have shown that a deletion (see *d25* in Fig. 1) that eliminates the *i*, *p* and *o* regions of *lac* largely (but not entirely) relieves catabolite repression (Yudkin, 1969b). As *d25* is a very long deletion, this result does not

implicate any particular region of the *i-p-o* system in determining sensitivity to catabolite repression. However, a paper from Silverstone, Magasanik, Reznikoff, Miller & Beckwith (1969) has focused attention specifically on the promoter. These workers report that a small deletion that runs from *i* into *p* (see L1 in Fig. 1) abolished catabolite repression of β -galactosidase synthesis, but that point mutations in *p* had no effect.

The experiments of Silverstone *et al.* (1969) did not include the internal controls, provided by the *trans* operons of diploid strains, that I have described above; I thought it would be useful to re-examine the effect of promoter mutations in strains that contained such control operons. This paper reports the results of experiments with the appropriate diploids. The experiments confirm that the L1 deletion significantly relieves repression, but show that the relief is only partial.

EXPERIMENTAL

Media. Most media have been described previously (Yudkin, 1969b). Minimal agar supplemented with 0.5% melibiose, incubated at 42°C, was used to select strains capable of expressing the *y* gene (Prestidge & Pardee, 1965). L broth, L soft agar and L agar were as described by Lennox (1955) and were supplemented with 2.5 mM-CaCl₂.

Parental organisms. The genotypes of these are given in Table 1. Strain 3000L8 has been mentioned previously by Moses & Yudkin (1968), strain 300U by Yudkin & Moses (1969) and strains RV, RM32 and XA8030/F'M15 by Yudkin (1969a).

Preparation of transducing lysates. Bacteriophage Plkc (kindly given by Dr S. Baumberg) was added to a young exponential culture of the required donor strain, growing in L broth, to give about 1 phage/50 bacteria. A portion (1 ml) of the mixture was added to 2 ml of L soft agar at 45°C and this was poured on to a warm L agar plate, which was then quickly returned to the 37°C incubator. After about 7 h the soft agar was scraped from the plate and centrifuged for 10 min at 3000g. The supernatant liquid containing the transducing phages was and removed stored over a few drops of chloroform.

Table 1. *Strains of E. coli*

Strain	Relevant genotype	Source
300U	<i>F⁺str⁺z⁺y⁻</i>	
RM32	<i>str⁻</i>	
RSY14	<i>lac-pro^{del}/F'lac-pro i^ai-p^{del}o⁺z⁺y⁺a⁺</i>	J. H. Miller
3000L8	<i>HfrH⁺p_{L8}o⁺z⁺y⁺a⁺</i>	
CA8019	<i>HfrH⁺p_{L29}o⁺z⁺y⁺a⁺</i>	K. Ippen
XA8030/F'M15	<i>str⁻z⁺y⁻/F'lac i⁺p⁺o⁺z^{del}_{M15}y⁺a⁺</i>	
RV	<i>F-str⁺lac^{del}</i>	
XA8001	<i>F-str-i-p_{L1}o⁺z⁺y⁺a⁺</i>	J. G. Scaife
L8/F'M15	<i>i⁺p_{L8}o⁺z⁺y⁺a⁺/F'lac i⁺p⁺o⁺z^{del}_{M15}y⁺a⁺</i>	
L29/F'M15	<i>i⁺p_{L29}o⁺z⁺y⁺a⁺/F'lac i⁺p⁺o⁺z^{del}_{M15}y⁺a⁺</i>	
L1/F'M15	<i>i-p_{L1}o⁺z⁺y⁺a⁺/F'lac i⁺p⁺o⁺z^{del}_{M15}y⁺a⁺</i>	
i ^a L1/F'M15	<i>i^ai-p_{L1}o⁺z⁺y⁺a⁺/F'lac i⁺p⁺o⁺z^{del}_{M15}y⁺a⁺</i>	

Transduction. The transducing lysate was freed from chloroform by shaking at 37°C for 30 min and was added to a young exponential culture of the required recipient strain, growing in L broth, to give about 5 phages/bacterium. After 20 min at 37°C the bacteria with adsorbed phages were collected by centrifugation and resuspended in an equal volume of minimal medium.

