Advances in Cyclic Nucleotide Research

Volume 7

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ADVANCES IN CYCLIC NUCLEOTIDE RESEARCH

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Preface

The number and variety of biological processes in which cyclic nucleotides have been implicated continues to increase at an undiminished rate. It has now become virtually impossible for any single investigator (or any pair of editors, for that matter) to keep up with all the advances that are currently being made in this area of research. The reviews in this volume of *Advances in Cyclic Nucleotide Research* summarize a number of important new developments and insights, including the important methodological advance represented by immunofluorescence techniques.

We hope our readers will be able to apply some of this information to their own specialized fields of interest, in addition to being brought up to date in the fields under discussion.

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Cyclic Nucleotides in Bacteria

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I. INTRODUCTION

The last 10 years have witnessed a tremendous increase in our understanding of the biology of cyclic nucleotides. Many of the most important studies in this area have been done in microbiological systems. Our perspective on the role of cyclic AMP in transcription and catabolite repression, although by no means complete, is now reasonably clear. The field has often been reviewed, as recently as 1974 (1). It is therefore my intention to cover the important areas of cyclic AMP biochemistry in bacteria, but with the primary emphasis on recent developments. The review undoubtedly reflects some of my biases, particularly with respect to the space allotted the enzymes of cyclic AMP metabolism and the mechanism of catabolite repression, as these have been the focus of my interests in cyclic nucleotide research.

II. DISTRIBUTION OF CYCLIC AMP IN BACTERIA

Cyclic AMP has been shown to be present in a number of bacterial species and related microorganisms. The recent review by Rickenberg (1) lists some of these sources and some new additions to the literature in this area have been made. *Vibrio cholerae* require cyclic AMP for the formation of flagella and somatic antigens (2). Mutagenesis with nitrosoguanidine followed by screening for pleiotropic sugar-negative strains led to the isolation of strains that require exogenous cyclic AMP for fermentation of sucrose, trehalose, fructose, maltose, and mannose. It was assumed that cyclic AMP-responding strains were adenylyl cyclase mutants.

Cohen (3) measured the levels of cyclic AMP in *Phycomyces* during a test of response to light. Within 1 min of light stimulation, cyclic AMP levels dropped to about 50% of the dark value. The sharp decrease in cyclic AMP suggested that the light receptor for *Phycomyces* is a nucleotide cyclase or a phosphodiesterase or, perhaps, is closely associated with one of these enzymes such that light could inhibit adenylyl cyclase or stimulate phosphodiesterase.

Adenylyl cyclase and cyclic AMP phosphodiesterase activities were identified in the photosynthetic algae *Euglena gracilis* (4). Whereas the adenylyl cyclase was associated with the membrane fraction, the cyclic AMP phosphodiesterase was associated with chloroplasts. There was no detectable guanylyl cyclase activity. Pyruvate (5 mM) had no effect on the adenylyl cyclase activity. The cyclic AMP content of whole organisms did not differ substantially in light- or dark-grown cells, thereby providing no evidence that cyclic AMP is involved in the control of chloroplast development.

In contrast to the growing literature documenting the presence of cyclic AMP in many microorganisms, there is also a developing literature indicating the absence of cyclic AMP in some bacteria. For example, Setlow (5) was unable to detect cyclic AMP in *Bacillus megaterium*. Using a protein kinase binding assay for cyclic AMP, this nucleotide could not be detected in either vegetative or sporulating cells of *Bacillus megaterium*. The conditions for purification, concentration, and assay of the samples would have detected a concentration in cells of 1×10^{-9} M. Attempts to demonstrate the activity of the enzymes of cyclic AMP metabolism (adenylyl cyclase and cyclic AMP phosphodiesterase) in extracts were also unsuccessful. Setlow concluded that cyclic AMP may not be a universal regulatory molecule and that it is not involved in the regulation of sporulation in *B. megaterium*. He suggested that some other small molecule must take the place of cyclic AMP in *Bacilli*.

