J. Biochem. 8, 357-369.
Cashion, L. M., and Stanley, Jr., W. M. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 436-440.
David, G. S., and Reisfeld, R. A. (1974), *Biochemistry 13,* 1014-1021.
Evans, G., and Rosenfeld, M. G. (1975), *Biochim. Biophys. Acta* (in press).
Gilbert, W., and Muller-Hill, B. (1970), in The Lactose Operon, Beckwith, J., and Zipser, D., Ed., Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, p 93.
Henshaw, E. C. (1968), *J. Mol. Biol. 36,* 401-411.
Ilan, J., and Ilan, J. (1973), *Nature (London), New Biol. 241,* 176-180.
Lebleu, B., Marbaix, G., Huez, G., Temmerman, J., Burny, A., and Chantrenne, H. (1971), *Eur. J. Biochem. 19,* 264-269.
Leytin, V. L., Podobed, O. V., and Lerman, M. I. (1970), *J. Mol. Biol. 51,* 727-721.
Neville, Jr., D. M. (1971), *J. Biol. Chem. 246,* 6328-6334.
Olsnes, S. (1970), *Eur. J. Biochem. 15,* 464-471.
Perry, R. P., and Kelley, D. E. (1968), *J. Mol. Biol. 35,* 37-59.
Petermann, M. L., Hamilton, M. G., and Pavlovec, A. (1972), *Biochemistry 11,* 2323-2326.
Roberts, B. E., and Paterson, B. M. (1973), *Proc. Natl. Acad. Sci. U.S.A. 70,* 2330-2334.
Schochetman, G., and Perry, R. P. (1972), *J. Mol. Biol. 63,* 577-590.
Sheldon, R., Jurale, C., and Kates, J. (1972), *Proc. Natl. Acad. Sci. U.S.A. 69,* 417-421.
Spohr, G., Granboulan, N., Morel, C., and Scherrer, K. (1970), *Eur. J. Biochem. 17,* 296-318.

Different Cyclic Adenosine 3′,5′-Monophosphate Requirements for Induction of β-Galactosidase and Tryptophanase. Effect of Osmotic Pressure on Intracellular Cyclic Adenosine 3′,5′-Monophosphate Concentrations†

Michel Piovant and Claude Lazdunski*

ABSTRACT: In this study we have tried to answer the following questions: (1) is it possible for different catabolite-repressible genes, although submitted to the same control, to be expressed selectively depending upon the growth conditions, and (2) what is the effect of increasing the osmolarity of the medium on the intracellular level of cAMP? Two conditions were found to cause a continuous variation of intracellular cAMP levels during growth. With different strains, higher cAMP levels are required for induction of the tryptophanase gene than are required for induction of the lactose operon. cAMP has also been provided externally in adenyl cyclase minus cells of a mutant that has been made permeable by EDTA treatment. Although external cAMP concentrations, 10 times higher than the usual intracellular levels, are required for induction of β-galactosidase and tryptophanase, the difference of requirements of cAMP is maintained. An increase in the osmolarity of the medium by sucrose addition causes a fourfold decrease in the intracellular cAMP level. As a consequence this prevents the induction of tryptophanase whereas β-galactosidase is still inducible. After pulse induction, a difference in the kinetics of expression of the tryptophanase and β-galactosidase genes was found. Its relationship with the previous results is discussed.

Many lines of evidence suggest that the major regulation of catabolite repression in *Escherichia coli* occurs through the interaction of cyclic adenosine 3′,5′-monophosphate (cAMP)[1] with a specific receptor protein (CRP) (Emmer et al., 1970; Riggs et al., 1971).

This complex binds to the promoters of catabolite-repressible genes and allows the initiation of transcription by the RNA polymerase (De Combrugghe et al., 1971; Beckwith et al., 1972). This functioning might be expected to be identical for all the genes submitted to this control. However, at least in one case, it has been shown that this is not the true situation. The levels of cAMP that are required to induce the positively controlled L-arabinose operon are higher than those needed to induce the negatively controlled lactose operon (Lis and Schleif, 1973). It is of interest to know if this is a unique case, or if other examples exist. In this report we describe a similar difference between the lactose operon and the tryptophanase gene. Four different systems showed that higher cAMP levels are required for induction of the tryptophanase gene than are required for induction of the lactose operon.