Constructed strains. (a) Strain 200U. Strain 300U was cured of the F factor with Acridine Orange by the method of Hirota (1960). The resulting female strain was transduced to streptomycin resistance with a lysate from strain RM32. After 15 h had been allowed for expression of the *str*⁻ mutation, the culture was plated on glycerol-minimal agar containing streptomycin. After two days at 37°C a transductant was picked and purified by restreaking three times on similar plates. The resulting strain, named 200U, is a Lac⁻ streptomycin-resistant female; it was used as a recipient in the construction by transduction of the three strains described next.

(b) Strain 2i⁹L1. This was constructed by transducing strain 200U with a lysate from strain RSY14. After adsorption of the phage and resuspension, a sample of the culture was spread on lactose-minimal plates. [Scaife & Beckwith (1966) have shown that strains can grow on lactose even though they express the *lac* operon at only 5% of the wild-type rate.] After 3 days at 37°C, several colonies were picked and grown overnight in lactose-minimal liquid medium. The concentration of β -galactosidase in the cultures was compared with that in a wild-type strain and with that in the donor strain, both of which had been similarly grown overnight in lactose-minimal medium. (This procedure was used to find a recombinant that had received *p*⁻ as well as *y*⁺ from the donor.) A culture showing the low concentration of enzyme characteristic of a promoter mutant (less than 10% of that in the wild-type) was purified by restreaking three times on lactose-minimal agar.

(c) and (d) Strains 2000L8 and 2000L29. These were constructed by transducing strain 200U with a lysate from strain 3000L8 or from strain CA8019, respectively. Selection and purification of the required transductants were as described for strain 2i⁹L1.

(e) Strain RV/F'M15. Young broth cultures of strains XA8030/F'M15 and RV were mixed in the proportion of 5:1 and shaken at 37°C for several hours. The mixture was diluted and plated on lactose-Eosin-Methylene Blue agar. After 30 h at 37°C, several Lac⁻ colonies were

picked into glycerol-minimal medium, grown up overnight, and subcultured into glycerol-minimal medium containing 1 mM-isopropyl β -D-thiogalactoside. After a few hours' growth at 37°C, samples were tested for the presence of acetylase. A culture that produced the enzyme (and had therefore received the F') was purified by restreaking three times on lactose-Eosin-Methylene Blue agar. The resulting strain is a Lac⁻ streptomycin-sensitive donor of F'*lac* *i*⁺*p*⁺*o*⁺*z*_{M15}^{del}*y*⁺*a*⁺. It was used as donor in the construction of the four strains described next.

(f) Strain i⁹L1/F'M15. This was constructed by mating strains RV/F'M15 and 2i⁹L1 as described for the construction of RV/F'M15. The mating mixture was diluted and plated on lactose-Eosin-Methylene Blue agar containing streptomycin. Several colonies were picked and tested (as described for strain RV/F'M15) for the production of acetylase. A culture that produced the enzyme was purified by restreaking three times on lactose-minimal agar.

(g) Strain L1/F'M15. This was constructed by mating strains RV/F'M15 and XA8001, as described above. The mating mixture was diluted and plated on melibiose-minimal agar containing streptomycin, and the plates were incubated at 42°C for 2 days. [The haploid strain carrying the L1 deletion cannot grow on melibiose-minimal medium at 42°C (Scaife & Beckwith, 1966).] A single colony was picked, and purified by restreaking twice on melibiose-minimal agar and once on lactose-minimal agar.

(h) Strain L8/F'M15. This was constructed by mating strains RV/F'M15 and 2000L8, as described above. The haploid strain carrying the L8 mutation cannot grow on melibiose-minimal medium at 42°C (Scaife & Beckwith, 1966); thus the required diploid strain was selected on melibiose-minimal-streptomycin plates, and purified by restreaking on melibiose-minimal and on lactose-minimal agar, as described for strain L1/F'M15.

(i) Strain L29/F'M15. This was constructed by mating strains RV/F'M15 and 2000L29. Selection and purification were exactly as described for strain L8/F'M15.

All of the last four strains are capable of donating to a *lac*^{del} strain the ability to grow on melibiose-minimal medium at 42°C but not to grow on lactose-minimal medium.