A search for cyclic AMP in *Lactobacillus plantarum* also suggested the absence of this nucleotide or related enzymes in this organism. No cyclic

AMP was detected in cells either starved or undergoing induction. Assay of extracts showed no evidence for adenylyl cyclase or cyclic AMP phosphodiesterase activities, nor was there any inhibitor in the extracts that depressed the respective enzyme activities demonstrable in *E. coli* extracts. Exposure of cell extracts to labeled ATP did not yield cyclic AMP (6). Studies of catabolite repression in *Lactobacillus plantarum* (7) indicated that glucose, but not α-methyl glucoside, inhibited β-galactosidase synthesis. Glucose exerted its effect through its ability to exclude galactose or lactose entry into the cell. Cyclic nucleotides did not relieve the glucose inhibition. Because glucose inhibited the induction of the enzymes required for lactose and galactose utilization at the level of inducer transport, the mechanism of the glucose effect was through catabolite inhibition rather than catabolite repression. The diauxic pattern of growth of *Lactobacillus plantarum* on a glucose–lactose mixture suggested that both compounds regulate the level of β-galactosidase. This was borne out by the findings that galactose or lactose induced the synthesis of the enzyme, whereas glucose inhibited its synthesis. Galactose or a metabolite thereof is the physiological inducer.

The genus *Bacteroides* include strictly anaerobic, gram-negative, nonspore-forming bacteria. Hylemon and Phibbs (8) presented the first evidence for the absence of cyclic AMP in a gram-negative bacterium. Cyclic AMP was not detected in whole cells, expended culture medium, or culture supernatant fluid of selected strains of *Bacteroides fragilis*. Neither adenylyl cyclase nor cyclic AMP phosphodiesterase activities were detected in cell extracts. Addition of dibutyryl cyclic AMP or sodium cholate to cultures growing in lactose did not significantly affect the specific activity of β-galactosidase measured in extracts. Nor was there a diauxic growth pattern in medium containing glucose and lactose.

Ullmann (9) recently argued that because cyclic AMP was demonstrated to have effect on catabolite repression does not necessarily indicate that an organism has a cyclic AMP–CRP system. Ullmann has used the *Bacillus megaterium* system as an example of a case in which neither cyclic AMP nor adenylyl cyclase, nor cyclic AMP phosphodiesterase has been demonstrated. This should be contrasted with the view proposed by Rickenberg (1) who argued that the cyclic AMP system is ubiquitous in prokaryotes and that wherever the formation of a bacterial protein is controlled by catabolite repression, cyclic AMP plays a regulatory role. Apparently, the ubiquity of cyclic AMP in bacteria is still an open question.

III. EFFECTS OF CYCLIC AMP ON GROWTH OF *E. coli*

Several recent reports of cyclic AMP effects on the growth of *E. coli* have appeared. At low streptomycin concentrations (2.5 μg/ml), *E. coli*

growing in a salts-glycerol medium lost viability following a 2½-hr lag period (10). Glucose repressed this bactericidal effect, and the addition of cyclic AMP reversed the glucose repression.

The question of whether cyclic AMP metabolism is coupled to cell division has been explored (11). Using a temperature-sensitive mutant of *E. coli*, the intracellular level of cyclic AMP was measured at the permissive and restrictive temperatures. It was found that the intracellular level of cyclic AMP is not altered when cell division stops. This situation is different from that found in certain mammalian cells in which, under contact inhibition or after exposure to prostaglandin E_1, there is an increase in endogenous cyclic AMP.

The growth of *E. coli* under anaerobic conditions is stimulated by cyclic AMP (12). The addition of cyclic AMP to a cyclic AMP-deficient strain of *E. coli* stimulated both growth and production of the formic hydrogenlyase system. It was also observed that CO_2 stimulated the anaerobic growth of the mutant in the absence of cyclic AMP. A more typical situation is found with respect to the effect of cyclic AMP on growth under aerobic conditions. Oxidative phosphorylation activity is repressed by growth on glucose and is derepressed by 5 mM cyclic AMP in the presence of glucose (13). It was also shown (14) that a cyclic AMP-requiring mutant of *E. coli* K-12 grows slowly on glucose and contains reduced levels of cytochrome b_1 and cytochrome oxidase *o*. Addition of exogenous cyclic AMP stimulated the synthesis of these cytochrome components and restored growth on glucose to the normal rate.