The effects of increasing the osmolarity of the medium on the intracellular cAMP level have been investigated. We have shown that a decrease of this level occurs and, as a consequence, this prevents the induction of tryptophanase whereas β-galactosidase induction is still possible.

† From the Centre de Biologie et de Biochimie Moléculaire, 31 chemin Joseph Aiguier, 13274 Marseille Cédex 2, France. *Received December 2, 1974.* This investigation was supported in part by la Délégation Générale á la Recherche Scientifique et Technique.

[1] Abbreviations used are: cAMP, cyclic adenosine 3′,5′-monophosphate; CRP, cAMP receptor protein; IPTG, isopropyl β-D-thiogalactoside; ONPG, *o*-nitrophenyl β-D-galactoside.

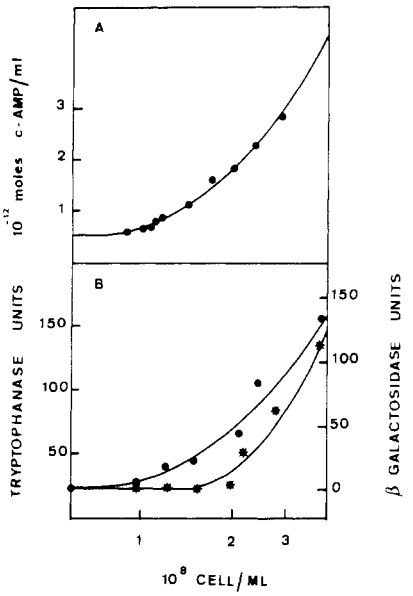

FIGURE 1: Relative dependence on intracellular cAMP concentration of β-galactosidase and tryptophanase synthesis. Aliquots were removed at intervals from the culture of the strain PA3097 and (A) cAMP was assayed as described. (B) The number of units of β-galactosidase (●) and tryptophanase (*) per milliliter of culture was determined at each cell density (measured by Coulter counts).

Materials and Methods

Chemicals. L-Tryptophan and pyridoxal phosphate were purchased from Merck; *p*-dimethylaminobenzaldehyde and indole were products of Eastman Organic Chemicals. Isopropyl β-D-thiogalactoside (IPTG), *o*-nitrophenyl β-D-galactoside (ONPG), and cyclic adenosine 3′,5′ monophosphate are from Sigma Chemicals.

Bacterial Strains and Media. The following strains were used throughout this work: P4X8, met^- a wild-type Hfr; PA3097 F^-, Thr^-, Leu^-, Arg^-, Thi^-, strs, DAP^-, Lys^-; CR34 F^-, Thr^-, Leu^-, B_1^-, Thy^-. These are generous gift from Dr Y. Hirota. CA8306 is an adenyl cyclase minus strain originally isolated by J. Beckwith. The bacterial strains were grown at 30 or 37° in minimal salt medium 63 (Monod et al., 1951), supplemented with 0.5% Casamino acids. Glycerol 0.4% was used as a carbon source. Yeast tryptone broth has been described (Lis and Scheif, 1973).

Assays. β-Galactosidase synthesis was induced by addition to the culture medium of 1 m*M* isopropyl β-D-thiogalactoside (IPTG). β-Galactosidase activity was assayed as outlined by Ullmann et al. (1965). One unit of β-galactosidase catalyzes the hydrolysis of 1 nmol of ONPG/min at 28°, pH 7. Tryptophanase was induced with 1.5 m*M* L-tryptophan and was assayed by the method of Bilezikian et al. (1967). One unit of enzyme activity is the amount that will produce 1 nmol of indole/min under these conditions. cAMP had no effect on the β-galactosidase assay but inhibits its tryptophanase at a concentration beyond 5×10^{-4} *M*. Our results have been corrected for these effects.