The genotypes of all the strains used in the experiments reported in the Results and Discussion section are given in

Table 2. Rates of enzyme synthesis of the strains of *E. coli* used in the present experiments

	Rates of enzyme synthesis*			
	Uninduced		Induced	
	β -Galactosidase	Acetylase	β -Galactosidase	Acetylase
L8/F'M15	0.1	<1	6	100
L29/F'M15	0.1	<1	4	100
L1/F'M15	0.1	<1	2	100
i ⁹ L1/F'M15	0.5	<1	5	100
XA8001	1.5		2	

* All rates are expressed as percentages of the fully induced wild type.

Table 1. The rates of synthesis of β -galactosidase and acetylase are given in Table 2.

Maintenance, growth and induction. These were as described by Yudkin (1969b).

Sampling, and assay of protein and of β -galactosidase. These were as described by Yudkin (1969b).

Assay of acetylase. This was as described by Leive & Kollin (1967). Each sample was assayed in duplicate.

Expression of results. The definition of 'percentage repression' by glucose is

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

Each experiment was done at least three times, with consistent results.

RESULTS AND DISCUSSION

In strain L8/F'M15, the chromosomal *lac* promoter contains the point mutation L8 (see Fig. 1), which restricts the expression of the chromosomal operon to about 6% of the wild-type rate. The episomal *lac* operon carries a deletion in the structural gene for β -galactosidase, but it synthesizes acetylase under the control of a wild type *i-p-o* system. Thus in this diploid all of the β -galactosidase is synthesized from the chromosome with the *p* mutation, whereas 95% of the acetylase is synthesized from the episome with the wild-type control system. If L8 alleviated catabolite repression, one would expect to find that in strain L8/F'M15 the synthesis of β -galactosidase was less repressed than the synthesis of acetylase. In fact, however, experiments with this strain showed that the enzymes suffered equal repression. Fig. 2 presents a typical result.

Strain L29/F'M15 is identical with strain L8/F'M15 except that the chromosomal *lac* promoter carries a different point mutation. In this strain, too, catabolite repression was equal for the chromosomal β -galactosidase and the episomal acetylase. We may conclude that neither L8 nor L29 alleviates catabolite repression.

Fig. 1. Diagram (not to scale) showing part of the *lac* region. The information is taken from Grodzicker & Zipser (1968), Jacob, Ullman & Monod (1965) and Miller, Ippen, Scaife & Beckwith (1968).

In strains carrying L8 or L29 the expression of the *lac* operon is still under the control of the *lac* promoter, albeit that promoter is mutated. The situation in strains carrying L1 is different. L1 crosses the boundary between *i* and *p* (see Fig. 1) and eliminates the 'stop signal' that normally terminates the transcription of *i* (Reznikoff, Miller, Scaife & Beckwith, 1969), so that in strains with this deletion the expression of *lac* comes (at least in

part) under the control of the promoter of the *i* gene. Thus L1 may be regarded as analogous not to the point mutations L8 and L29 but rather to the deletions that fuse the *lac* operon to other operons (Jacob, Ullman & Monod, 1964, 1965). I have previously shown that one such deletion (see *d25* in Fig. 1) partially relieves catabolite repression (Yudkin, 1969b). If this is a general property of fusion deletions one should expect that L1 too would partially relieve catabolite repression.

Fig. 2. Catabolite repression in *E. coli* strain L8/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 acetylase (a) and of β -galactosidase (b). $\circ$, Glycerol medium; $\bullet$, glucose medium. In this experiment acetylase was repressed 69% and β -galactosidase 66%.

This expectation is confirmed by experiments with strain L1/F'M15. In this strain the severity of catabolite repression suffered by β -galactosidase was much less than that suffered by acetylase (Fig. 3). In several experiments the synthesis of β -galactosidase was 27–31% repressed but that of acetylase was 71–80% repressed.

These results show that the L1 deletion considerably relieves catabolite repression. One might argue, however, that this effect is due not to the fusion of *lac* with *i* but to the fact that the rate of expression of the chromosomal *lac* operon in strain L1/F'M15 is so low. Admittedly L8 and L29 severely restrict the rate of expression of *lac* without relieving catabolite repression, but that point is not conclusive since strain L1/F'M15 synthesizes β -galactosidase at less than half the rate of the other two strains. To be sure that the effect of L1 is not due to the restriction of expression of *lac*, one would have to test a strain that retains L1 but carries in addition a second mutation that increases the rate of synthesis of β -galactosidase at least twofold.