The growth of wild-type *E. coli* K-12 was inhibited when the cells were grown in the presence of cyclic AMP and such carbon sources as D-xylose, L-arabinose, or D-glucose-6-phosphate (15). The mechanism of the inhibition was correlated with the accumulation of toxic levels of methylglyoxal. It was suggested that cyclic AMP leads to the induction of enzymes for xylose utilization, which subsequently results in excessive production of methylglyoxal.

Studies carried out in Torres' laboratory have considerably enhanced our knowledge of the mechanism by which cyclic AMP affects the growth of *E. coli*. *Escherichia coli* Hfr 3000 were growth-inhibited by cyclic AMP when the carbon source was glucose or pyruvate. In the presence of 5 mM cyclic AMP (when glucose was used as carbon source), the generation time changed from 60 min in the absence of cyclic AMP to 90 min in the presence of cyclic AMP. Comparable concentrations of 5′-AMP, ADP, ATP, or adenosine had no effect. When the carbon source was succinate, malate, or glycerol, the growth rate was not affected by cyclic AMP. It was argued (16) that the intracellular cyclic AMP level during growth on these carbon sources was already high; therefore, no effect of added cyclic AMP was seen. The effect of cyclic AMP was reversed by washing cells and resuspending in medium without cyclic AMP, suggesting that the cyclic AMP

effect was not of a toxic nature. Although cyclic AMP showed a growth-inhibiting effect on a wild-type strain, it did not inhibit the growth of a CRP⁻ strain (17). This suggested that the growth inhibition exerted by cyclic AMP was at the transcriptional level and not caused by an effect on an existing system. However, it should be noted that CRP⁻ strains have high cellular cyclic AMP levels. Therefore, the absence of an effect of added cyclic AMP on a CRP⁻ strain may be a reflection of the already high cyclic AMP levels. In agreement with this model is the observation that CRP⁻ strains grew slower than their parents, even in the absence of added cyclic AMP.

When grown on glucose-casamino acids, an adenylyl cyclase deletion required 2 mM cyclic AMP for optimal growth (see Fig. 1A); but higher concentrations of cyclic AMP were shown to inhibit growth. The parental strain showed a different response to added cyclic AMP (see Fig. 1B). Fastest growth was observed in the absence of added cyclic AMP. Growth inhibition was maximal at approximately 5 mM cyclic AMP. Whereas wild-type strains showed no effect of added cyclic AMP on growth when grown in glycerol, the adenylyl cyclase mutant was strongly inhibited by concentrations of cyclic AMP greater than 0.1 mM.

It has been suggested that cyclic AMP can inhibit the growth of *E. coli* by affecting the transport of metabolites (18). In media containing 5 mM cyclic AMP, the incorporation of uracil was lowered about threefold. This inhibition was not an immediate effect of cyclic AMP addition, but required