Measurements of cAMP Levels. For cAMP assay, samples (4.5 ml) of the culture were filtered rapidly through membrane filters (Millipore HA 0.45 μm). The filters were suspended immediately without washing, in 1 ml of 1 *N* hot perchloric acid and kept for 10 min at 100°. For assaying cAMP, the heated extract was homogenized with a micropotter and centrifuged. The supernatant was collected, neutralized with potassium carbonate, and then appropriate dilutions were used for the assay. A highly sensitive radioimmunoassay of cAMP was employed. In this new assay reported by Cailla et al. (1973), cAMP is first converted into 2-*O*′-succinyl-cAMP. Like an earlier assay (Steiner et al., 1969) this one is based on the competition between iodinated and cold antigen for the binding site of an antibody directed against succinyl-cAMP. The data obtained from the auto γ scintillation counter on a perforated tape were analyzed by a computer. Duplicate assays differed rarely by more than 5%. Calculation of intracellular concentrations of cAMP in units of molarity is based on an accessible volume of 7.5×10^{-13} ml/bacterium.

Measurements of Cell Numbers. Cell numbers were counted in a Coulter counter, Model F (Coultronic France). Samples were diluted 1000 times in a buffer 0.1 *M* sodium phosphate (pH 7) containing 1 g/l. of sodium citrate and 0.4% formaldehyde, filtered through a Millipore filter (0.1 μm).

Permeabilization of Cells to cAMP. The cells were treated with EDTA; the only difference between our method and that of Leive (1965) was that 1 m*M* EDTA was used in this study.

Results

Previous studies hinted that induction of tryptophanase and the lactose operon might require different levels of cAMP. McFall and Mandelstam (1963) had found that pyruvate repressed tryptophanase synthesis more effectively than β-galactosidase synthesis. It had been also shown by Moses and Prevost (1966) that the time of recovery from transient repression by glucose was four times as long for tryptophanase synthesis than for β-galactosidase synthesis. In 1969, De Crombrugghe et al. mentioned that the half-maximal effect of cAMP on glycerol-grown strains repressed with 1×10^{-2} *M* glucose was obtained at about 8×10^{-4} *M* for β-galactosidase and at 2×10^{-3} *M* for tryptophanase. The implications of these results seemed to be important enough to warrant a detailed investigation of this topic.

Relative Dependence on Intracellular cAMP Levels of Tryptophanase and β-Galactosidase Synthesis. For this study we had to find a medium that causes (1) a low intracellular concentration of cAMP at the beginning of growth, and (2) a slow increase of this level. We found that addition of 0.5% Casamino acids to the minimal medium used fitted perfectly those conditions. This is shown in Figure 1A. The endogenous molarity of cAMP can be evaluated by monitoring the number of cells per milliliter of culture. Using 7.5×10^{-13} ml/bacterium as the accessible volume, one can calculate that the molarity at the beginning of growth (0.8×10^{-5} *M*) is not sufficient for the induction of β-galactosidase (Buettner et al., 1973). Such a result is found as shown in Figure 1B. During growth the catabolites responsible for repression are progressively used up and the endogenous cAMP level is increased (Figure 1A). Above a cell density of 1×10^8 cells/ml, the synthesis of β-galactosidase is initiated. The synthesis of tryptophanase under the same conditions was initiated only at a cell density of about 2×10^8 cells/ml. This suggests a difference of sensitivity to catabolite repression between these two enzymes. By comparing the corresponding cAMP levels (Figure 1A) one finds roughly a requirement in cAMP fourfold higher for tryptophanase induction than for β-galactosidase induction. In fact, with the Hfr strain P4X8 similar results are obtained when a simple yeast extract, tryptone broth, is used: for a cell density of 3×10^8 cells/ml the endogenous level of

