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Regulation of *lac* Operon Expression: Reappraisal of the Theory of Catabolite Repression

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The physiological state of *Escherichia coli* with respect to (permanent) catabolite repression was assessed by measuring the steady-state level of β-galactosidase in induced or in constitutive cells under a variety of growth conditions. Four results were obtained. (i) Catabolite repression had a major effect on fully induced or constitutive expression of the *lac* gene, and the magnitude of this effect was found to be dependent on the promoter structure; cells with a wild-type *lac* promoter showed an 18-fold variation in *lac* expression, and cells with the *lacP37* (formerly *lac-L37*) promoter exhibited several hundred-fold variation. (ii) Exogenous adenosine cyclic 3',5'-monophosphoric acid (cAMP) could not abolish catabolite repression, even though several controls demonstrated that cAMP was entering the cells in significant amounts. (Rapid intracellular degradation of cAMP could not be ruled out.) (iii) Neither the growth rate nor the presence of biosynthetic products altered the degree of catabolite repression; all variation could be related to the catabolites present in the growth medium. (iv) Slowing by imposing an amino acid restriction decreased the differential rate of β-galactosidase synthesis from the wild-type *lac* promoter when bacteria were cultured in either the absence or presence of cAMP; this decreased *lac* expression also occurred when the bacteria harbored the catabolite-insensitive *lacP5* (formerly *lacUV5*) promoter mutation. These findings support the idea that (permanent) catabolite repression is set by the catabolites in the growth medium and may not be related to an imbalance between catabolism and anabolism.

The presence of glucose in growth media often has a severe effect on catabolic enzyme expression—a phenomenon originally referred to as the glucose effect (5). Two decades ago Neidhardt and Magasanik (14) and later Mandelstam (9) demonstrated that many catabolizable substrates were capable of eliciting repression of catabolic enzyme synthesis. An hypothesis accounting for this repression was originally formulated by Neidhardt and Magasanik (13). Briefly, this hypothesis states that growth conditions that lead to an excess of catabolism over anabolism will reduce the synthesis of catabolic enzymes (8). The term "catabolite repression" was coined to describe this general control (8), which now is known to be involved in the regulation also of some enzymes not involved in carbon and energy source metabolism (16). Whereas most early studies of catabolite repression dealt with the permanent phase of catabolite repression, a much more severe type of repression referred to as transient repression (10, 15, 22) occurs after addition of glucose to a growing culture. Whether or not transient repression and permanent catabolite repression are controlled by the same mechanism(s) remains uncertain.

Adenosine cyclic 3',5'-monophosphoric acid (cAMP) was found to abolish transient repression and to increase the level of catabolic enzyme expression under conditions of permanent catabolite repression (18, 25). Subsequently, both in vivo and in vitro evidence demonstrated a role for cAMP and a protein factor known as CAP (catabolite gene activator protein [19]), or cAMP regulator protein (4) for high-level expression of the catabolite repression-sensitive *lac* operon in *Escherichia coli*. This control was shown to act at the promoter site on the DNA, and mutations in the *lac* promoter have been isolated which make the *lac* operon insensitive to catabolite repression (21).

Although cAMP and CAP are necessary for high-level *lac* expression both in vivo and in vitro, their role as exclusive mediators of catabolite repression is not settled (24). Also, because catabolite repression has come into general use as a term to designate inhibited synthesis of

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inducible enzymes, the task remains to determine whether or not all the diverse phenomena called catabolite repression actually share a cAMP-CAP mechanism. Re-examination of the original concept of catabolite repression in the light of new information about cAMP is now appropriate.

We have directed this paper towards defining catabolite repression in the cell under conditions where either the medium composition, the growth rate, or both are varied. We have done this by using *lac* expression controlled by different *lac* promoters as a probe into the physiological state of the cell.

(A preliminary report of part of this work was presented at the Annu. Meet. Am. Soc. Microbiol. New York, N.Y., 27 April to 2 May 1975).