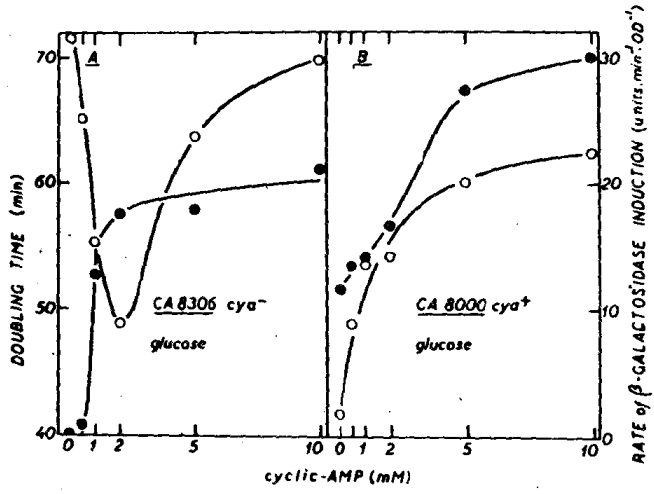

FIG. 1. Effect of cyclic AMP concentration on the doubling times (○) and the rate of β-galactosidase synthesis (●) after induction with 1 mM isopropyl β-D-thiogalactoside of CA 8306 (cya⁻) and CA 8000 (cya⁺) cultures. Cells were grown in AG minimal medium, supplemented with 50 mM glucose, 0.1% vitamin-free casaminoacids, thiamine, and the indicated concentrations of cyclic AMP. Reproduced from De Robertis et al. (17) with permission from Academic Press.

about one generation time (approximately 105 min). This delayed effect of cyclic AMP suggested a cyclic AMP-dependent change in membrane structure. The cyclic AMP-dependent inhibition of transport could be demonstrated either as inhibition of uracil uptake into total cells or into acid-soluble or insoluble fractions. Cyclic AMP also inhibited uracil uptake in the presence of rifampicin, where RNA synthesis was inhibited. The effect was specific since ATP, ADP, or AMP did not replace cyclic AMP. The maximum inhibition was at 5 mM, whereas half-maximal inhibition of uracil uptake was seen at 1.5 mM. Cyclic AMP inhibited uracil uptake when cells were grown on glucose but not on malate or succinate. The suggestion was made that growth on succinate or malate leads to high intracellular levels of cyclic AMP, and therefore any effect of exogenous cyclic AMP would not be expected. The inhibition of cellular growth by cyclic AMP may therefore be explained at least in part by the cyclic AMP-dependent inhibition of the transport of metabolic precursors.

IV. EFFECTS OF CYCLIC AMP ON DIFFERENTIATION AND DEVELOPMENT

Tetrahymena pyriformis is a unicellular organism that synthesizes and stores glycogen when the culture medium contains glucose. The finding that the organism contains catecholamines and that its metabolism of glycogen is affected by adrenergic drugs suggested that its carbohydrate metabolism may be under cyclic AMP regulation. The organisms indeed contain cyclic AMP, adenylyl cyclase, and cyclic AMP phosphodiesterase (19). The concentration of cyclic AMP was high in early logarithmic phase, declined rapidly during growth, and then slowly increased again during the stationary phase. The adenylyl cyclase activity of cells at various periods paralleled the cyclic AMP concentration, whereas the cyclic AMP phosphodiesterase activity showed an inverse correlation, the highest phosphodiesterase activity being present in early stationary phase when the cyclase was lowest. The glycogen concentration in cells reached a maximum when the cells entered early stationary phase. Cultures grown in the presence of glucose had higher concentrations of glycogen, lower concentrations of cyclic AMP, lower levels of adenylyl cyclase, and higher levels of cyclic AMP phosphodiesterase. It was proposed that cyclic AMP levels remained low during exponential growth to allow synthesis of glycogen. At late stationary phase, when glycogen reserves were utilized, cyclic AMP levels went up and the phosphorylase system was stimulated to degrade glycogen.

The sporulating ability of *Saccharomyces cerevisiae* was repressed by glucose (20). This glucose repression of sporulation could be reversed by cyclic AMP, theophylline, or caffeine. It was therefore concluded that cyclic AMP plays a role in some aspect of the sporulation-inducing mechanism.

Cyclic AMP has been implicated in the control of fruiting body formation in the fungus *Coprinus macrorhizus* (21,22). Mycelia of strains that form fruiting bodies contained both adenylyl cyclase and cyclic AMP phosphodiesterase, whereas some strains that do not form fruiting bodies lacked either of these enzyme activities. Growth in high-glucose medium prevented the burst in cyclic AMP levels and also the formation of fruiting bodies. Mycelia grown in high-glucose media had lower amounts of adenylyl cyclase and cyclic AMP phosphodiesterase than did mycelia grown in low-glucose media. The regulation in this system may not be similar to that of a classic catabolite repression system, as the effect of glucose on fruiting body formation was not relieved by exogenous cyclic AMP.