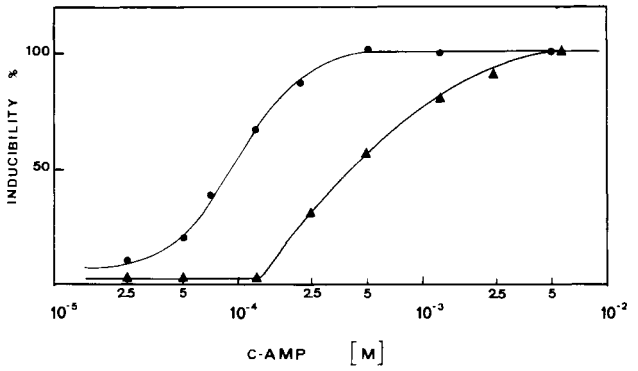

FIGURE 2: Relative dependence on exogenously supplied cAMP of β-galactosidase and tryptophanase. Adenyl cyclase-minus cells (strain CA8306) were grown on M9 minimal salts medium plus 0.2% succinate, containing 1 m*M* cAMP to decrease the growth advantage of cyclase-plus revertants. At 5 × 10^8 cells/ml, the cells were harvested, resuspended in 5 volumes of prewarmed M9/succinate medium, and grown for two doublings. Cells were then centrifuged and washed once with sterile 0.12 *M* Tris-HCl buffer (pH 8) before submitting them to the permeabilization treatment of Leive (1965) with EDTA. To stop EDTA action the incubation mixture was diluted 10 times with warmed M9 succinate, bringing the cell density to 10^8 cells/ml; 0.5 ml of cells was added to culture tubes containing varying amounts of cAMP and incubated for 6 min. L-Tryptophan and IPTG were added to each tube to a final concentration of 1 m*M*. After 20 min of induction at 37°, the cells were assayed for β-galactosidase (●) and tryptophanase (▲).

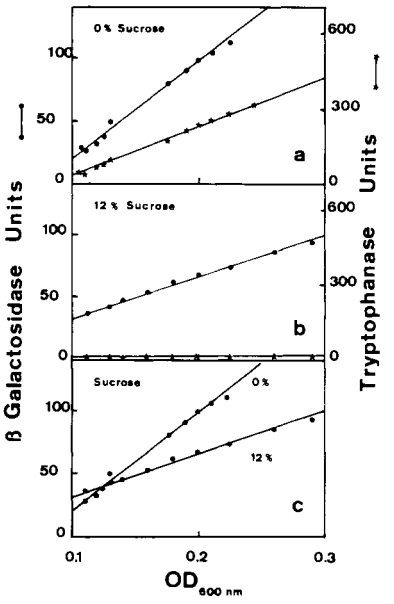

FIGURE 3: Differential effect of osmotic pressure on the induction of β-galactosidase and tryptophanase synthesis. The differential levels of synthesis of β-galactosidase (●) and tryptophanase (★) were determined in cultures of strain CR34 growing on M9/glycerol (0.4%) medium: (a) without addition of sucrose or (b) with addition of 12% sucrose. (c) Comparison of the differential levels of β-galactosidase synthesis in cells grown with or without sucrose.

cAMP is such that the inducibility of tryptophanase is about 50% the maximum, whereas the inducibility of β-galactosidase is 100%.

Response of Tryptophanase and β-Galactosidase Synthesis to cAMP Provided Externally. It was interesting to confirm the above results by another approach. For this purpose we have used a mutant which cannot synthetize its own cAMP. Adenyl cyclase minus cells, made permeable by EDTA treatment (Leive, 1965), were preincubated with varying amounts of exogenously supplied cAMP and then induced for both enzymes simultaneously. Tryptophanase and β-galactosidase levels were then measured after 20 min of induction (Figure 2). At 1 × 10^{-4} *M* cAMP the inducibility of β-galactosidase is 50% whereas tryptophanase is not induced. To obtain the same inducibility (50%) with tryptophanase four times more cAMP (4 × 10^{-4} *M*) is required. Thus, these results are consistent with the previous ones.

Effect of Osmotic Pressure on the Induction of β-Galactosidase and Tryptophanase Synthesis. We found that addition of 12% sucrose to a minimal salts medium supplemented with 0.4% glycerol causes a decrease of the intracellular cAMP molarity from 4 × 10^{-5} to 10^{-5} *M* (averages of eight different measurements during logarithmic growth) in the F^- strain used for these experiments. This is reflected by a dual effect: (1) the differential rate of β-galactosidase synthesis is decreased by a factor of 2 (Figure 3c) and (2) tryptophanase synthesis cannot be induced at all in the presence of sucrose whereas β-galactosidase is still inducible (Figure 3b). The control is shown in Figure 4a where both enzymes have been induced in the absence of sucrose. This situation is not specific for the strain used since the same result was obtained in an Hfr strain P4X8 (Figure 5). In this case the effects of adding 0.5% Casamino acids to the minimal salts medium was superimposed on the effect of sucrose. The catabolite effect of Casamino acids on β-galactosidase synthesis was not detectable in this strain at

