THE ROLE OF THE LAC PROMOTOR LOCUS IN THE REGULATION OF β-GALACTOSIDASE SYNTHESIS BY CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE

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We have recently reported that cyclic 3′,5′-adenosine monophosphate (cyclic 3′,5′-AMP) stimulates the production of β-galactosidase and tryptophanase in *Escherichia coli.*[1] Investigation of the effect of cyclic 3′,5′-AMP on β-galactosidase synthesis revealed that the nucleotide acts at the level of transcription to increase the synthesis of β-galactosidase messenger RNA (mRNA).[2] This effect of cyclic 3′,5′-AMP is distinct from the well-characterized repressor-operator control of the *lac* operon,[3] since cyclic 3′,5′-AMP is active in mutants of both the repressor and the operator loci. Further, cyclic 3′,5′-AMP overcomes the transient repression of β-galactosidase synthesis produced by the addition of glucose to glycerol-grown *E. coli.*[2] We have now studied the regulation of β-galactosidase synthesis in mutants of the *lac* promotor region. These mutants have recently been isolated and described by Scaife and Beckwith.[4] They respond to inducers of the *lac* operon, but when fully induced make only about 5 per cent as much of the *lac* enzymes as does their parent strain, *E. coli* K12 3000. We find that cyclic 3′,5′-AMP does not stimulate β-galactosidase synthesis in one of these promotor mutants, strain L8. In addition, while glucose produces a normal permanent repression of β-galactosidase synthesis in this mutant, it does not produce a transient repression of β-galactosidase synthesis when added to glycerol-grown cells. The studies with strain L8, reported in this paper, suggest that the site at which cyclic 3′,5′-AMP acts to stimulate β-galactosidase synthesis is the *lac* promotor, and that transient repression of the *lac* operon is mediated by changes in the concentration of cyclic 3′,5′-AMP.

Materials and Methods.—The following strains of *E. coli* K12 were used: 3000 (the parent strain, *lac* $i^+p^+o^+z^+y^+a^+$); L8 and L29, two promotor mutants that make about 5% as much β-galactosidase as does 3000;[4, 5] 64, a revertant of L8 that makes normal amounts of β-galactosidase and that is genetically indistinguishable from 3000;[6] 82 and N27, two other revertants of L8 that contain a second mutation within the promotor locus, and that make, respectively, 15–20% and 50–70% as much β-galactosidase as does the parent strain;[6] o^c_{15} and o^c_{307}, two operator-constitutive mutants;[5] and i^-_{522}, a constitutive *i*-gene mutant.[5] Strain 3000 was a gift from Dr. E. Steers; all other strains were given to us by Dr. J. Beckwith.

Bacteria were grown aerobically at 37°C in medium A, a minimal medium,[7] supplemented with thiamine. Glucose (0.5%) or glycerol (0.5%) served as carbon sources. Growth was measured turbidimetrically at 560 nm in a Coleman Jr. spectrophotometer. All strains grew with doubling times of 50–60 min on glucose and 70–80 min on glycerol; cyclic 3′,5′-AMP had no significant effect on their growth rates. β-Galactosidase was induced by the addition of 10^{-3} *M* isopropyl-1-thio-β-D-galactoside (IPTG) and was assayed in toluene-treated cells by the method of Pardee, Jacob, and Monod.[8] Tryptophanase was induced by the addition of 2.5×10^{-4} *M* tryptophan and was assayed in toluene-treated cells by the method of Bilezikian *et al.*[9] Other materials and methods have been described previously.[1, 2]