The green alga *Chlamydomonas reinhardtii*, when exposed to methylxanthines, showed inhibition of the function of regeneration of flagella (23,24). To test the hypothesis that these effects involve cyclic AMP, a search was made for the presence of cyclic AMP and enzymes of cyclic AMP metabolism. Small amounts of cyclic AMP (about 25 pmoles/g dry weight) were found. After 1 hr of treatment with 5 mM theophylline, the cyclic AMP level increased more than 10-fold. It was therefore concluded that cyclic AMP levels in this organism are partly controlled by a cyclic AMP phosphodiesterase that is sensitive to theophylline.

Late exponential and stationary phase is the time during which cyclic AMP levels generally rise in *E. coli*. This is also the period in which there is the highest level of competence for transformation in *Hemophilus influenzae*. This correlation suggested that cyclic AMP may stimulate transformation (25). Addition of exogenous cyclic AMP to exponential cultures indeed increased the competence for transformation as much as 10,000-fold. Cyclic AMP added to late exponential or stationary phase cells did not stimulate their already high degree of competence for transformation. It was concluded that *Hemophilus influenzae* probably contain high cyclic AMP levels at late growth stages and that some aspect of competence for transformation is regulated by cyclic AMP, probably in the same manner as cyclic AMP has been shown to affect many other operons.

A number of recent studies presented convincing evidence that cyclic AMP metabolism in *E. coli* and *Salmonella* is important in viral infections. The model that temperate phages use the cyclic AMP system of bacteria in the decision between lysis or lysogeny was presented by Ames and his collaborators (26). When the energy supply is low and cyclic AMP levels are high, phages are integrated into the bacterial DNA to give the lysogenic response. When the energy supply is high (associated with low cyclic AMP levels), synthesis of complete phage is favored to give the lytic response. Mutants of *Salmonella* in the cya, crp, or RNA polymerase genes are lysogenized at reduced frequency by phage P22. The suggestion was made that the cyclic AMP–CRP system is necessary for transcription of those phage operons connected with the synthesis of phage repressor, an element essen-

tial for the lysogenic pathway of phage development. That the effect of cyclic AMP in establishing lysogeny was not specific for phage P22 was evidenced by the observation that cyclic AMP metabolism was also involved in lysogeny of phages MG40 and MG178 (27). Infection of *E. coli* by the lambda phage results in an inhibition of the capacity of the host to respond to inducers of the galactose operon. The idea that this might be an example of catabolite repression was supported by the observation that cyclic AMP reversed the repression of β-galactosidase synthesis due to lambda (28) or bacteriophage N4 (29). Although lambda can influence cyclic AMP metabolism and thereby the metabolism of the host, no evidence could be found that cyclic AMP was necessary in lambda growth. An adenylyl cyclase-deficient mutant supported lytic infections normally. Echols and his coworkers (30) have attempted to test the idea that cyclic AMP plays a direct regulatory role in the lysogenic pathway. In this study, cyclic AMP levels were varied in different ways after infection rather than during prior growth. When the experiments were carried out with this format, no evidence could be obtained for an effect of added cyclic AMP on the synthesis of lambda repressor, as contrasted with an effect on β-galactosidase synthesis. Neither the presence of cyclic AMP in the culture of a cya mutant nor the presence of glucose in the culture of a normal strain had a significant effect on lambda repressor synthesis. It was therefore concluded that changes in cyclic AMP levels do not play an important regulatory role in lambda infection. The mechanism by which lambda infection leads to the inhibition of β-galactosidase synthesis apparently involves a depletion of intracellular cyclic AMP (H. Echols, *personal communication*). Because variations in cyclic AMP levels during infection apparently are not of critical importance to the virus, it was speculated that lowered cyclic AMP levels may be a cellular response to viral infection that serves as a defense mechanism.

