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Catabolite and Transient Repression in *Escherichia coli* Do Not Require Enzyme I of the Phosphotransferase System

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Transient and catabolite repression with changes in intracellular concentrations of cyclic adenosine 3',5-monophosphate is produced by glycerol and by glucose-6-phosphate in a strain with a partial deletion of the structural gene for enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system.

Catabolite repression is the term used to describe nonspecific control by the carbon and energy source of the expression of inducible enzymes in bacteria (8, 9). Transient repression is a related regulatory phenomenon observed when a new carbon source is added to a growing culture (11). Both of these types of repression are mediated by changes in the cell concentration of cyclic AMP (cAMP) and affect operons whose expression is dependent on cAMP and the cAMP-binding protein (5, 12, 14). In exponential growth, the phase of growth generally used to study this type of repression, cAMP levels are regulated primarily by controlling the rate of cAMP synthesis (15, 26).

The phosphoenolpyruvate:sugar phosphotransferase system (PTS) has an essential role in mediating repression by substances metabolized by the PTS. Enzyme I, which is required for all phosphorylation by the PTS, is essential for repression by PTS substrates. Tight enzyme I mutants show no repression (19, 22), while leaky enzyme I mutants are hypersensitive to repression by analogs such as α-methylglucoside (13, 19), which are phosphorylated and transported but not further metabolized by cells with a functional PTS. The membrane-bound sugar-specific enzymes II of the PTS are also required to obtain repression. A PTS substrate does not repress in a mutant lacking enzyme II activity for that substrate (7, 22). These and other findings have led Peterkofsky (6, 16) and Saier (18) to propose models for the regulation of adenylate cyclase. Phosphorylation of a regulatory protein by enzyme I is a central feature of both models.

The extensive use of PTS substrates in studying repression and the pleiotropic effects of enzyme I mutants on repression have tended to suggest that enzyme I of the PTS plays an essential role in all regulation of adenylate cyclase. To determine whether regulation can occur in the absence of enzyme I, we have tested the ability of 2 non-PTS substrates to produce transient and permanent repression in an enzyme I deletion mutant.

A *ptsI* deletion was identified in a survivor able to grow at 42°C of an *Escherichia coli* strain (23) in which λcI857 is integrated into *ptsI*, the structural gene for enzyme I (3). The deletion used here, Δ*ptsI15*, does not recombine with six *ptsI* point mutations which recombine with each other, but does recombine with nine other *ptsI* point mutations, showing the deletion removes part but not all of the *ptsI* gene. The deletion complements two *ptsH* point mutations tested, showing it does not extend into the adjacent *ptsH* gene. The strain does not grow on maltose, a phenotype of *crr*$^+$ *ptsI* strains (21). Isogenic *ptsI15* and *pts*$^+$ strains were obtained by P1*kc*-mediated transduction using *dsdA*$^+$ as the selected marker. The strains used are constitutive for the glycerol catabolic enzymes (*glpR*) and for glucose-6-phosphate transport (*uhpR*^{c} from strain HP6R of R. Kadner) to permit growth of the mutants on glycerol and production of transient repression by glycerol and glucose-6-phosphate. Glycerol produces transient repression only in strains expressing the glycerol catabolic enzymes at a high rate (24). We find that constitutive expression of glucose-6-phosphate transport allows this compound to produce transient repression. The synthesis of β-galactosidase, a process very sensitive to catabolite and transient repression, was used to measure repression.

Catabolite repression was measured during the exponential phase of growth on five carbon sources that *pts* mutants can utilize. The *pts* mutant exhibits catabolite repression control (Table 1). Carbon sources most repressing in the *pts*$^+$ strain are also strongly repressing in the mutant. The major difference is that the extent

TABLE 1. *Levels of β-galactosidase and cAMP in ptsI15 mutants and pts⁺ strains*[a]

Growth conditions	*ptsI15*		*pts⁺*
	β-Galactosidase[b] U/mg, dry wt	cAMP[c] μM	β-Galactosidase[b] U/mg, dry wt
Exponential phase growth in carbon source excess:			
Gluconate, 20 mM	4.7	0.26 ± 0.07 (6)	13
Glycerol, 40 mM	7.3		13.7
Glucose-6-phosphate, 20 mM	7.3	0.34 ± 0.22 (5)	16.8
Pyruvate, 20 mM	7.4		17.5
Casamino Acids, 4 g/liter	12.9	0.66 ± 0.25 (10)	20.3
Chemostat[d]			
Glucose-6-phosphate	27.1	1.0 ± 0.2 (4)	25.1
Transient repression by:			
Glycerol[e]	1.5	0.10 ± 0.03 (9)	

