Effect of Point Mutations in the *lac* Promoter on Transient and Severe Catabolite Repression of the *lac* Operon of *Escherichia coli*

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1. Experiments were devised to show whether the point mutations L8 and L29 in the *lac* promoter alleviate transient repression. 2. Several recombinants were picked from matings between a single F^-p^+ strain and Hfr strains carrying mutations L8 and L29. All of the 19 p^- recombinants tested proved to suffer no transient repression, whereas all of the eight p^+ recombinants tested suffered prolonged transient repression. 3. A diploid strain was constructed in which more than 90% of the thiogalactoside transacetylase is synthesized from the episome with a wild-type *lac* promoter, whereas 100% of the β -galactosidase is synthesized from the chromosome with a promoter carrying mutation L8. In this diploid the synthesis of thiogalactoside transacetylase suffered transient repression but the synthesis of β -galactosidase did not. 4. Exactly similar results were obtained with a diploid strain in which the chromosomal promoter carried mutation L29. 5. The same diploid strains were used in experiments to show whether mutations L8 and L29 alleviate the severe catabolite repression caused by growth in glucose plus gluconate. In both strains glucose + gluconate repressed the synthesis of β -galactosidase much less than the synthesis of thiogalactoside transacetylase. 6. These and previously reported results can be explained by assuming (a) that both mutations L8 and L29 render the *lac* promoter partially, but not completely, insensitive to catabolite repression, and (b) that transient repression is an exceptionally severe form of catabolite repression.

When wild-type strains of *Escherichia coli* are growing in glycerol-minimal medium, they can be induced by the addition of certain galactosides to synthesize the proteins of the *lac* $\dagger$ operon at a rapid rate. If glucose is added to such induced cultures, the rate of synthesis of the *lac* proteins falls almost to zero for some minutes or hours; thereafter synthesis is resumed at the rate that is characteristic of cultures growing exponentially in minimal medium containing glycerol and glucose. The temporary, extremely severe, repression that is evoked by the addition of glucose is called transient repression.

Pastan & Perlman (1969) reported that the point

mutation L8, which maps in the *lac* promoter, *p*, abolished transient repression, but that mutation L29, another point mutation in *p*, had no effect. Since these two mutations are extremely closely linked, and since their effect in restricting maximum expression of *lac* is almost identical, the difference between them reported by Pastan & Perlman (1969) is rather surprising. The apparent difference might in fact be due to unrecognized strain differences between the organism carrying mutation L8 and that carrying mutation L29. Changes in genotype that are selected by repeated subculture in minimal medium can abolish transient repression from a strain that previously possessed it, and conversely repeated subculture in rich medium can cause transient repression to appear in a strain from which it was previously absent (Yudkin, 1969a).

These factors obviously make it difficult to determine whether a particular mutation in *p* does or does not relieve transient repression. The difficulty may be overcome by constructing and testing diploid strains of bacteria (Yudkin, 1970a). In a diploid of the genotype $p^-z^+a^+/F'p^+z^-a^+$ all of the β -galactosidase is synthesized under the direction of the *z* gene *cis* to a mutant promoter, but 95%

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$\dagger$ Abbreviations and genetic symbols: *lac*, lactose locus; *Lac* $^+$, ability to ferment lactose; acetylase, thiogalactoside transacetylase; *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; *thr* $^-$, *leu* $^-$, *arg* $^-$, mutations leading to auxotrophy for threonine, leucine, arginine.

of the acetylase is synthesized under the direction of the *a* gene *cis* to a wild-type promoter. If in such a strain the synthesis of acetylase suffers transient repression but the synthesis of β -galactosidase does not, one may conclude that the *p*-mutation has specifically relieved transient repression of the operon that lies adjacent to it.

Experiments with diploids of this sort show that both mutations L8 and L29 do in fact specifically abolish transient repression.

EXPERIMENTAL

Media. Most media have been described previously (Yudkin, 1969b, 1970b). Glucose–gluconate–minimal medium contained 0.5% glucose and 0.5% sodium gluconate. For the growth of strain MS 1054 and its derivatives media were supplemented with 100 μ g each of L-threonine and L-leucine and 20 μ g of L-arginine/ml.

