TRANSIENT REPRESSION OF THE *LAC* OPERON – THE EFFECT OF A *LAC* PROMOTER DELETION

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Experiments have been done to show whether the *lac* promoter delection L1, which partly alleviates catabolite repression, also affects transient repression of *lac*. In stain L1/F'M15 all of the β-galactosidase is synthesized from a chromosomal gene *cis* to L1, whereas 98% of the thiogalactoside transacetylase is synthesized from an episomal gene *cis* to an intact *i-p-o* region. The addition of glucose to induced cultures of strain L1/F'M15 growing in glycerol medium caused extensive transient repression of transacetylase but almost no transient repression of β-galactosidase. In control experiments with a diploid stain of genotype $p^+z^+a^-/F'p^+z^-a^+$ the two enzymes suffered equal transient repression. Thus L1 substantially relieves transient repression.

1. Introduction

When cultures of *Escherichia coli* that are synthesizing β-galactosidase in glycerol-minimal medium are supplemented with glucose, synthesis of the enzyme usually suffers a period of very severe repression. After some minutes this phase ends spontaneously, and the bacteria then resume enzyme synthesis at the rate that is characteristic of cultures growing exponentially in minimal medium containing both glycerol and glucose. This temporary, exceptionally severe, repression is called transient repression.

Transient repression has many features in common with catabolite repression (for example, both forms of repression appear to affect the synthesis of β-galactosidase and of thiogalactoside transacetylase coordinately), and a simple interpretation is that both operate through the same mechanism [1, 2]. According to this view, the addition of glucose to a culture that is growing in glycerol-minimal medium results in an exceptional accumulation of catabolites, which is responsible for the exceptional degree of repression. After a period of growth the concentration of catabolites returns to a new steady state, intermediate between the original concentration and that obtained during transient repression, and the rate of enzyme synthesis similarly returns to an intermediate level. (A more modern formulation would be that during transient repression the concentration of 3',5'-cyclic AMP is exceptionally low and that in catabolite repression it is at an intermediate level [3–5].)

On the other hand, Tyler, Loomis and Magasanik [6] and Tyler and Magasanik [7] have suggested that there are important differences between transient and catabolite repression and believe that the two do not share the same mechanism.

One piece of evidence that would speak in favour of the view that the two forms of repression have the same mode of action, would be that a genetic lesion that affects sensitivity to catabolite repression similarly affects sensitivity to transient repression. It is known that catabolite repression is alleviated by deletions of the *lac*-promoter [8–10]. The smallest such deletion recognized hitherto is L1 [11], which crosses the boundary between the *lac* regulator gene *i* and the *lac* promoter *p*, and brings the expression of *lac* (at least in part) under the control of the promoter of *i* [12]. L1 has been shown to relieve catabolite repression of *lac* to a substantial extent [8, 10]; and if transient repression operates by the same mechanism one would expect L1 to relieve transient repression too.

Unfortunatelty, the sensitivity of strains to transient repression is greatly affected not only by the na-

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ture of their *lac* genes but also by their general genetic background [13]. For this reason, even if one could show that a strain carrying L1 suffered no transient repression, that would not be sufficient to establish that L1 specifically alleviates repression. I have suggested that a difficulty of this sort can be overcome by constructing a diploid strain that synthesizes β-galactosidase exclusively from one *lac* operon and thiogalactoside transacetylase exclusively from the other [14]. One can arrange for one of the two operons to carry L1; and if this lesion does indeed alleviate transient repression one would expect the enzyme synthesized by that operon to suffer much less transient repression than the enzyme synthesized by the other operon with an intact *i-p-o* region.

2. Materials and methods

Strain XA8030/F'M15 ($i^-p^+o^+z^+y^{-\text{polar}}$/F'*lac* $i^+p^+o^+z^{\text{del}}y^+a^+$) has been descibed by Yudkin [14], and strain L1/F'M15 ($i^-p^{\text{del}}_{\text{L1}}o^+z^+y^+a^+$/F'*lac* $i^+p^+o^+z^{\text{del}}y^+a^+$) by Yudkin [10]. They were grown at 37° in glycerol-minimal medium and induced with isopropyl β-D-thiogalactoside [9]. To establish transient repression, glucose was added to give a final concentration of 1%. Sampling and the estimation of bacterial protein and of β-galactosidase were as described by Yudkin [9]. Thiogalactoside transacetylase was estimated in triplicate by the method of Leive and Kollin [15].