MATERIALS AND METHODS

Media. Morpholinopropane sulfonic acid medium was used for all growth studies and prepared as described previously (12). Carbon sources and supplements were added as listed in Table 1 of reference 27. All components were filter sterilized. Unless specified otherwise, cAMP when present was added at 5 mM.

Bacterial strains. Most strains used in this study were derivatives of our reference strain *E. coli* NC3. This strain is an unmutagenized derivative of *E. coli* B/r which lacks the B-type restriction system. Its derivation has been given previously (27). *E. coli* AS19, an *E. coli* B strain which contains several mutations that increase the permeability of the membrane (20), was used in a few experiments. It was obtained from K. Gausing. *E. coli* NP4, an *E. coli* B strain maintained in this laboratory for several years, was also used in a few experiments.

Genetics. All genetic methods were described previously (27). The *lacP37* (formerly *lac-L37*) catabolite-sensitive *lac* promoter mutation was transduced into a proline auxotroph of our reference strain NC3 by using bacteriophage P1 and selecting for prototrophy. *E. coli* CA8005, which harbors the *lacP37* lesion, was obtained from B. Magasanik. Auxotrophs were isolated by penicillin enrichment at 42°C as described previously (27). Auxotrophs were screened for temperature-sensitive auxotrophy by replica plating at 30 and 42°C. A strain harboring the *leuA*(Ts) lesion was isolated after mutagenesis with *N*-methyl-*N'*-nitrosoguanidine. Bacteriophage P1 transduction was used to demonstrate genetic linkage of this allele to the *ara* operon. By in vitro assay, the strain harboring this *leuA*(Ts) lesion contains a thermolabile 2-isopropylmalate-synthetase. The *leuA*(Ts) allele was transferred into a leucine auxotroph of NC3 by using bacteriophage P1 transduction. Prototrophs were selected at permissive temperature (30°C). Transductants were purified, and, when checked for temperature-sensitive auxotrophy, more than 95% were temperature sensitive. Other auxotrophs were isolated without mutagenesis. The *trpE*(Ts) allele was so designated because the auxotrophy could be satisfied with anthranilate or tryptophan. The *trpE*(Ts) allele was linked to *galU* in P1 transduction. The *trpS*(Ts) allele was shown to be linked to the *aroB* gene. The *met*(Ts) allele was not mapped.

Bacterial growth and enzyme assays. Cultures were grown and assayed as described previously (27). β-Galactosidase assays were performed on sonic supernatant fluids, because this procedure was previously shown to give the most reliable data. Units are micromoles of *o*-nitrophenol produced per minute at 28°C. Growth rates are expressed in terms of the specific growth rate constant k, as calculated from the expression $k = (\ln 2)/(\text{mass doubling time [hours]})$. An optical density at 420 nm of 1.0 is equivalent to 3×10^8 to 3.5×10^8 cells per ml for a logarithmic culture of *E. coli* NC3 growing in glucose minimal medium. The culture conditions used support logarithmic growth to an optical density at 420 nm of 7.0.

Analytical methods. The protein concentration of cell extracts was determined colorimetrically by the method of Lowry et al. (7).

Reagents. Morpholinopropane sulfonic acid, tricine [*N*-tris(hydroxymethyl)methylglycine], cAMP, isopropyl-β-D-thiogalactoside, glycyl-L-proline, 5-bromo-4-chloro-3-indolyl-β-D-galactoside, phenyl-β-D-galactoside (φGal), and *o*-nitrophenyl-β-D-fucoside were purchased from Sigma Chemical Co. Radioactive amino acids were obtained from New England Nuclear Corp. or Schwarz/Mann. *o*-Nitro-β-D-thiogalactoside was purchased from Cyclo Chemical. cAMP was also kindly donated by Asahi Chemical Co. (Nobeoka, Japan).

RESULTS

Effect of medium composition on β-galactosidase synthesis controlled by a wild-type promoter. A great variation in expression of the *lac* operon can be seen in cells under different growth conditions. This variation, which is independent of inducer, is shown in Fig. 1A and B. The specific activity of β-galactosidase in bacteria which are either constitutive or fully induced and carry the wild-type *lac* promoter region is shown in Fig. 1A.