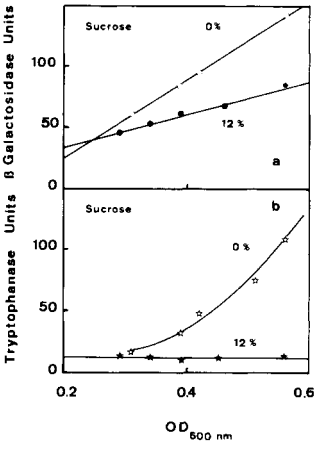

FIGURE 4: Superimposed effects of Casamino acids and sucrose addition. (a) The differential levels of synthesis of β-galactosidase were compared in cultures of strain P4X8 growing on M9/glycerol medium supplemented with 0.5% Casamino acids without sucrose addition (○) or with 12% sucrose addition (●). (b) The differential levels of synthesis of tryptophanase were compared under similar conditions: without sucrose addition (☆), or with 12% sucrose addition (★).

cell density beyond 1.2 × 10^8 cells/ml ($OD_{600\,nm}$ = 0.2) whether or not sucrose was present (Figure 5a). However, once again the differential rate of synthesis is decreased by a factor of 2 when the osmolarity of the medium is increased (Figure 5a). Conversely, both the catabolite effect of Casamino acids and the osmotic effect of sucrose are effective on tryptophanase induction (Figure 5b). The maximal rate of synthesis is only reached at a cell density beyond 1.8 × 10^8 cells/ml ($OD_{600\,nm}$ = 0.3) when sucrose is not added. This difference with β-galactosidase synthesis is consistent with the results presented in Figure 1. In the presence of 12% sucrose, tryptophanase synthesis cannot be induced at all. This difference reinforces the evidence for a high requirement of cAMP for tryptophanase induction.

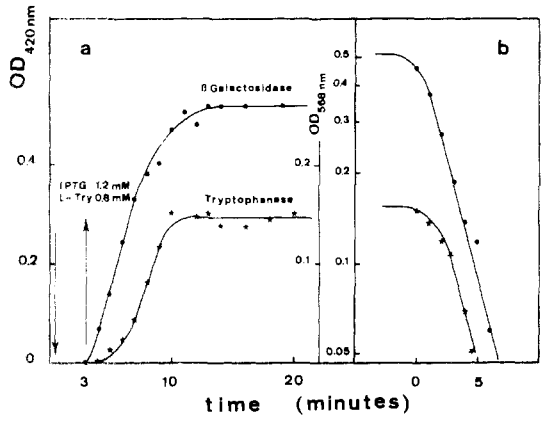

FIGURE 5: Early kinetics of expression of β-galactosidase and tryptophanase genes. A culture of P4X8 growing exponentially in medium 63 glycerol was filtered, washed, and resuspended in 0.1 volume of the same medium containing 1.2 mM IPTG and 0.8 m*M* L-tryptophan for 3 min. (a) The inductions of β-galactosidase (●) and tryptophanase (★) were stopped by a tenfold dilution in 63 medium and enzyme levels were determined at intervals. (b) Semilogarithmic representation of the difference between β-galactosidase (●) and tryptophanase (★) values at the plateau and at time *t*.

The osmotic effect of sucrose seems to be a threshold phenomenon since only 1% sucrose added in the minimal salts medium is sufficient to prevent the induction of tryptophanase, whereas the differential rate of β-galactosidase synthesis is decreased in a stepwise manner. This rate was 75% in the presence of 4–8% sucrose and 43% in the presence of 12% sucrose, as compared to the rate observed in the absence of sucrose.