Strain i^aL1/F'M15 is of this kind. The chromosome contains L1, but it also has the i^a mutation

(Müller-Hill, Crapo & Gilbert, 1968), which increases the rate of expression of the *i* gene and therefore (owing to the presence of L1) of *lac*. In this strain β -galactosidase and acetylase are synthesized from the chromosome at about 5% of the wild-type rate. The strain harbours the same episome as the strains previously described; thus all of the β -galactosidase in this strain comes from the *z* gene *cis* to i^aL1, but 95% of the acetylase comes from the *a* gene *cis* to a wild-type *i*-*p*-*o* system.

In strain i^aL1/F'M15 the catabolite repression suffered by β -galactosidase was, once again, much less than that suffered by acetylase. In this strain one effect of L1 (the fusion of *lac* to the *i* gene) has been retained, whereas the other effect of L1 (restriction of the expression of *lac* to a rate even lower than that in the promoter point mutants) has been abolished. We may therefore conclude that the alleviation of catabolite repression in strains with L1 is due to the fusion. In other words, bringing *lac* under the control of a new promoter has substantially relieved catabolite repression; this result is consistent with the reports that *lac* suffers much

Fig. 3. Catabolite repression in *E. coli* strain L1/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 acetylase (a) and of β -galactosidase (b). ○, Glycerol medium; ●, glucose medium. In this experiment acetylase was repressed 79% and β -galactosidase 31%.

Fig. 4. Catabolite repression in *E. coli* strain XA8001. The bacteria were grown for many generations in glycerol- or glucose-minimal medium and the cultures were divided. Isopropyl β -D-thiogalactoside was added to (b) but not to (a). Samples were taken for the measurement of bacterial density (E_{600}) and of β -galactosidase. ○, Glycerol medium; ●, glucose medium. In this experiment the uninduced culture was repressed 40% and the induced culture 41%.

less repression if it is placed under the control of the *trp* promoter (Silverstone *et al.* 1969) or the *purE* promoter (Yudkin, 1969b) than if it is controlled by its usual *p* region.

Even the strains carrying L1, however, still consistently suffered some 30% catabolite repression (Fig. 3). One possible reason for this residual repression might be the presence in the diploid strains of an *i*⁺ gene, which produces a repressor that could act *trans*. This possibility was excluded by doing experiments with strain XA8001 (the haploid strain corresponding to strain L1/F'M15), which contains no *i*⁺ gene. The synthesis of β -galactosidase in this strain suffered at least as much catabolite repression as in strain L1/F'M15 (Fig. 4). Thus the residual repression observed in the strains with L1 probably does not involve the interaction of the repressor with the operator, and it therefore appears to be altogether independent of the *i-p-o* system. These findings are entirely consistent with the fact that a similar residual repression occurs in a strain carrying the deletion *d25* (Yudkin, 1969b), for that deletion eliminates not only the *i* gene but also the operator, and thus leaves no site at which a repressor could act. This result with deletion *d25* also speaks against another possible interpretation of the residual repression in strains carrying L1, namely that residual repression involves a part of the *lac* promoter that L1 has not deleted.

In summary, then, these results lend support to the notion that there are two forms of catabolite repression for *lac* (Yudkin & Moses, 1969; Yudkin, 1969a,b). One of these (previously called 'transcriptional repression') involves the *lac* promoter, and presumably depends on a specific interaction between the promoter, an effector molecule and the RNA polymerase (Silverstone *et al.* 1969); the specificity of this interaction appears to be unaffected by promoter point mutations that diminish the rate of expression of *lac* by 95%. The other form of catabolite repression may be observed even when the *lac* operon is brought under the control of a

foreign promoter. This residual repression is variable in extent [it was absent from some of the strains described by Silverstone *et al.* (1969) but is evident in each of the three strains carrying L1 studied in the present work; cf. Yudkin, (1969b)], and its mode of action is more obscure.

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