The most noteworthy observations are the following. (i) An 18-fold variation in the specific activity of β-galactosidase was found over a 5.6-fold range in growth rate. (ii) An overall trend of decreasing enzyme activity with increasing growth rate was found, but with large variations in enzyme activity in media supporting similar growth rates. Additional information in Fig. 1A can be summarized by examining four sets of related growth conditions. (iii) The effect of single carbon sources in minimal medium was found to be as follows. If one assigns a value of 100 to the highest specific activity (found in D-alanine minimal medium [no. 23]), then the levels in the other media are 88 in succinate (no. 22), 85 in L-aspartate (no. 19), 74 in pyruvate (no. 20a), 69 in acetate (no. 16), 55 in glycerol (no. 9), 40 in D-serine (no. 21), and 12 in glucose (no. 1). (iv) The effect of a second carbon source in minimal medium was found to be as follows.

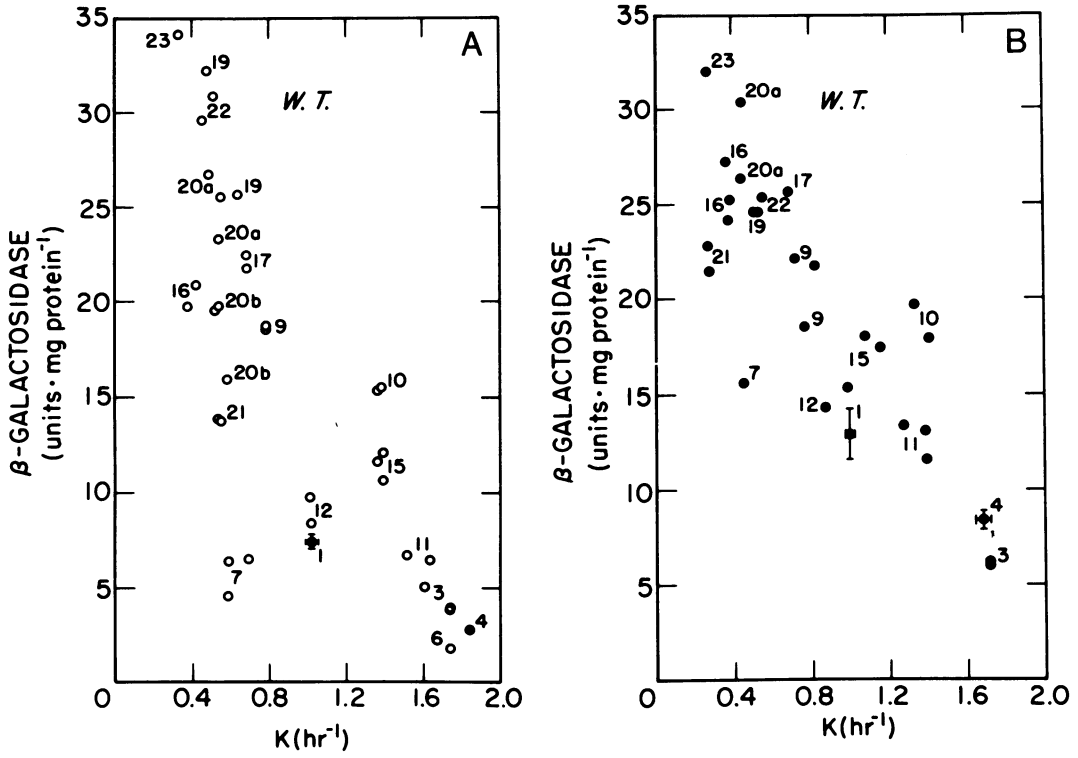

FIG. 1. *β-Galactosidase activity in E. coli NC3 harboring the wild-type lac promoter region. The bacteria were maintained in logarithmic growth at 37°C in the various media for at least 10 generations before removing portions for assays. Both wild-type and full-level constitutive strains were used. Inducible strains were grown in media containing 1 mM isopropyl-β-D-thiogalactoside. The media are identified by number in the body of the figure and are described in Table 1 of reference 27. At an optical density at 420 nm of approximately 0.3, 0.6, and 1.0, samples were taken into prechilled tubes containing sufficient chloramphenicol to give a final concentration of 100 μg/ml in an ice-water bath. After washing by pelleting and suspending the cells, the samples were sonically treated, and the supernatant fluids were assayed for protein and β-galactosidase activity as described previously (27). Each point represents the average value for three samples. Reproducibility of results was always within 10%. Open circles represent fully induced level of β-galactosidase activity from the lac promoter in a cell with wild-type background grown in media without cAMP; closed symbols are for those strains grown in the presence of 5 mM cAMP. When a particular medium was used more than three independent times, the standard deviation is represented by a vertical or horizontal bar drawn through each appropriate point. (A) Cultures grown without exogenous cAMP: medium no. 1 was used for 15 cultures, and medium no. 4 was used for 4 cultures. (B) Cultures grown with a 5 mM cAMP: medium no. 1 was used for five cultures, and medium no. 4 was used for four cultures.*