Parental organisms. The genotypes of these are given in Table 1. Strains RV/F'M15, UL8/F'M15 (previously called L8/F'M15), XA8030/F'M15, 3000 L8 and CA 8019 have been mentioned by Yudkin (1970b), and strain MS 1054 by Yudkin (1969c).

Constructed strains. (i) Strains MS L8, MS L29 and MS Lac⁺. A young broth culture of strain MS 1054 was mixed in equal proportions with a young broth culture of either strain 3000 L8 or strain CA 8019. After incubation without shaking at 37°C for 20 min, the mixture was diluted and plated on lactose–minimal–streptomycin agar supplemented with threonine, leucine and arginine. After 3 days at 37°C colonies were picked and streaked on to lactose–tetrazolium agar (Signer, Beckwith & Brenner, 1965). The large majority of recombinants gave red colonies, and these were found after purification and testing to synthesize β -galactosidase at the restricted rate characteristic of their male parent; they therefore contain a *p*[−] mutation and are called MS L8 and MS L29. A small minority of the recombinants selected on lactose gave white colonies on lactose–tetrazolium agar, and these were found after purification and testing to synthesize β -galactosidase at the wild-type rate. These recombinants therefore retain the *p*⁺ allele of strain MS 1054, and must

be derived from crossing over between *p* and the *z*-proximal end of the *y* deletion in strain MS 1054; they are called MS Lac⁺.

(ii) Strains MS L8/F'M15 and MS L29/F'M15. One of the MS L8 and one of the MS L29 strains were chosen, and each of these was mated with strain RV/F'M15. Diploids were selected on melibiose–minimal–streptomycin agar supplemented with threonine, leucine and arginine. This procedure is described fully by Yudkin (1970b).

The genotypes of all strains are given in Table 1.

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

Transient repression. To an induced culture growing exponentially in glycerol–minimal medium, glucose was added from a 40% (w/v) solution to give 1% final concentration.

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

Assay of acetylase. This was as described by Yudkin (1970b).

RESULTS AND DISCUSSION

In strain UL8/F'M15 the chromosomal *lac* region contains the promoter mutation L8, which restricts the expression of the chromosomal *z* and *a* genes to about 6% of the wild-type rate. The episome carries a deletion in *z*, but it synthesizes acetylase under the control of a wild-type *p* region and therefore at 100% of the wild-type rate. Thus all of the β -galactosidase synthesized by this diploid comes from the *z* gene *cis* to mutation L8, whereas 95% of the acetylase comes from the *a* gene *cis* to the wild-type promoter. If mutation L8 relieved transient repression, one might expect that in strain UL8/F'M15 acetylase would suffer transient repression whereas β -galactosidase would not be repressed. In fact, however, experiments showed that neither enzyme suffered transient repression (Fig. 1).

In some strains of *E. coli* transient repression is not observed even when the *lac i-p-o* region is of the wild type (Yudkin, 1969a). One such strain is 300U, from which strain UL8/F'M15 is remotely descended (for genealogy see Yudkin, 1970b). Therefore it seems likely that the absence of transient repression from strain UL8/F'M15 is due to unknown characteristics of this strain's genotype, rather than to any specific feature of its *lac* regions. This result, of course, does not prove that mutation L8 does not relieve transient repression; it shows only that no conclusion whatever about the effect of L8 on transient repression can be drawn from experiments with strain UL8/F'M15.

Thus in order to show whether mutation L8 has any alleviating effect, it is necessary to introduce the mutation into a strain whose genetic background is such that transient repression can normally be observed. One suitable strain is MS 1054, which carries a wild-type promoter and which normally suffers prolonged transient repression