Results.—Transient repression: When glucose is added to cultures of *E. coli* growing on another carbon source (such as glycerol), a transient, severe repression of β-galactosidase synthesis ensues.[10] During the period of transient repression, which lasts for about half a generation, almost no β-galactosidase is made. Following this period, β-galactosidase synthesis resumes at the rate characteristic of glucose-grown cultures. The effects of glucose on β-galactosidase synthesis in a glycerol-grown culture of strain 3000 are illustrated in Figure 1. In this experiment, β-galactosidase synthesis was induced for ten minutes before the addition of glucose alone or of glucose plus cyclic 3′,5′-AMP (*arrow*). Following the addition of glucose, there is an abrupt cessation of β-galactosidase synthesis. After a lag period of about half a generation (20–30 min), β-galactosidase synthesis resumes at a decreased rate, reflecting the permanent form of glucose repression (see below). Cyclic 3′,5′-AMP completely abolishes the transient repression and has a small effect on the permanent repression. Similar results are obtained when glucose and cyclic 3′,5′-AMP are added at the same time as the inducer.[2] However, the degree of permanent repression is quite variable from experiment to experiment, depending, presumably, on how well adapted the cells are to growth on glycerol. Indeed, in cultures that grow as rapidly on glycerol as on glucose, the addition of glucose still produces transient repression but causes little or no permanent repression.[2]

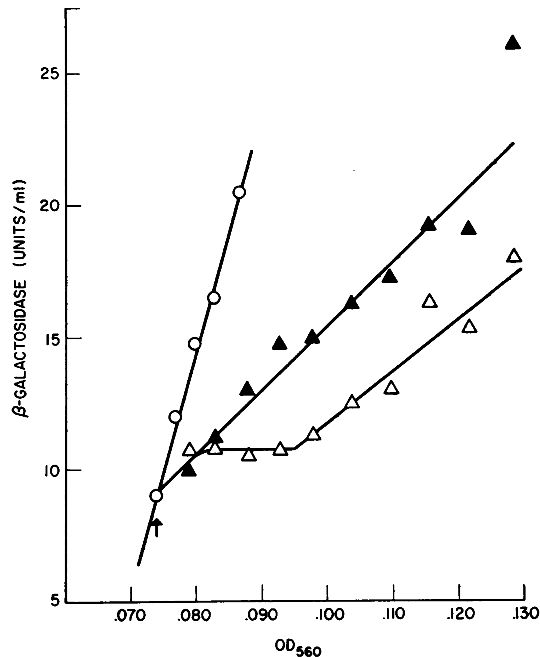


FIG. 1.—Effects of glucose and cyclic 3′, 5′-AMP on β-galactosidase synthesis in strain 3000. Cells growing on glycerol were incubated with 10^{-3} *M* IPTG. Ten minutes later, the culture was divided into three parts, which received the following additions: (O), none; (△), 0.025 *M* glucose; (▲), 0.025 *M* glucose + 10^{-3} *M* cyclic 3′, 5′-AMP.

Figure 2 shows the effects of glucose on β-galactosidase synthesis in a glycerol-grown culture of a promotor mutant, strain L8. In this strain, glucose does not cause a transient repression of enzyme synthesis. Instead, following the addition of glucose, the rate of β-galactosidase synthesis rapidly decreases to the perma-

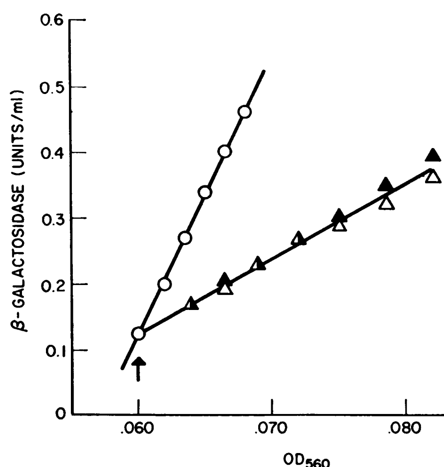


FIG. 2.—Effects of glucose and cyclic 3'5'-AMP on β-galactosidase synthesis in strain L8. Experimental procedure and symbols are the same as in Fig. 1.