In all cases examined (no. 7 and 12, and data not shown), the presence of two carbon sources leads to a lower enzyme level than with either single carbon source. (v) The effect of additional carbon sources in rich medium was found to be as follows. The cells were grown in rich medium with any of four carbon sources: acetate, glycerol, glucose, or L-serine. The addition of either glucose (no. 4) or glycerol (no. 11) as a second carbon source to L-serine-rich medium (no. 15) produced a greater repression than that found in rich medium with the single carbon source. (Unlike most amino acids, L-serine is rapidly catabolized in rich media, even in the presence of glucose). The addition of both ribose and fructose to glucose-rich medium led to a further repression of 36% (no. 6 versus no. 4). (vi) The effects of medium supplementation were found to be as follows. Supplementation of acetate, glycerol, or glucose minimal medium cultures with the mixture of amino acids (lacking L-serine), nucleic acid bases, and vitamins in all cases increased the growth rate. Supplementation of acetate minimal medium led to a 70% increase in growth rate and a 9% increase in enzyme activity. For glycerol medium (no. 10 versus no. 9), a near doubling of the growth rate (76% increase) was accompanied by an 18% decrease

in enzyme activity, whereas for glucose medium (no. 3 versus no. 1), a 52% increase in growth rate occurred with a 28% decrease in enzyme activity.

Effect of exogenous cAMP. High-level expression of the lactose operon requires cAMP. Expression of *lac* in the presence of exogenous cAMP was examined, and the results are shown in Fig. 1B. Four general effects of the presence of 5 mM cAMP are evident. (i) The apparent inverse correlation of enzyme level with growth rate seen in the absence of cAMP (Fig. 1A) was more pronounced in its presence. This resulted from a partial quenching of medium-specific effects. (ii) The presence of cAMP usually increased the relative rate of β-galactosidase synthesis, but in a few instances (media no. 9, 17, 19, and 23) appeared to have very little effect. (iii) Media in which there was marked growth rate inhibition by cAMP (media no. 4, 11, 12, 15, and 21) also showed large stimulation of β-galactosidase synthesis; a growth rate inhibition, however, was not a necessary condition for an increase in the enzyme activity (e.g., no. 1 and 10).