3. Results

In strain L1/F'M15, the chromosome carries the L1 deletion [11], which reduces the rate of expression of the *lac* operon to about 2% of the wild-type rate. The strain harbours the episome F'*lac*M15, which synthesizes thiogalactoside transacetylase under the direction of a wild-type *i-p-o* system but has a deletion in the structural gene for β-galactosidase. Thus all of the β-galactosidase in this strain comes from the chromosome with L1, and 98% of the transacetylase comes from the episome with an intact promoter. If L1 relieves transient repression, one would expect the synthesis of β-galactosidase to suffer much less repression than the synthesis of transacetylase.

Fig. 1 shows that the addition of glucose to a culture of strain L1/F'M15 produced a transient repression of transacetylase that lasted for well over one generation, whereas β-galactosidase suffered hardly any transient repression.

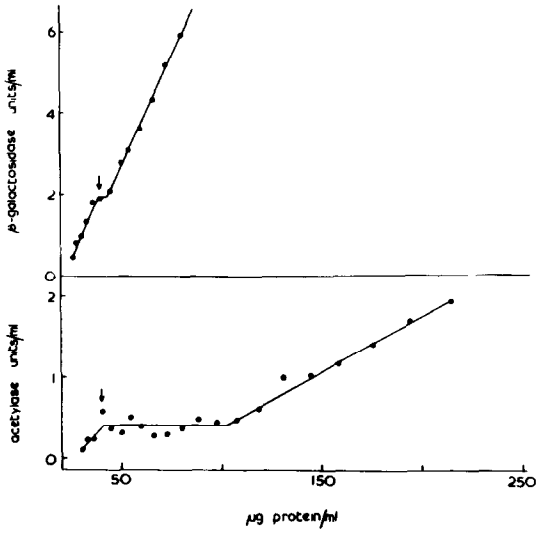

Fig. 1. Transient repression in strain L1/F'M15. Glucose was added at the point indicated by the arrows to an induced culture growing in glycerol-minimal medium.

At first sight this result suggests that L1 does indeed greatly alleviate transient repression. But it might be argued that the difference in response of the two enzymes is due to the fact that one is synthesized from the chromosome and the other from the episome. This explanation was ruled out by experiments with strain XA8030/F'M15. This strain harbours the same episome as strain L1/F'M15; and its chromosome directs the synthesis of β-galactosidase under the control of a wild-type *i-p-o* system but produces only negligible quantities of transacetylase. Thus in this strain the two enzymes are synthesized from separate operons each with an intact promoter; fig. 2 shows that they suffered approximately equal transient repression.

4. Discussion

The results make it clear that L1 largely abolishes transient repression, and strongly suggest that transient repression involves the *lac* promoter. Catabolite

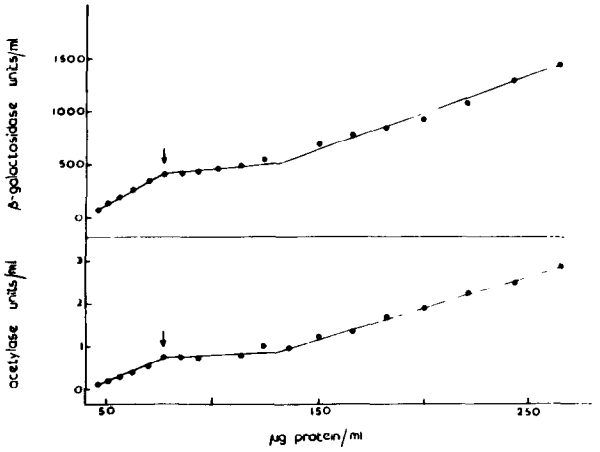

Fig. 2. Transient repression in strain XA8030/F'M15. Glucose was added at the point indicated by the arrows to an induced culture growing in glycerol-minimal medium.

repression also involves the promoter [8], at least to some extent [10]; and it therefore seems very likely that the two forms of repression share the same mode of action.

It has been proposed that transient repression is mediated through the interaction of the repressor with the operator [16]. The present results speak against that view. In strain L1/F'M15, although the chromosomal *i* gene is partly deleted the operator is intact, and the chromosomal operon is fully repressed by the product of the episomal *i* gene. Nonetheless the chromosomal operon escapes from transient repression, notwithstanding the fact that there is at the same time a prolonged transient repression of the synthesis of thiogalactoside transacetylase from the episome. These facts suggest that interaction of the repressor with the operator is not involved in transient repression.

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