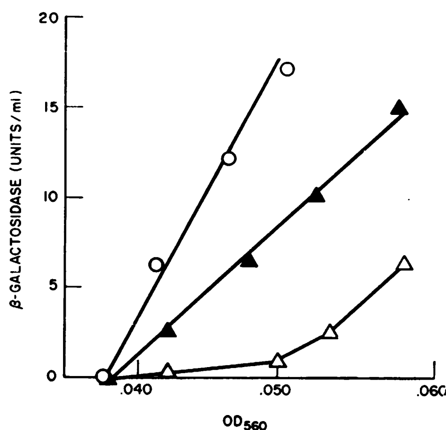


FIG. 3.—Effects of glucose and cyclic 3',5'-AMP on β-galactosidase synthesis in strain 64. Experimental procedure and symbols are the same as in Fig. 1, except that glucose and cyclic 3',5'-AMP were added at the time of the inducer, rather than 10 min later.

nently repressed rate. Further, cyclic 3',5'-AMP has little or no effect on β-galactosidase synthesis in this strain. In Figure 2, the rates were plotted for only half a generation, but were linear for two generations.

Strain L8 is a mutant of strain 3000, and is thought to contain only a single mutation in the *lac* operon.[4] It is unlikely that strain L8 carries an additional mutation affecting the uptake or metabolism of cyclic 3',5'-AMP. To investigate this possibility, however, we examined the effect of the nucleotide on tryptophanase synthesis in strain L8. In the experiment shown in Table 1, cells treated with tris(hydroxymethyl)aminomethane (Tris) and ethylenediaminetetraacetate (EDTA) were used.[1] The nucleotide stimulates tryptophanase production in both the mutant and the parent strain. Strain L8, therefore, does not contain a mutation affecting its responsiveness to cyclic 3',5'-AMP in general. Rather, it contains a specific defect in the response of its *lac* operon to the nucleotide.

Further evidence that the mutation in the promotor locus was responsible for the lack of transient repression and cyclic 3',5'-AMP stimulation of β-galactosidase synthesis in strain L8 was obtained from studies with strain 64, a revertant of strain L8 that makes normal amounts of β-galactosidase. As shown in Figure 3, glucose produces a normal transient repression of β-galactosidase synthesis in

TABLE 1. *Stimulation of tryptophanase production by cyclic 3',5'-adenosine phosphate.*

Cells	Tryptophanase Formed (U/ml/min)	
	Control	Cyclic 3',5'-AMP
L8	0.027	0.055
3000	0.028	0.083

Cells were treated with EDTA (10^{-3} *M*) as previously described, and induced with tryptophan (2.5×10^{-4} *M*) for 20 min in the presence of glucose (2.5×10^{-2} *M*) with or without cyclic AMP (10^{-3} *M*). Rates of enzyme synthesis are calculated from assays 10 and 20 min after induction.

this strain, and cyclic 3′,5′-AMP overcomes this repression. The fact that reversion at the promotor locus is associated with a resumption of normal responsiveness to glucose and cyclic 3′,5′-AMP strongly suggests that a single gene controls all these functions.

Experiments with two partial revertants of strain L8, strains 82 and N27, support the hypothesis of a relationship between the promotor locus, transient repression, and cyclic 3′,5′-AMP stimulation of β-galactosidase synthesis. Both of these revertants have regained glucose repression and cyclic 3′,5′-AMP stimulation of β-galactosidase production (Table 2). In growth experiments not shown, the glucose repression was found to be transient.

We have studied one other promotor mutant, L29, whose mutation maps at a site distinct from L8. In contrast to L8, however, this strain displays both transient repression and cyclic 3′,5′-AMP stimulation of β-galactosidase synthesis (Fig. 4, Table 2). The explanation of this difference may be that the promotor locus may have two distinct functions, the control of *lac* operon expression and the response to glucose and cyclic 3′,5′-AMP. Some promotor mutations might interfere with one of these functions, and others might interfere with both. It should be noted that, while it is possible to quantitate the promotor function of the promotor locus (by measuring the maximal rate of β-galactosidase synthesis), it is not possible to quantitate either transient repression or cyclic 3′,5′-AMP stimulation of β-galactosidase synthesis. We have only been able to describe these latter functions qualitatively as either present or absent. Studies on other promotor mutants will be necessary to clarify these aspects of promotor function.