If the differences in the level of β-galactosidase expression shown in Fig. 1A are caused by catabolite repression and if cAMP is the sole mediator of catabolite repression, then these effects should have been eliminated in the experiments presented in Fig. 1B. Obviously, this was not the case, but there could be several reasons for the failure of cAMP to overcome the restrictions observed. Several lines of evidence suggest that the ability of cAMP to abolish catabolite repression is not limited by a restriction of entry into the cell. First, the addition of 1 mM cAMP was shown to be sufficient to saturate these cells with respect to β-galactosidase synthesis under many growth conditions examined; *lac* expression was no higher in 10 than 1 mM cAMP in media no. 1, 2, and 16 (data not shown). Second, an adenyl cyclase-deficient mutant of *E. coli* NC3 was examined. Such a mutant grows very poorly; the addition of exogenous (1 mM) cAMP completely restored the growth rate. Finally, under some growth conditions, very little effect of exogenous cAMP was observed, and the possibility that cAMP does not enter the cells was tested with the glucose analog, α-methylglucoside, to induce transient repression (21). Significant repression of β-galactosidase synthesis was observed when α-methylglucoside was added to cultures of NC3 growing in three different media, and the repression was abolished in all cases by the simultaneous addition of cAMP (data not shown). Nevertheless, it is possible that exogenous cAMP is not saturating for the *lac* operon if a higher concentration is needed for *lac* expression than for a normal growth rate and for the relief of transient repression and if this concentration cannot be reached during growth in some media containing cAMP.

Effect of medium composition on β-galactosidase synthesis controlled by a low-level catabolite-sensitive promoter. Data obtained for the *lacP37* promoter, a "down" promoter mutation in the CAP interaction region, are presented in Fig. 2. Significant observations include the following. (i) With the *lacP37* promoter, there was a dramatic decrease in β-galactosidase activity when the growth rate was increased. (ii) The addition of cAMP to glucose minimal medium increased this level over 50%. (iii) The response of the *lacP37* promoter to media of different composition was qualitatively similar to that of the wild type.

Effect of biosynthetic restriction on β-galactosidase synthesis controlled by the wild-type *lac* promoter. A strain of *E. coli* with a wild-type *lac* promoter and a temperature-sensitive first enzyme in leucine biosynthesis was grown in glucose minimal medium containing the gratuitous inducer isopropyl-β-D-thiogalactoside at 37°C (a permissive temperature). Portions were then placed at a variety of restricting temperatures from 38.5 to 40.8°C. These temperature shifts caused an abrupt change to lower growth rates and were accompanied by a severe inhibition of β-galactosidase synthesis for approximately half a mass dou-

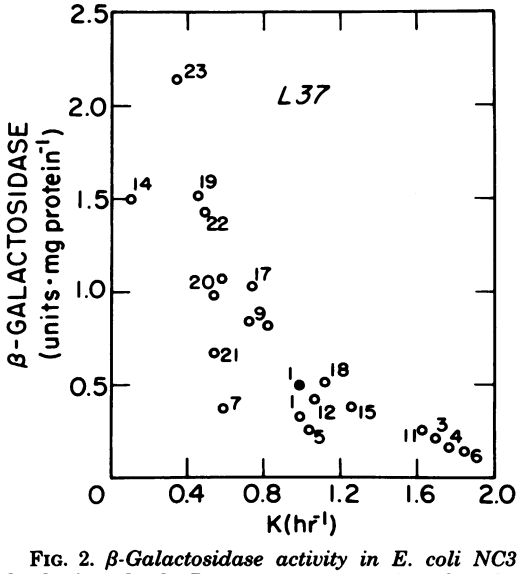

FIG. 2. *β-Galactosidase activity in E. coli NC3 harboring the lacP37 promoter mutation. See the legend to Fig. 1 for key to the media, methods, and symbols used.*

bling. The more pronounced the growth inhibition, the more severe was this temporary inhibition (data not shown). After this transition period, the differential rate of β-galactosidase synthesis increased, but the final steady-state rates during restricted growth were lower than that during unrestricted growth. The more severe the growth restriction (i.e., the higher the temperature), the lower was the final differential rate of enzyme synthesis. Control cultures grown with leucine at the same temperatures showed the normal rate of β-galactosidase synthesis, demonstrating that the lower growth rate during leucine restriction was responsible for the depressed rates of enzyme synthesis.