[a] Strain JK116 (F⁻ *lacI3 glpR uhpR^c ptsI15 rpsL*) and isogenic *pts⁺* strain JK114 were grown with shaking at 37°C in K115 medium (4) containing the indicated carbon source. At intervals over a period of at least two generations, cell mass was estimated from turbidity measurements and a calibration curve, and samples were taken for β-galactosidase assay. The enzyme was assayed after toluene treatment as previously described (2).

[b] One unit of β-galactosidase liberates 1 μmol of *o*-nitrophenol from *o*-nitrophenyl-β-D-galactoside per min at 37°C.

[c] cAMP was measured as previously described (5); results are given as average, standard deviation, and number of determinations.

[d] Cells were grown at 37°C in a chemostat at a dilution rate of 0.1/h, about 10% of the growth rate in glucose-6-phosphate excess. Data shown was obtained after 3.5 days of growth when the specific activity of β-galactosidase remained constant with time.

[e] From an experiment similar to that of Fig. 1 in which cells were grown in Casamino Acids. Differential rate of β-galactosidase synthesis is shown for the period of 35 min after the addition of glycerol in *lacI⁺* strain JK115 which is otherwise isogenic with strain JK116.

of repression in a given medium is greater in the mutant than in the isogenic *pts⁺* strain. Catabolite repression is markedly reduced or abolished in wild-type strains when the availability of the carbon source becomes rate limiting for growth (10). Glucose-6-phosphate-limited growth in a chemostat completely abolished repression in both *pts* mutant and *pts⁺* strain (Table 1). We conclude that catabolite repression does occur in a *ptsI* deletion mutant.

Transient repression in the *ptsI* mutant growing on pyruvate is shown in Fig. 1. Similar results were obtained when the mutant was grown on Casamino Acids (data not shown). Addition of glucose-6-phosphate or glycerol produced marked transient repression in the *ptsI* mutant (Fig. 1A) as well as in the *pts⁺* strain (Fig. 1B). The duration of repression is very similar for the two strains when expressed in terms of the fractional increase in mass shown in the lower scale for the abscissa of Fig. 1. Transient repression by glycerol ends after a 50% increase in mass, while repression by glucose-6-phosphate ends when cell mass has doubled. Addition of cAMP largely reversed repression in both strains. The PTS substrate α-methylglucoside had no effect in the *ptsI* mutant, the curve being indistinguishable from the control which in this experiment showed a short accelerating phase of enzyme synthesis lasting 1/10 generation before reaching a constant rate. In the *pts⁺* strain α-methylglucoside produced transient repression lasting 1/3 generation. In similar experiments with Casamino Acids-grown cells, there was no lag in either control and α-methylglucoside had no effect in the mutant but very similar transient repression in the *pts⁺* strain (data not shown).

Isopropyl-β-D-thiogalactoside at 1 mM concentration enters cells to achieve saturating concentrations for induction in the presence of many carbon sources (5, 9) so that inducer exclusion is unlikely to account for the results of Fig. 1. To confirm this point, similar experiments were performed in strains expressing β-galactosidase constitutively where no inducer is needed. For cells growing on Casamino Acids, glucose-6-phosphate and glycerol produce transient repression in the *ptsI15* mutant and the *pts⁺* strain (Fig. 2).

Intracellular cAMP concentrations in the mutant vary with the degree of repression as in wild-type strains. Measurements for five selected conditions are shown in Table 1. cAMP in the mutant varied over a 10-fold range, from a very low value during transient repression to a high value during chemostat growth on glucose-6-phosphate. The relationship of the degree of repression, measured by the specific activity of β-galactosidase, to intracellular cAMP in the mutant is similar to that for *pts⁺* strain X9250. cAMP levels in strain X9250 corresponding to the five degrees of repression for which cAMP was measured in the mutant were interpolated from Fig. 2 of reference 5. For β-galactosidase specific activities of 1.5, 4.7, 7.3, 12.9, and 27.1 U/mg, dry weight, intracellular cAMP concentrations are 0.09, 0.25, 0.45, 0.78, and 1.0 μM, respectively. The first four values are in good agreement with the data for the mutant in Table 1. There is marked divergence only for chemostat-grown cells where the mutant has only 40% of the cAMP concentration seen in the wild type at this low degree of repression.