Mutations that affect the development of lysogeny can be expressed in other ways. A colicin-tolerant mutant of *E. coli* with a strong bias toward lysogeny has been described (31,32). The mutant favors the lysogenic pathway even though it has reduced cellular concentrations of cyclic AMP. It was proposed that this mutant has an altered RNA polymerase that gives less rightward transcription of the lambda genome with leftward transcription remaining unaffected. The ratio of leftward to rightward transcription may influence the degree of lysogenization. As a result of this change in transcription specificity, the cyclic AMP regulation seen in the parent was not observed in the mutant. Other types of phenomena involving the repression of transcription occur during prophage induction (33). On a rich medium, gal operon expression is reduced as a result of catabolite repression even in the presence of the inducer fucose. Thermal induction of lysogens containing prophages for λh80 or φ80 leads to a significant escape from repression of gal operon expression. This derepression effect is specific for the gal operon and therefore is not caused by an increase in cyclic AMP

levels. In fact, lambda phage production has been shown to lower cyclic AMP levels. It was proposed that phage multiplication leads to production of a factor that can substitute for cyclic AMP in activation and transcription of the gal operon. The situation has been further complicated by the observation that lysogeny of *E. coli* by bacteriophage lambda requires cyclic AMP and CRP or the λcIII gene function (34). The greatest degree of lysogenization occurs when both systems are operative. In the absence of both systems, there is no lysogenization. When the hfl (high frequency of lysogenization) gene is mutant, neither system is required for effective lysogenization. The model was proposed that the hfl gene product antagonizes the action of both the λcIII gene product and the cyclic AMP-CRP system.

Cyclic AMP is also involved in the replication of plasmids (35–37). Glucose inhibits the production of colicins in *E. coli*. The observations that synthesis of colicins requires cyclic AMP and CRP and that the glucose inhibition of colicin synthesis is reversed by exogenous cyclic AMP indicate that colicin synthesis is under the control of catabolite repression. The natural occurrence of plasmids as "relaxation complexes" of supercoiled DNA and protein prompted the question as to whether cyclic AMP stimulated the synthesis of the DNA or the protein component of the complex. It was shown that cyclic AMP addition to cells growing in glucose media resulted in a 6- to 10-fold increase in the rate of synthesis of the protein components and a 3- to 5-fold increase in the rate of synthesis of colicin E1DNA. The stimulation of colicin E1DNA synthesis was independent of protein synthesis but sensitive to rifampicin, an inhibitor of RNA synthesis. This led to the hypothesis that the cyclic AMP stimulation of colicin synthesis occurred through the classic effect of cyclic AMP on RNA synthesis. The finding that replication of colicin E1DNA in the presence of chloramphenicol led to the synthesis of supercoiled molecules containing a stretch of about 25 ribonucleotides inserted into the supercoiled DNA further implicated RNA synthesis in colicin DNA replication. It was therefore suggested that cyclic AMP stimulates the synthesis of colicin-specific primer RNA, a requirement for plasmid DNA replication.

V. REGULATION OF CARBON METABOLISM

Whereas cyclic AMP does not appear to be essential for viability of *E. coli* when the cells are grown in glucose-ammonia medium, it is a necessary cofactor for the transcription of genes for many dispensable functions. The classic report of Perlman and Pastan (38) showed that a strain of *E. coli* isolated by mutagenesis with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine was deficient in adenylyl cyclase activity. This strain could not ferment or grow on lactose, maltose, arabinose, mannitol, or glycerol, and grew slowly on glucose, fructose, and galactose. All of these deficiencies in growth and fermentation properties were corrected by cyclic AMP (2×10^{-3} M). Synthesis

of β-galactosidase (induced by IPTG) in the mutant required the presence of cyclic AMP. It was concluded that cyclic AMP is necessary for the synthesis of many induced enzymes essential to the fermentation of sugars.