Table 1. *Strains of E. coli*

Strain	Genotype
RV/F'M15	<i>str</i> ⁺ /F' <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ^{del} <i>y</i> ⁺ <i>a</i> ⁺
UL8/F'M15	<i>i</i> ⁺ <i>p</i> _{L8} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺ /F' <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ^{del} <i>y</i> ⁺ <i>a</i> ⁺
XA 8030/ F'M15	<i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ⁺ <i>p</i> _o ^{lar} /F' <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ^{del} <i>y</i> ⁺ <i>a</i> ⁺
MS 1054	F- <i>str</i> ⁺ <i>thr</i> ⁺ <i>leu</i> ⁺ <i>arg</i> ⁺ <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ^{del} <i>a</i> ⁺
3000 L8	HfrH <i>str</i> ⁺ <i>i</i> ⁺ <i>p</i> _{L8} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺
CA 8019	HfrH <i>str</i> ⁺ <i>i</i> ⁺ <i>p</i> _{L29} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺
MS L8	F- <i>str</i> ⁺ <i>thr</i> ⁺ <i>leu</i> ⁺ <i>arg</i> ⁺ <i>i</i> ⁺ <i>p</i> _{L8} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺
MS L29	F- <i>str</i> ⁺ <i>thr</i> ⁺ <i>leu</i> ⁺ <i>arg</i> ⁺ <i>i</i> ⁺ <i>p</i> _{L29} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺
MS Lac ⁺	F- <i>thr</i> ⁺ <i>leu</i> ⁺ <i>arg</i> ⁺ <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺
MS L8/ F'M15	<i>thr</i> ⁺ <i>leu</i> ⁺ <i>arg</i> ⁺ <i>i</i> ⁺ <i>p</i> _{L8} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺ /F' <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ^{del} <i>y</i> ⁺ <i>a</i> ⁺
MS L29/ F'M15	<i>thr</i> ⁺ <i>leu</i> ⁺ <i>arg</i> ⁺ <i>i</i> ⁺ <i>p</i> _{L29} ^o <i>z</i> ⁺ <i>y</i> ⁺ <i>a</i> ⁺ /F' <i>i</i> ⁺ <i>p</i> ⁺ <i>o</i> ⁺ <i>z</i> ^{del} <i>y</i> ⁺ <i>a</i> ⁺

Fig. 1. Lack of transient repression in *E. coli* strain UL8/F'M15. A culture growing exponentially in glycerol-minimal medium was induced with isopropyl β -D-thiogalactoside. Glucose was added at the point indicated by the arrows. Samples were taken for the measurement of bacterial density (E_{600}), of acetylase (a) and of β -galactosidase (b).

(results not shown). From a mating of strain MS 1054 with the p^- strain 3000 L8, one can select a recombinant (strain MS L8) in which mutation L8 has been inserted into a chromosome that otherwise retains most of the genes of strain MS 1054. Fig. 2(a) shows that the recombinant suffered no transient repression.

There are two obvious explanations for this result: that mutation L8 does indeed relieve transient repression, or that strain MS L8 has adventitiously acquired from strain 3000 L8 some other gene that is responsible for abolishing transient repression. To prove that the absence of transient repression from strain MS L8 is in fact due specifically to the p^- mutation, one can make two predictions and test these experimentally. First, by picking enough, say several dozen, streptomycin-resistant Lac⁺ recombinants from a mating between strain MS 1054 and strain 3000 L8 it should be possible to find some that have retained the p^+ allele of the recipient and some that have acquired the p^- allele from the donor; then all of the first class should suffer transient repression and all of the second class should not. Secondly, by inserting into strain MS L8 ($p^-z^+a^+$) an episome of the geno-

type F'*lac p⁺z^{del}a⁺* it should be possible to obtain a diploid strain whose notional genotype is similar to that of strain UL8/F'M15; however, since the genotype of this diploid is largely that of the recipient strain MS 1054, acetylase should suffer transient repression but β -galactosidase should not.

These predictions were borne out by experimental results. Of nine p^- recombinants picked from a mating between strains MS 1054 and 3000 L8, all nine were similar to the original MS L8 (Fig. 2a) in showing no transient repression. Moreover the p^- mutation L29 was found to have exactly the same effect: ten out of ten p^- recombinants picked from a mating between strain MS 1054 and strain CA 8019 also suffered no transient repression. By contrast eight out of eight p^+ recombinants picked from these two matings (five with strain 3000 L8 as donor and three with strain CA 8019 as donor) suffered prolonged transient repression (Fig. 2b). Transient repression in p^+ strains still occurred even when the rate of expression of the operon was diminished (by decreasing the concentration of inducer) twenty-fold, showing that the absence of transient repression in the p^- strains was not due simply to the low level of expression.