In Fig. 3 the final steady-state rates of β-galactosidase synthesis are plotted as a function of the specific growth rate constant of the restricted and unrestricted cultures. Figure 3 also displays the results obtained when cAMP was added at the time of the temperature shift. The

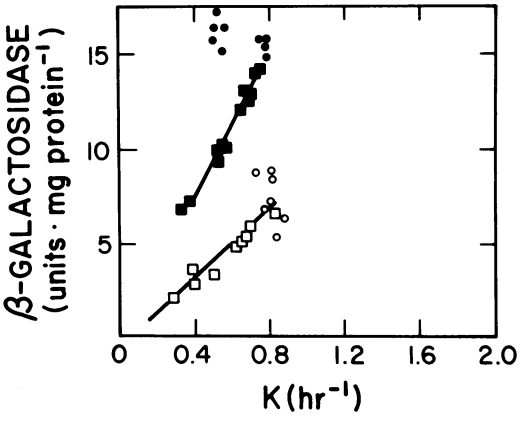

FIG. 3. *β-Galactosidase activity in E. coli NP4 harboring the wild-type lac promoter region and a temperature-sensitive first enzyme in leucine biosynthesis. The bacteria were maintained in logarithmic growth at 37°C in glucose minimal medium containing isopropyl-β-D-thiogalactoside for several generations. A portion of each culture was shifted to a growth-restricting temperature between 38.5 and 40.8°C. A control culture containing leucine was simultaneously shifted. Each point shown represents the slope from a differential plot of the enzyme activity per milliliter of culture versus the amount of protein per milliliter of culture between an optical density at 420 nm of 0.1 and 1.0. At least five portions of the culture were assayed over this interval. Open symbols represent the level of β-galactosidase activity in a cell grown in media without cAMP; closed symbols are for cultures incubated in the presence of 5 mM cAMP. Circles represent unrestricted cultures, and squares represent amino acid-restricted cultures. Solid lines connect points plotted for steady-state cultures.*

temporary, severe inhibition of enzyme synthesis was eliminated, and the final steady-state rate during restricted growth was increased, but the permanent decrease in *lac* expression during leucine restriction was little affected by the presence of cAMP. Several experiments were repeated with a derivative strain that has a full level of constitutive synthesis of β-galactosidase. Identical results were obtained.

The effect of leucine restriction on fully induced *lac* expression was examined in cultures during growth on lactose and on glycerol minimal media. In each case a severe inhibition of β-galactosidase synthesis immediately followed the temperature shift. Afterwards, a more moderate decrease in *lac* expression ensued (data not shown). The addition of cAMP abolished any temporary severe repression and increased the level of β-galactosidase synthesis in the steady-state restricted culture. However, the final steady-state rate in the cAMP-containing restricted culture was always reduced when compared with the cAMP-containing unrestricted control culture.

To test the generality of the results obtained with leucine restriction, strains with a temperature-sensitive tryptophanyl-tRNA synthetase [*trpS*(Ts)] or a temperature-sensitive methionine biosynthetic enzyme were employed. The results from these experiments can be summarized briefly. Shifting each of the three strains from its permissive to a restrictive temperature caused a temporary, severe inhibition of β-galactosidase synthesis for about half a generation. In each case this period was followed by a lowered steady-state rate of enzyme synthesis during prolonged growth at the restricted rate. The addition of cAMP eliminated the severe transient inhibition for methionine limitation as for leucine limitation, but had no effect on the tryptophanyl-tRNA-limited culture. The final differential rate of β-galactosidase synthesis in the amino acid-restricted cultures containing cAMP in all cases represented a great reduction from the rate in the control cAMP-containing unrestricted culture (data not shown).