We have demonstrated that catabolite and

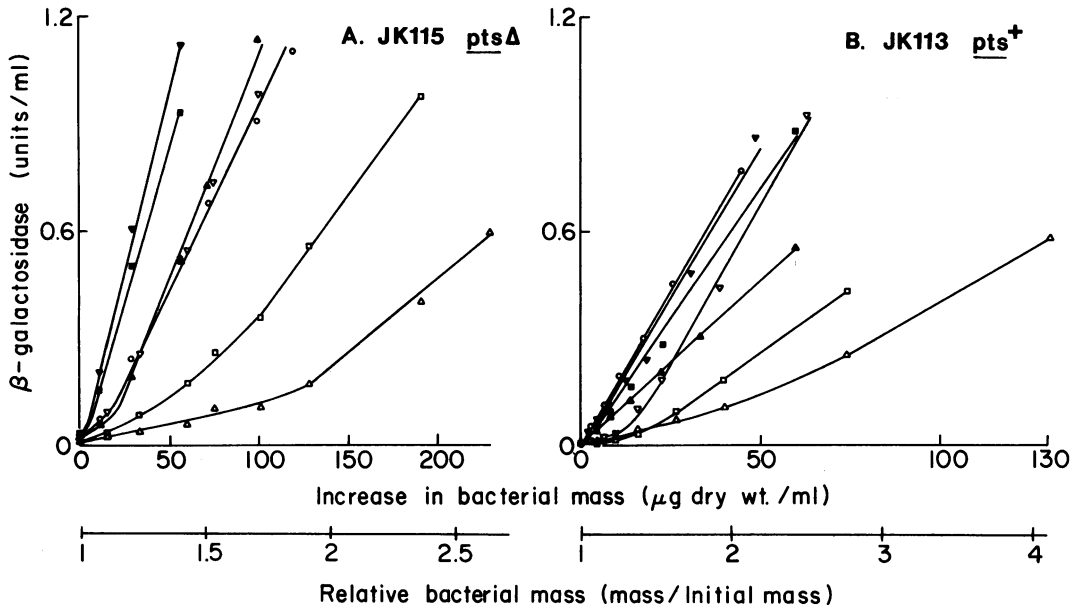

FIG. 1. *Transient repression in a pts deletion mutant and an isogenic pts⁺ strain. Lactose inducible (lacI⁺) strains JK115 (A) and JK113 (B), isogenic except for lacI genotype with strains JK116 and JK114 of Table 1, respectively, were grown with shaking at 37°C in K115 medium containing 20 mM sodium pyruvate. When the cultures contained approximately 130 μg (dry weight) per ml for strain JK115 and 40 μg (dry weight) per ml for strain JK113, they received 1 mM of the gratuitous inducer isopropyl-β-D-thiogalactoside and were immediately divided into five lots to receive the indicated additions: ○, none; □, 20 mM glycerol; △, 10 mM glucose-6-phosphate; ▽, 10 mM α-methylglucoside; ■, 20 mM glycerol + 5 mM cAMP; ▲, 10 mM glucose-6-phosphate + 5 mM cAMP; ▼, 10 mM α-methylglucoside + 5 mM cAMP. Cell mass and β-galactosidase activity were measured as described in Table 1. Data are plotted in terms of bacterial dry weight; the lower scale on the abscissa is in terms of cell mass at the time of inducer addition.*