The conclusion that mutations L8 and L29 do relieve transient repression was confirmed by experiments with strains MS L8/F'M15 and MS L29/F'M15. In these strains, as in strain UL8/F'M15, all of the β -galactosidase is synthesized under the direction of a mutant promoter, and 95% of the acetylase under the direction of a wild-type promoter. Since these organisms are derived from strain MS 1054, which shows prolonged transient repression, one would expect that their synthesis of acetylase would suffer transient repression but that their synthesis of β -galactosidase would not. Fig. 3 shows this result for strain MS L8/F'M15. Strain MS L29/F'M15 behaved identically. By contrast, in a diploid strain in which the two operons are both under the control of wild-type promoters, the length of the transient repression was the same for the two operons (Yudkin, 1970a).

These results leave no doubt that mutations L8 and L29 almost completely abolish transient repression of *lac* operons to which they lie *cis*. However, this conclusion appears rather paradoxical in view of the fact that these mutations have little or no effect on the (comparatively mild) catabolite repression that occurs during exponential growth on glucose (Yudkin, 1970b). That the two forms of repression have much in common is now certain: the small promoter deletion L1, which largely relieves catabolite repression (Silverstone, Magasanik, Reznikoff, Miller & Beckwith, 1969; Yudkin, 1970b) also abolishes transient repression (Yudkin, 1970a). Moreover the addition of 3':5'-cyclic AMP

Fig. 2. Effect of glucose in *E. coli* strain MS L8 (a) and strain MS Lac⁺ (b). Cultures growing exponentially in glycerol-minimal medium were induced with isopropyl β -D-thiogalactoside. Glucose was added at the points indicated by the arrows. Samples were taken for the measurement of bacterial density (E_{600}) and of β -galactosidase.

to bacterial cultures overcomes both catabolite repression (Ullman & Monod, 1968) and transient repression (Perlman & Pastan, 1968). These facts make it clear that transient repression and catabolite repression are closely related, and are not distinct phenomena as was previously claimed (Tyler, Loomis & Magasanik, 1967; Tyler & Magasanik, 1969; but see Silverstone, Arditti & Magasanik, 1970). Consequently one would expect mutations L8 and L29, since they substantially abolish transient repression, to affect catabolite repression.

Inspection of Fig. 1 suggests a solution to this paradox. After the addition of glucose to an induced culture of strain UL8/F'M15 growing exponentially in glycerol the rate of acetylase synthesis fell much more than the rate of β -galactosidase synthesis. (A similar, rather less pronounced, effect is shown in Fig. 3.) It seems as if the fairly severe catabolite repression observed when bacteria are growing in the presence of both glycerol and glucose can be partially relieved by mutation L8. In the light of this result, one might expect that if bacteria were grown exponentially in a medium that caused catabolite repression that is nearly as severe as transient repression, this too might be considerably alleviated by mutation L8.

When strain MS L8/F'M15 was induced in glucose-gluconate-minimal medium, the rate of

synthesis of acetylase was only 2–4% of that in glycerol-minimal medium, but the rate of synthesis of β -galactosidase was 25–35% of that in glycerol-minimal medium. In control experiments the effect of glucose plus gluconate was tested in strain XA 8030/F'M15. In this diploid, all of the β -galactosidase is synthesized from the chromosome and more than 95% of the acetylase from the episome, but both operons are under the control of wild-type promoters. The rate of synthesis of both enzymes in glucose-gluconate-minimal medium was 10–15% of that in glycerol-minimal medium (catabolite repression in this organism is rather less severe than in strain MS L8/F'M15).

We can summarize the above results, together with those previously described (Yudkin, 1970b) in the following way. Some strains of *E. coli* show no transient repression of *lac* irrespective of their *p* allele. In those strains that do suffer transient repression, the repression is almost completely abolished by mutations L8 or L29. The same mutations also relieve catabolite repression provided that it is quite severe, but the milder the catabolite repression the less relief is afforded by mutations L8 or L29.