Expression of the *lacP5* promoter. The *lacP5* (formerly *lacUV5*) promoter of the *lac* operon is insensitive to catabolite repression. In a previous study we have shown that the level of *lac* expression from this promoter is unaffected by cAMP and is virtually constant at different growth rates (27). The effects of biosynthetic restriction on the function of this decontrolled operon were examined with the temperature-sensitive *leuA* enzyme. The results (Fig. 4) were that slow, biosynthetically restricted growth led to decreased rate of β-galactosidase synthesis both in glucose minimal me-

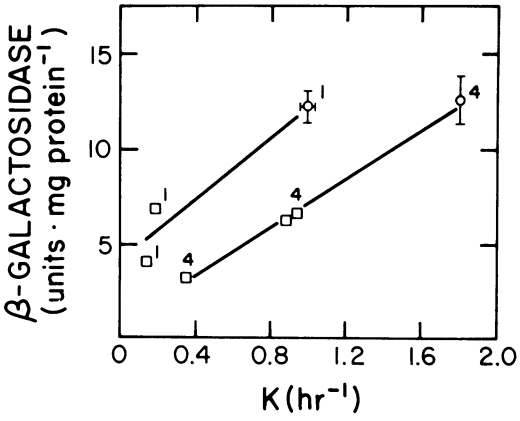

FIG. 4. *β-Galactosidase activity in E. coli NC3 harboring the lacP37 lacP5 promoter mutation and a temperature-sensitive first enzyme in leucine biosynthesis. Bacteria were cultured as in Fig. 1 at a variety of temperatures between 37 and 40.8°C to impose amino acid restriction. The bacteria were grown in either glucose minimal (no. 1) or glucose-rich medium (no. 4), except lacking leucine. The circles represent unrestricted cultures, and the squares represent amino acid-restricted cultures.*

dium and in glucose-rich medium (lacking leucine). An analogous experiment examined the effect of tryptophan restriction on β-galactosidase synthesis controlled by the *lacP5* promoter by using a strain carrying a *trpE*(Ts) allele. During tryptophan restriction a decreased differential rate of β-galactosidase synthesis was also observed (data not shown).

DISCUSSION

We have used several tactics to alter the balance between catabolism and anabolism: changing the carbon source, supplying additional carbon sources, supplementing the medium with biosynthetic products, and restricting the synthesis of individual amino acids. To facilitate discussion of our results, we first propose to define catabolite repression as an active inhibition of the differential rate of catabolic gene expression that (i) is independent of regulatory factors unique to a particular operon and, thus, is exerted on a variety of operons coding for catabolic enzymes; (ii) is exerted in the promoter region of catabolic operons and, thus, is sensitive to promoter structure; and (iii) is normally brought about by catabolites in the medium. (According to this definition, repression that is affected by the cAMP-CAP system would constitute one form of catabolite repression, which we might simply designate as cAMP-mediated catabolite repression.) In short, catabolite repression is a general effect, acting on initiation of transcription and brought about by catabolites.

Growing *E. coli* in different culture media resulted in an 18-fold variation in the level of constitutive β-galactosidase synthesis. Most, if not all, of this variation is caused by catabolite repression, as established by several observations. (i) The variation is present whether the *lac* operon is fully induced or the *lacI* gene repressor is inactivated by mutation. (ii) Practically no variation in β-galactosidase synthesis is observed when a cell carrying a catabolite repression-insensitive *lac* promoter is grown under identical conditions (27); most of the variation is eliminated in a cell carrying a deletion of the CAP interaction region of the promoter (27). Therefore, this variation is promoter specific. (iii) The variation is dependent upon the presence of catabolites in the growth medium, but is not dependent upon the presence of biosynthetic products.