A listing of many proteins whose synthesis is regulated by cyclic AMP was recently presented (1). These proteins range from the well-studied lac and gal operon products to GMP reductase to proteins of flagella and the enzymes that modify some antibiotics. Some recent further examples of involvement of cyclic AMP in gene regulation have appeared. The observations generally made are that the synthesis of the enzyme in question is inhibited in the presence of glucose and that the glucose inhibition is overcome by added cyclic AMP. *Pseudomonas aeruginosa*, when grown in the presence of acetylcholine, synthesizes a catabolite-repressible acetylcholinesterase (39). The enzymes of fatty acid degradation in *E. coli* are regulated by cyclic AMP and CRP (40). Synthesis of D-serine deaminase (41) and the biodegradative L-threonine deaminase (42) is repressed by glucose.

A unique observation at the interface between carbon and nitrogen metabolism was made by Ullmann and Monod (43). Studies of β-galactosidase induction in *E. coli* showed that glucose repressed about 30% as compared to succinate when the nitrogen source was ammonia. Although the enzyme level in the presence of succinate was not affected by cyclic AMP, the glucose repression could essentially be completely overcome by 5×10^{-3} M cyclic AMP. The interesting observation was made that with glucose as the carbon source and a dipeptide (e.g., his-glu) as the nitrogen source, the repression was far more severe than if the nitrogen source was ammonia. About 100 times more repression was observed with the dipeptide as compared to that produced with ammonia. With dipeptide as the nitrogen source, 5×10^{-3} M cyclic AMP gave about a 50-fold stimulation of β-galactosidase synthesis.

The mechanism of catabolite repression in yeast may be more complex. The results of studies on *Rhodotorula gracilis* (44) were interpreted to indicate that glucose repression of the synthesis of enzymes for xylose metabolism was caused by prevention of xylose uptake. No monosaccharide tested other than D-glucose repressed the induction of xylose metabolic enzymes. D-Fructose, a hexose which, after phosphorylation, flows into the pathway of glucose metabolism, was ineffective both in preventing xylose uptake and in repressing induced enzyme synthesis. However, when xylose-grown cells were preloaded with xylose, glucose did not interfere with xylose metabolism. It was concluded from these studies that the locus of glucose action is the cell membrane. Competition experiments indicated that both xylose and glucose are taken up by the same membrane carrier, with glucose uptake being highly preferred.

Valine inhibits the growth of *E. coli* K-12. The mechanism of this inhibition is explained by feedback inhibition by valine on an enzyme necessary for isoleucine biosynthesis–acetohydroxyacid synthetase activity. This in-

hibition leads to a condition of isoleucine starvation. It has been recently shown (45) that the enzyme is sensitive to catabolite repression. The addition of exogenous cyclic AMP prevented the valine-induced growth inhibition when cells were grown under catabolite repression conditions. Cells grown in acetate as a carbon source grew rapidly and showed no inhibition by valine. Thus, valine may act competively with cyclic AMP at the level of transcription for acetohydroxyacid synthetase activity.

Maltose utilization in *E. coli* requires the presence of three enzymes—maltose permease, amylomaltase, and maltodextrin phosphorylase. The three enzymes do not constitute a single operon for maltose permease maps at 79 min, whereas the other two enzymes map at 66 min on the *E. coli* chromosome. Nevertheless, all three enzymes are subject to catabolite repression, and cyclic AMP overcomes the repression (46).

L-Arabinose isomerase synthesis is inhibited by ultraviolet irradiation in *E. coli* B/r (47). The inhibition is partially reversed by cyclic AMP, with maximal reversal occurring at a concentration of about 4×10^{-3} M. The effect of cyclic AMP in reversing UV irradiation-induced inhibition of enzyme synthesis was rapid and inhibited by rifampicin. These data indicate that the cyclic AMP effect involved transcription.