We can rationalize these findings by making a number of assumptions. We assume first that catabolite-repressible operons are under the control

Fig. 3. Transient repression in *E. coli* strain MS L8/F'M15. A culture growing exponentially in glycerol-minimal medium was induced with isopropyl β -D-thiogalactoside. Glucose was added at the points indicated by the arrows. Samples were taken for the measurement of bacterial density (E_{600}), of acetylase (a) and of β -galactosidase (b). The difference in behaviour between the two enzymes, although not very great, was reproducible from one experiment to another.

of promoters that initiate transcription only when 3':5'-cyclic AMP is present, whereas catabolite-insensitive promoters initiate transcription irrespective of the presence of cyclic AMP (Zubay & Lederman, 1969). We further assume that the rate of transcription of catabolite-repressible operons is related to the concentration of cyclic AMP up to a maximum value. Thirdly, we assume that during exponential growth of cultures in glycerol-minimal medium, the concentration of cyclic AMP is close to that at which the rate of transcription of those operons is at its maximum; that in conditions of transient repression the concentration of cyclic AMP is so low that almost no transcription can occur; and that in conditions of more or less severe catabolite repression the availability of cyclic AMP is more or less restricted but always between these two extremes. Finally, we assume that mutations L8 and L29 alter the *lac* promoter so as to make it partially, but not completely, independent of cyclic AMP; in other words, that the mutant promoters can initiate transcription at a low rate in the absence of cyclic AMP but can initiate transcription at their maximum rate only in the presence of the maximum concentration of cyclic AMP.

To illustrate the effect of these assumptions, we may suggest two further assumptions which are not strictly necessary to the above argument but which are introduced so that the quantitative implications can be discussed. The first of these is that the promoters carrying mutations L8 and L29 are 20% independent of cyclic AMP, i.e. that in the complete absence of cyclic AMP they initiate transcription at 20% of their maximum rate. The second is that the rate of initiation of transcription from those promoters that are dependent on cyclic AMP (and of that fraction of initiation from the L8 and L29 promoters that is dependent on cyclic AMP) is directly proportional to the concentration of cyclic AMP, at least up to the concentration at which the rate of transcription is half maximum.

Using these hypothetical figures, we can now calculate the rate of expression under various conditions of the two *lac* operons in a strain with the genotype $p^{-}z^{+}a^{+}/F'p^{+}z^{\text{del}}a^{+}$. In transient repression the concentration of cyclic AMP is effectively zero; thus the operon *cis* to p^{+} will not be expressed at all, and the operon *cis* to the mutant promoter will be expressed at its minimum rate, which is 20% of its maximum rate. If after recovery from transient repression the concentration of cyclic AMP rises again to 10% of its maximum, the operon *cis* to p^{+} will be expressed at 10% of its maximum rate, and the other operon at (20+10% of 80)%, or 28%, of its maximum rate. This situation corresponds approximately to that observed towards the end of the experiment illustrated in Fig. 3. In mild catabolite repression, if the concentration of cyclic AMP is half maximum, the operon *cis* to p^{+} will be expressed at 50% of its maximum rate and the other operon at (20+50% of 80)%, or 60%, of its maximum rate; the difference between these rates will probably be too small to be detected, and it will therefore appear that the promoter mutation has no effect on mild catabolite repression (Yudkin, 1970b). Finally, in very severe catabolite repression such as that occurring in growth on glucose plus gluconate, if the concentration of cyclic AMP is 2% of maximum, the operon *cis* to p^{+} will be expressed at 2% of its maximum rate and the other at (20+2% of 80)%, or 22%, of its maximum rate. This situation corresponds to that described above for strain MS L8/F'M15.

The important points that emerge are, first, that mutations L8 and L29 are indistinguishable in their effects on catabolite repression and transient repression of *lac*; secondly, that both mutations render the *lac* promoter only partially insensitive to repression; and thirdly, that transient repression can be treated in its effects merely as an exceptionally severe form of catabolite repression.

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