Kinetics of Expression of β-Galactosidase and Tryptophanase Genes. Early kinetics of induced β-galactosidase and tryptophanase synthesis have been determined (Jacquet and Kepes, 1969). An exponential culture of a wild-type strain P4X8, growing on medium 63 glycerol was submitted to a 3-min pulse induction by 1.2 m*M* IPTG and 0.8 m*M* L-tryptophan (Figure 5). The induction was stopped by dilution. The time course of expression of messenger RNA initiated during the pulse was followed by sampling on chloramphenicol (100 μg/ml) at intervals and measuring β-galactosidase and tryptophanase activities. It can be seen that following the deinduction process, enzyme accumulation proceeds for approximately 10 min in both cases. This is in agreement with the results previously published by Bilezikian et al. (1967) and Jacquet and Kepes (1969). But we find that the maximal rate of synthesis is reached 3 min later with tryptophanase than with β-galactosidase. This result is in agreement with early experiments of Pardee and Prestidge (1961) where the deinduction process was not applied. However, this observation is at variance with the results reported by Bilezikian et al. (1967). Although there is a difference in the kinetics of expression of the two enzymes (Figure 5a), the half-life of the coding capacity of messenger RNA was exactly the same in both cases, as expected (Figure 5b).

Discussion

Four types of experiments demonstrated that induction of tryptophanase requires higher cAMP levels than the lactose operon. A similar difference was recently observed between the L-arabinose operon and the lactose operon (Lis and Schleif, 1973). However, in this study, the endogenous levels of cAMP were not measured. Here we found (Figure 1) a fourfold difference in the cAMP requirements for induction of tryptophanase and β-galactosidase. Whereas full induction is obtained with intracellular concentrations of about 3×10^{-5} *M* cAMP for β-galactosidase (Figure 1), much higher levels are required (2.5×10^{-4} *M*) when cAMP is provided externally (Figure 2). The experiments described in Figures 1 and 3 have been carried out with different strains and comparisons of concentrations might not be totally meaningful. However, this points to a barrier of permeability for cAMP when provided from outside the cell. This has been previously emphasized by different authors (Lis and Schleif, 1973; Ullmann, 1971). We have noticed that the fourfold difference for the cAMP requirements, found in Figure 1, is again found in Figure 2. This might not be a simple coincidence but may reflect a true situation where the existence of the permeability barrier would just shift the absolute concentrations without modifying the difference of requirement. To our knowledge, it has never been shown before that an increase in the osmolarity of the medium causes a decrease in the intracellular cAMP level. This level depends upon cAMP synthesis, its degradation, and its release from the cell. cAMP phosphodiesterase is not the primary element of control (Buettner et al., 1973; Perlman and Pastan, 1971). Since adenylate cyclase activity is not very likely to change upon sucrose addition, the decrease in cAMP concentration is probably achieved by a partial release of the cAMP synthesized. The rate of exit of this nucleotide would then increase with the osmolarity of the medium. The mechanism of cAMP exit is not at present understood. However, the relationship between the osmotic pressure and the rate of exit of cAMP points to a simple diffusion process across the cell envelope in *E. coli*. This would be very consistent with the recent results of Potter et al. (1974). These authors have shown that CRP^- mutants (deficient in cAMP receptor protein) excrete cAMP at an abnormally high rate compared to the wild-type strain (CRP^+), which can be correlated with the abnormally high intracellular levels of cAMP in CRP^- cells.

Several hypotheses can be presented for explaining the fact that different genes submitted to the same control, by the same effectors (cAMP and CRP), respond differently.[2] The most simple explanation would be that the difference resides in the promoters involved. This in turn would imply that the affinity of promoters for the cAMP-CRP complex is variable. To our knowledge this has never been shown. However, it has been reported (Perlman and Pastan, 1971) that in one strain with a point mutation in the lac promoter L8 (Scaife and Beckwith, 1966) an increased concentration of cAMP was required to overcome glucose repression of β-galactosidase synthesis (Perlman and Pastan, 1971). Furthermore, low-efficiency promoters do exist as described in the tryptophan operon of *E. coli* (Morse and Yanofsky, 1968). We do not know if the slower kinetics of elementary wave of tryptophanase compared to β-galactosidase is related at all to the difference of cAMP requirement. However,

[2] Earlier work from Pastan and Perlman ((1969), *J. Biol. Chem. 244*, 2226–2232) suggested that cAMP increases the accumulation of tryptophanase even when added to cultures in which RNA synthesis had been inhibited by proflavine or actinomycin D. After further work, these authors mentioned that "the conclusions reached in these studies must be tested with the more sophisticated methods that are now available for studying gene regulation" (Perlman and Pastan, 1971). These early results were disregarded in the interpretation of our studies.

it is tempting to suggest that a lower frequency of initiation causes this latency during the first minutes of induction. Then the same steady state of the frequency of initiation for tryptophanase and β-galactosidase is reached.