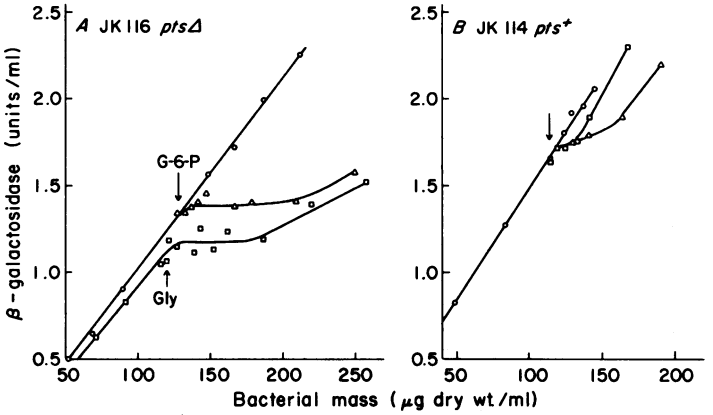

FIG. 2. *Transient repression in lac constitutive strains. Strains JK116 (A) and JK114 (B) were grown at 37°C in K115 medium containing Casamino Acids, 4 g/liter. When the cultures attained about 40 μg (dry weight) per ml, they were divided among three flasks. At the culture density indicated by the arrows, the following compounds were added: ○, none; □, 20 mM glycerol; △, 10 mM glucose-6-phosphate. Other experimental details are as for Fig. 1.*

transient repression correlated with changes in intracellular cAMP concentrations are produced by non-PTS substrates in a strain with a partial deletion of the structural gene for enzyme I. The effects observed are qualitatively very similar to those in wild-type strains, although not always quantitatively the same. Repression by non-PTS substrates has also been observed in several

presumably tight enzyme I point mutants (data not shown). Earlier data suggesting that *ptsI* function is needed for transient repression (24) and recent results suggesting that enzyme I deletion mutants had low, fixed levels of cAMP (17) were obtained in cells growing on strongly repressing substances, gluconate in the first case and nutrient broth supplemented media in the second. Transient repression is readily demonstrated during growth on less repressive substances such as pyruvate (Fig. 1) and Casamino Acids (Fig. 2), while regulation of intracellular cAMP concentrations is seen when cells grown on different substances are examined.

Is there any direct role of enzyme I in repression by non-PTS substrates? Our results do not absolutely exclude it, because the fragment of enzyme I remaining in the mutant may have some function. We believe this to be very unlikely, because our genetic analysis suggests that approximately 40% of the gene has been deleted, and we find no evidence that enzyme I functions in the mutant. The enhanced repression by α-methylglucoside characteristic of leaky enzyme I mutants is not seen; our mutant is not repressed by this analog.

Catabolite repression is a complicated phenomenon which has so far defied a simple, mechanistic explanation (25). Our study has dealt primarily with the role of cAMP because this compound can be measured, while other possible factors remain to be identified. We believe that our findings are best explained as showing regulation of adenylate cyclase in *ptsI* deletion strains. Excretion and degradation are important in determining intracellular and extracellular concentrations of this nucleotide (1, 20), but we know of no evidence to suggest that either process is regulated under the conditions of the experiments shown here (5). If we are correct in considering regulation of adenylate cyclase to occur in these mutants, then we must conclude that regulation of this enzyme does not necessarily require the function of enzyme I. What relationship does the mechanism seen in the mutants have to regulation by PTS substrates which does require enzyme I? The two mechanisms may be distinct, sharing only the enzyme being regulated. We prefer to believe that a single regulatory interaction has adapted to multiple needs rather than postulate the development of two totally separate regulatory mechanisms. If there is only a single regulatory mechanism, then our results show that the function of enzyme I is not a part of the machinery common to all regulation. This view would require some revision of current models to place enzyme I and its action on a path signaling from PTS substrates to the common regulatory machinery. A choice between these two alternatives, a single mechanism versus two or more, is not possible at present because available data can be explained by either scheme.