Although inducer exclusion may be an important mechanism in catabolite repression in yeast, regulation of cyclic AMP levels also appears to be an important factor. Studies on *Schizosaccharomyces pombe* indicated an inverse correlation between glucose concentration in the medium and intracellular cyclic AMP levels (48). Because glucose represses the synthesis of various induced enzymes in this organism, it was concluded that the catabolite repression is due to a decrease in cellular cyclic AMP. The mechanism by which glucose leads to lowered cyclic AMP levels in yeast is not clear.

VI. REGULATION OF NITROGEN METABOLISM

As indicated by the studies that were pointed out previously, there is substantial evidence that the regulation of the synthesis of enzymes that utilize carbon sources (e.g., sugars) is primarily controlled in a positive sense by cyclic AMP and is therefore influenced by catabolite repression. The elegant studies carried out by Magasanik and his co-workers on the regulation of nitrogen metabolism in *Klebsiella aerogenes* have provided an example in which an element other than cyclic AMP participates in the regulation of enzyme synthesis. The utilization of amino acids as energy sources provides a unique element of metabolic complexity in the sense that not only can the amino acids be used as carbon sources, but they can also be used as nitrogen sources. A great selective advantage is given to *Klebsiella aerogenes* because of its ability to synthesize histidase, the enzyme that degrades histidine, in the presence of glucose when histidine is the sole source of nitrogen.

Obviously, if the histidase were glucose-repressed when histidine was the sole nitrogen source the cells could not grow. However, in the presence of excess ammonia, histidine is not essential and glucose can repress the synthesis of histidase. *Salmonella typhimurium* does not show such selective properties because its histidase is subject to catabolite repression by glucose in both the presence and absence of ammonia in excess.

In *Klebsiella aerogenes*, both histidase and β-galactosidase synthesis are repressed by glucose when a good source of nitrogen such as ammonia is present. Under conditions of nitrogen limitation, as when histidine is used as nitrogen source, the catabolite repression of histidase is relieved while the repression of β-galactosidase remains. In the presence of excess ammonia, the repression of both histidase and β-galactosidase can be relieved by cyclic AMP. However, in limiting ammonia, the repression of histidase synthesis is relieved even in the absence of cyclic AMP, whereas the repression of β-galactosidase is relieved only in the presence of cyclic AMP. It was shown that cyclic AMP is not required for the synthesis of histidase when histidine is used as a nitrogen source. A mutant deficient in adenylyl cyclase was able to make histidase in the absence of added cyclic AMP, whereas the synthesis of β-galactosidase did require the presence of cyclic AMP (49).

Prival et al. (50) presented evidence that glutamine synthetase was the cytoplasmic factor necessary for the cyclic AMP-independent synthesis of catabolite-sensitive enzymes that degrade nitrogenous compounds (histidase and proline oxidase). They showed that despite the relief of catabolite repression of histidase by nitrogen limitation in wild-type strains of *Klebsiella aerogenes*, glutamine-requiring mutants did not show such a relief of catabolite repression under nitrogen-limitation conditions. A number of experiments done in varying strains and different media suggested that high histidase levels were typically accompanied by high levels of glutamine synthetase. Further support for the proposition that glutamine synthetase can play a role in the regulation of histidase synthesis was provided by studies of *in vitro* transcription of the hut DNA (histidine utilization operon) (51). These studies showed that unadenylated glutamine synthetase stimulated hut transcription. The rate of hut transcription was the same whether unadenylated glutamine synthetase or CRP plus cyclic AMP was used to stimulate the system. The unadenylated form of glutamine synthetase is the major form of the enzyme during nitrogen limitation. The adenylated inactive form of glutamine synthetase, which is present primarily during nitrogen excess, had very little stimulatory effect on hut transcription.

These studies suggested that the regulation of the transcription of enzymes for utilization of amino acids such as histidine can operate in two ways, depending on the environmental conditions. In the presence of a good carbon source and a source of excess ammonia, the concentration of glutamine synthetase is low and that of glutamate dehydrogenase is high. Under such