Finally it must be emphasized that cAMP and CRP might not be the only effectors of catabolite repression, as suggested by recent results of Ullmann (1974) and Wayne and Rosen (1974). For instance, besides cAMP, a negative effector might exist. In fact it has been recently reported that intracellular cGMP concentration directly antagonizes the variation of cAMP levels during growth of *E. coli* (Bernlohr et al., 1974) and that cGMP inhibits the synthesis of β-galactosidase and tryptophanase in this organism (Artman and Werthamer, 1974). Thus the difference of cAMP requirements that we observed for the induction of two catabolite-repressible enzymes might in fact reflect a more complex situation than the hypothesis presented.

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References

Artman, M., and Werthamer, S. (1974), *J. Bacteriol. 120,* 980–983.

Beckwith, I., Grodzicker, T., and Arditti, R. (1972), *J. Mol. Biol. 69,* 155–160.

Bernlohr, R. W., Haddox, M. K., and Golberg, N. D. (1974), *J. Biol. Chem. 249,* 4329–4331.

Bilezikian, J. P., Kaempfer, R. O., and Magasanik, B. (1967), *J. Mol. Biol. 27,* 495–506.

Buettner, M. J., Spitz, E., and Rickenberg, H. V. (1973), *J. Bacteriol. 114,* 1068–1073.

Cailla, H. L., Racine-Weissbuch, M. S., and Delaage, M. A. (1973), *Anal. Biochem. 56,* 394–407.

De Crombrugghe, B., Chen, B., Anderson, W., Nissley, P., Gottesman, M., Pastan, I., and Perlman, R. (1971), *Nature (London), New Biol. 231,* 139–142.

De Crombrugghe, B., Perlman, R. I., Varmus, H. E., and Pastan I. (1969), *J. Biol. Chem. 244,* 5828–5835.

Emmer, M., De Crombrugghe, B., Pastan, I., and Perlman, R. (1970), *Proc. Natl. Acad. Sci. U.S.A. 66,* 480–487.

Jacquet, M., and Kepes, A. (1969), *Biochem. Biophys. Res. Commun. 36,* 84–92.

Leive, L. (1965), *Proc. Natl. Acad. Sci. U.S.A. 53,* 745–750.

Lis, J. T., and Schleif, R. (1973), *J. Mol. Biol. 79,* 149–162.

McFall, E., and Mandelstam, J. (1963), *Biochem. J. 89,* 391–397.

Monod, J., Cohen-Bazire, G., and Cohn, M. (1951), *Biochim. Biophys. Acta 7,* 585–591.

Morse, D. E., and Yanofsky, C. (1968), *J. Mol. Biol. 38,* 447–451.

Moses, V., and Prevost, C. (1966), *Biochem. J. 100,* 336–353.

Pardee, A. B., and Prestidge, L. S. (1961), *Biochem. Biophys. Res. Commun. 36,* 84–92.

Perlman, R. L., and Pastan, I. R. (1971), *Curr. Top. Cell. Regul. 3,* 117–134.

Potter, K., Chaloner-Larsson, G., and Yamazaki, H. (1974), *Biochem. Biophys. Res. Commun. 57,* 379–385.

Riggs, A. D., Reiness, G., and Zubay, G. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 1222–1225.

Scaife, J., and Beckwith, J. R. (1966), *Cold Spring Harbor Symp. Quant. Biol. 31,* 403–410.

Steiner, A. L., Kipnis, D. M., Utiger, R., and Parker, C. (1969), *Proc. Natl. Acad. Sci. U.S.A. 64,* 367–373.

Ullmann, A. (1971), *Biochimie 53,* 3–6.

Ullmann, A. (1974), *Biochem. Biophys. Res. Commun. 57,* 348–352.

Ullmann, A., Perrin, D., Jacob, F., and Monod, J. (1965), *J. Mol. Biol. 12,* 918–923.

Wayne, P. K., and Rosen, O. M. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1436–1440.