Much of the variation was eliminated by growing *E. coli* in the presence of cAMP, and this component we call cAMP-mediated catabolite repression. Still, a very large repression (greater than fivefold) which is promoter dependent and carbon source dependent, and which apparently is growth rate related, remains when the bacteria are cultured in the presence of cAMP. Substantial evidence indicates that this repression is catabolite repression. Whether it is mediated by cAMP is uncertain. The repression cannot be reversed by adding cAMP, but our data do not rigorously exclude a failure of exogenous cAMP to produce a sufficiently high internal level to saturate the *lac* operon. Much better information on this point comes from the more recent work of Dessein et al. (1) who showed that mutants of *E. coli* deleted for the adenyl cyclase gene and possessing a second mutation in either the *lac* region or the CAP protein still exhibit repression during nitrogen limitation and enhanced expression of the *lac* operon during carbon source limitation. Related work by Dessein et al. (2) provides evidence that the catabolite modulator factor described by Ullmann et al. (26) may be an additional effector in catabolite repression, possibly acting on the CAP protein. Our results are consistent with this hypothesis and also with the work of Epstein et al. (3) who reported a few exceptions to the overall close correlation of internal cAMP levels and *lac* expression in different media. The possibility that this variation is caused by changes in the amount of CAP in the cell has been ruled out by direct measurements of the CAP protein in the strain used here under a variety of growth conditions (Smith and Neidhardt, unpublished data).

Enrichment of the growth medium with amino acids and nucleic acid bases leads to a very severe repression in β-galactosidase synthesis, and much of this repression was eliminated by addition of cAMP. Several individual amino acids could be added, however, with very little change in *lac* expression (data not shown). Once the rapid catabolism of the amino acid L-serine in rich medium was recognized, most of the amino acid-induced repression could be eliminated simply by removing L-serine from the supplementation mixture. We also demonstrated that L-proline is catabolized, albeit more slowly, in rich medium (data now shown). These data support the idea that supplementation only with catabolizable amino acids leads to repression of *lac* gene expression. Little, if any, repression occurs when amino acids and nucleic acid bases are added which are not substantially catabolized, i.e., in rich medium, any catabolite imbalance seems to be due to the presence of catabolizable supplements.

When a strain carrying the catabolite-sensitive *lacP37* promoter mutation was examined, qualitatively similar results were obtained. Comparison of the *lacP37* data with that obtained for the *lacP1* deletion-bearing strain reported previously (27) reveals a major component of β-galactosidase gene activity in the *lacP37*-carrying strain which is not dependent upon catabolite repression, particularly at faster growth rates, and we presume that this represents activity of the *lacP1* promoter. By subtracting the level of enzyme synthesized in the *lacP1* strain from that in the *lacP37* strain, a several hundred-fold variation in gene activity in the different media is revealed. Therefore, the sensitivity of an operon to catabolite repression is dependent upon the promoter structure and can be greatly enhanced by mutations within the CAP interaction site.

When the growth rate was slowed below that of minimal medium by restricting the rate of synthesis of a required amino acid, a repression of β-galactosidase was found. A similar repression was seen in both the presence and absence of cAMP as well as in strains harboring the catabolite repression-insensitive *lacP5* promoter. This latter repression is therefore not catabolite repression because it does not meet the criterion of promoter specificity. Although the behavior of amino acid-restricted cultures superficially fits the predictions of the original catabolite repression hypothesis (8), deeper analysis reveals the possibility of different underlying mechanisms. Hansen et al. (6), for example, have described the synthesis of incomplete polypeptide chains of β-galactosidase during restricted protein synthesis, and have ascribed this to endonucleolytic cleavage of mRNA during slow or interrupted ribosome travel along the molecule. At this point, the detailed mechanism is not entirely clear. The temporary severe inhibition upon amino acid restriction at least in some cases has a cAMP involvement, although this may be an indirect one. In this context, we can distinguish the severe temporary repression observed during amino acid restriction from glucose-induced transient repression (23). Transient repression does not occur in cells bearing the *lacP5* promoter, but temporary repression brought on by leucine restriction does (this laboratory, unpublished data).

Finally, it should be noted that the extensive range of *lac* operon expression presented in this study provides a measure of the catabolic state of the cell, because catabolite-insensitive promotion of this operon virtually eliminates the differences seen here (27). This measure (of what we might call the catabolite potential) will be useful in examining the expression of other inducible, catabolic operons and also in the study now underway of the pattern of regulation of *E. coli* proteins resolved on two-dimensional polyacrylamide gels (11, 17). Discovering what fraction of the cell's protein is regulated in this manner is necessary for an understanding of how the composition of the growth medium determines the bacterial growth rate.

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