We have reported previously that cyclic 3′,5′-AMP overcomes the transient glucose repression of β-galactosidase synthesis in an operator mutant, o^c_{67}, and in an *i*-gene mutant, 3300.[2] We have now studied two more operator mutations, o^c_{15} and o^c_{307}, and one other *i*-gene mutant, i_{522}. In each of these strains, glucose represses β-galactosidase synthesis, and this repression is overcome by cyclic 3′,5′-AMP (Table 2). Experiments with these mutants provide further evidence that the effect of cyclic 3′-5′-AMP is distinct from the repressor-operator control of the *lac* operon.

TABLE 2. *Effect of glucose and cyclic 3′,5′-adenosine phosphate on β-galactosidase synthesis in various mutants of E. coli 3000.*

	β-Galactosidase (U/ml/10⁸ Cells)			
		Units Made in 30-Min Induction		
Cells	Initial	Control	Glucose	Glucose and cyclic 3′,5′-AMP
3000	0.0	2.4	0.7	2.0
L8	0.0	0.08	0.02	0.02
64	0.0	2.8	0.8	2.1
N27	0.0	1.1	0.24	0.77
82	0.0	0.8	0.07	0.19
L29	0.0	0.15	0.04	0.18
o^c_{15}	6.22	1.1	0.15	1.5
o^c_{307}	4.65	0.5	0.0	1.1
i_{522}	13.5	2.4	0.0	2.4

Log-phase cells (1–4 × 10^8 cells/ml) were induced with IPTG (10^{-3} *M*) with or without glucose (0.025 *M*) and cyclic 3′,5′-AMP (10^{-3} *M*). IPTG was not added to constitutive strains (o^c_{307}, i_{522}).

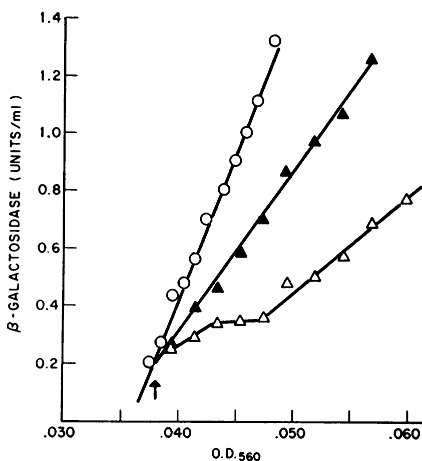
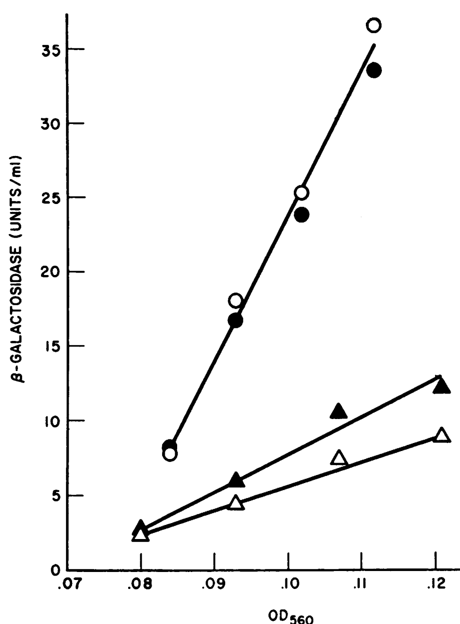


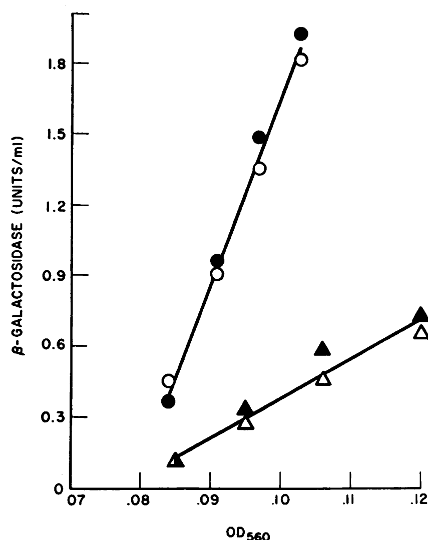
FIG. 4.—Effects of glucose and cyclic 3′,5′-AMP on β-galactosidase synthesis in strain L29. Experimental procedure and symbols are the same as in Fig. 1.