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LITERATURE CITED

1. **Buettner, M. J., E. Spitz, and H. V. Rickenberg.** 1973. Cyclic adenosine 3',5'-monophosphate in *Escherichia coli*. J. Bacteriol. **114**:1068–1073.
2. **Epstein, W.** 1967. Transposition of the *lac* region of *Escherichia coli*. IV. Escape from repression in bacteriophage-carried *lac* genes. J. Mol. Biol. **30**:529–543.
3. **Epstein, W., S. Jewett, and C. F. Fox.** 1970. Isolation and mapping of phosphotransferase mutants in *Escherichia coli*. J. Bacteriol. **104**:793–797.
4. **Epstein, W., and B. S. Kim.** 1971. Potassium transport loci in *Escherichia coli* K-12. J. Bacteriol. **108**:639–644.
5. **Epstein, W., L. B. Rothman-Denes, and J. Hesse.** 1975. Adenosine 3',5'-cyclic monophosphate as mediator of catabolite repression in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2300–2304.
6. **Gonzalez, J. E., and A. Peterkofsky.** 1977. The mechanism of sugar-dependent repression of synthesis of catabolite enzymes in *Escherichia coli*. J. Supramol. Struct. **6**:495–502.
7. **Harwood, J. P, C. Gazdar, C. Prasad, A. Peterkofsky, S. J. Curtis, and W. Epstein.** 1976. Involvement of the glucose enzymes II of the sugar phosphotransferase system in the regulation of adenylate cyclase by glucose in *Escherichia coli*. J. Biol. Chem. **251**:2462–2468.
8. **Magasanik, B.** 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249–254.
9. **Magasanik, B.** 1970. Glucose effects: inducer exclusion and repression, p. 189–219. *In* J. R. Beckwith and D. Zipser (ed.), The lactose operon. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
10. **Mandelstam, J.** 1962. The repression of constitutive β-galactosidase in *Escherichia coli* by glucose and other carbon sources. Biochem. J. **82**:489–493.
11. **Moses, V., and C. Prevost.** 1966. Catabolite repression of β-galactosidase synthesis in *Escherichia coli*. Biochem. J. **100**:336–353.
12. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**: 527–551.
13. **Pastan, I., and R. L. Perlman.** 1969. Repression of β-galactosidase synthesis by glucose in phosphotransferase mutants of *Escherichia coli*: repression in the absence of glucose phosphorylation. J. Biol. Chem. **244**: 5836–5842.
14. **Perlman, R. L., B. de Crombrugghe, and I. Pastan.** 1969. Cyclic AMP regulates catabolite and transient repression in *E. coli*. Nature (London) **223**:810–812.
15. **Peterkofsky, A., and C. Gazdar.** 1974. Glucose inhibition of adenylate cyclase in intact cells of *Escherichia coli* B. Proc. Natl. Acad. Sci. U.S.A. **71**:2324–2328.
16. **Peterkofsky, A., and C. Gazdar.** 1975. Interaction of enzyme I of the phosphoenolpyruvate:sugar phosphotransferase system with adenylate cyclase of *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **72**:2920–2924.
17. **Peterkofsky, A., J. E. Gonzalez, and C. Gazdar.** 1978. The *Escherichia coli* adenylate cyclase complex. Regulation by enzyme I of the phospho*enol*pyruvate:sugar phosphotransferase system. Arch. Biochem. Biophys. **188**:47–55.
18. **Saier, M. H., Jr.** 1977. Bacterial phosphoenolpyruvate: sugar phosphotransferase systems: structural, functional, and evolutionary interrelationships. Bacteriol. Rev. **41**:856–871.

19. **Saier, M. H., Jr., and B.U. Feucht.** 1975. Coordinate regulation of adenylate cyclase and carbohydrate permeases by the phosphoenolpyruvate:sugar phosphotransferase system in *Salmonella typhimurium*. J. Biol. Chem. **250**:7078–7080.
20. **Saier, M. H., Jr., B. U. Feucht, and M. T. McCaman.** 195. Regulation of adenosine 3′,5′-cyclic monophosphate excretion in *Escherichia coli* and *Salmonella typhimurium*. J. Biol. Chem. **250**:7593–7601.
21. **Saier, M. H., Jr., and S. Roseman.** 1976. Sugar transport. The *crr* mutation: its effect on repression of enzyme synthesis. J. Biol. Chem. **251**:6598–6605.
22. **Saier, M. H., Jr., R. D. Simoni, and S. Roseman.** 1976. Sugar transport. Properties of mutant bacteria defective in proteins of the phosphoenolpyruvate:sugar phosphotransferase system. J. Biol. Chem. **251**:6584–6597.
23. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1972. Prophage lambda at unusual chromosomal locations. II. Location of the secondary attachment sites and properties of the lysogens. J. Mol. Biol. **63**:483–503.
24. **Tyler, B., and B. Magasanik.** 1970. Physiological basis of transient repression of catabolic enzymes in *Escherichia coli*. J. Bacteriol. **102**:411–422.
25. **Wanner, B. L., R. Kodaira, and F. C. Neidhardt.** 1978. Regulation of *lac* operon expression: reappraisal of the theory of catabolite repression. J. Bacteriol. **136**:947–954.
26. **Wayne, P. K., J. Fetell, and O. M. Rosen.** 1975. Measurement of cyclic 3′,5′-adenosine monophosphate synthesis in growing *Escherichia coli*. Biochem. Biophys. Res. Commun. **64**:81–88.