Permanent repression: When cultures of either strain 3000 or strain L8 are grown for many generations on either glucose or glycerol and then induced with IPTG, the differential rate of β-galactosidase synthesis in the glucose-grown cells is very much less than that in the glycerol-grown cells. This is the phenomenon of permanent repression.[11] As mentioned before, the absolute amount of this repression is variable; in the experiments shown in Figures 5 and 6, the glucose-grown cells of both strains made only about 25 per cent as much β-galactosidase as did the glycerol-grown cells. Evidently, strain L8 displays normal permanent repression. Cyclic 3′,5′-AMP increased the rate of β-galactosidase synthesis in glucose-grown cultures of strain 3000 by about 10 per cent, but did not restore this rate to that in the glycerol-grown cultures. Cyclic 3′,5′-AMP had very little effect on β-galactosidase synthesis in glucose-grown cultures of strain L8 or in glycerol-grown cultures of either strain. Apparently, cyclic 3′,5′-AMP plays only a small role in the permanent repression of β-galactosidase synthesis by glucose.

Discussion.—We have previously reported that in *E. coli* 3000 the transient repression of β-galactosidase synthesis produced by glucose is overcome by cyclic 3′,5′-AMP.[2] Since glucose lowers the cyclic 3′,5′-AMP concentration in *E. coli*,[7] we proposed that transient repression is due to a fall in cyclic 3′,5′-AMP concentration. We have now found that in strain L8, a mutant of the *lac* promotor, glucose does not produce a transient repression of β-galactosidase synthesis, and cyclic 3′5′-AMP does not stimulate β-galactosidase production. Further, we have found that return of the promotor function toward normal, in three revertants of strain L8, is associated with a restoration both of transient repression and of cyclic 3′,5′-AMP stimulation of β-galactosidase synthesis. We propose that transient repression of the *lac* operon is mediated by the *lac* promotor and that the promotor is the site at which cyclic 3′,5′-AMP acts, directly or indirectly, to stimulate β-galactosidase synthesis. We have previously shown that the nucleotide has little effect on glucose oxidation in *E. coli*, and that stimulation of β-galactosidase mRNA formation by cyclic 3′,5′-AMP does not require protein synthesis.[2]

Our finding that the nucleotide increases the synthesis of β-galactosidase mRNA is in agreement with the hypothesis of Ippen *et al.*,[5] that is, that the promotor controls the rate of transcription, rather than the rate of translation, of the *lac* operon. These authors have proposed that the promotor controls the maximal rate of *lac* operon expression, and that it is the site of attachment of RNA





(*Left*) FIG. 5.—Permanent repression of β-galactosidase synthesis in strain 3000. Cells growing on glycerol (*circles*) or glucose (*triangles*) were incubated with 10^{-3} *M* IPTG, in the presence (●, ▲) and absence (○,△) of 10^{-3} *M* cyclic 3',5'-AMP. β-Galactosidase assays were begun 10 min after the addition of IPTG, by which time the rates of β-galactosidase synthesis have become constant.

(*Right*) FIG. 6.—Permanent repression of β-galactosidase synthesis in strain L8. Experimental procedure and symbols are the same as in Fig. 5.

polymerase to the *lac* operon DNA. Our results suggest that cyclic 3',5'-AMP is also required for the maximal rate of *lac* operon expression.

Summary.—β-Galactosidase synthesis in a mutant of the *lac* promotor, strain L8, is not subject to transient repression by glucose and is not stimulated by cyclic 3',5'-AMP. In both the parent organism and in revertants of this strain, cyclic 3',5'-AMP overcomes the transient repression, but not the permanent repression, of β-galactosidase synthesis. We propose that transient repression of the *lac* operon by glucose is mediated by the *lac* promotor, and that the promotor is the site at which cyclic 3',5'-AMP acts, directly or indirectly, to stimulate β-galactosidase synthesis.

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