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Cyclic Nucleotides in Procaryotes

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INTRODUCTION

Cyclic nucleotides, particularly adenosine 3',5'-phosphate (cAMP) and guanosine 3',5'-phosphate (cGMP) are found in a variety of cells (10, 71, 142, 210). An understanding of cyclic nucleotide action is a question fundamental to biology. However, as this review will demonstrate, our understanding of these nucleotides in procaryotes is very limited. The mechanisms involved and some of the physiological consequences of changes in levels of cAMP and cGMP are not at all certain, even in the familiar *Escherichia coli*. In most other procaryotes, little is known except that the nucleotides can be isolated and identified, and, if added exogenously, that they can influence some physiological activity.

This review surveys what is known about cyclic nucleotides in procaryotes and is the first general review of the subject since the reviews of Rickenberg (206) and Peterkofsky (181). The review also includes a survey of the literature with respect to cAMP and cGMP metabolism in *E. coli* since the review of Pastan and Adhya (176). Metabolism of cyclic nucleotides in eucaryotic microorganisms will not be reviewed. This was partially reviewed by Peterkofsky (181).

CYCLIC NUCLEOTIDES IN *ESCHERICHIA COLI* AND *SALMONELLA TYPHIMURIUM*

Mechanism of Action of Adenosine 3',5'-Phosphate (cAMP)

The currently accepted model for the mechanism of action of cAMP in *E. coli* and other enteric coliforms was first proposed by Pastan and Perlman (177). This model has been elaborated (176), particularly with respect to the *lac* operon, and is supported by a variety of physiological, genetic, and biochemical evidence.

Briefly, cAMP acts through a cAMP receptor protein (termed CRP or CAP), a dimer having two identical subunits each capable of binding one molecule of cAMP. The CRP has two distinct domains: the N-terminal portion binds to cAMP, and the carboxy terminal end binds to deoxyribonucleic acid (DNA). In the presence of cAMP, the protein undergoes a large allosteric transition (56, 126). In this active conformation, the protein preferentially binds to specific portions of DNA, enabling ribonucleic acid (RNA) polymerase to bind and to initiate transcription at a second distinct site 30 to 50 nucleotides distal to the CRP binding region. The structure of the CRP has been determined by X-ray crystallography (158). Model building suggests that the protein binds to DNA in the atypical left-handed B structure, and this could account for its promoter activity.

The DNA sequences for the relevant region in the *lac* (49), *ara* (76, 134, 239), and *gal* (45, 246) operons have been determined. The DNA sequences of the regions that the CRP binds in these three operons are similar but not identical. It has been proposed that the *ara* operon has two CRP binding regions, one for the promoter for the *araBAD* genes (the structural genes) and one for the promoter for the *araC* gene (the regulatory protein). The *araC* gene product, when acting as a repressor, blocks both of these CRP binding sites, precluding transcription of both *araBAD* and *araC* (154). In the presence of arabinose, the *araC* gene product no longer blocks these CRP binding sites, and transcription is initiated. Apparently the *ara* repressor acts by preventing the CRP from binding, thus precluding initiation of transcription, rather than by binding to a site distal to the initiation site and preventing movement of RNA polymerase down the operon as is the case in the *lac* operon (168). This model is not yet certain. An alternative model has been proposed in which there is a single CRP binding site that affects the activity of both promoters (134).

The galactose operon has two promoters. Initiation of transcription at one of these promoters is inhibited by a CRP-cAMP complex, whereas initiation at the other promoter is stimulated (5, 45, 211). There is a physiological rationale for this. The galactose operon has both catabolic and biosynthetic functions. One promoter responds to the catabolic needs of the cell, and one responds to the biosynthetic requirements. Presumably, the promoter region that is stimulated by the CRP-cAMP complex responds to the catabolic needs of the cell.

The interaction of the CRP-cAMP complex with the *lac* promoter has been defined in considerable detail (205, 209, 226), using both genetic studies of *lacP* mutations and protection of the relevant sequences from chemical modification by the CRP-cAMP complex. However, even in this familiar operon, it is not clear how the binding of the CRP-cAMP complex to a distinct region of the promoter permits RNA polymerase to bind and to initiate transcription.

In vitro, the CRP binds nonspecifically to calf thymus DNA (224). The CRP binds to DNA restriction fragments including the *lac* promoter with only 10- to 100-fold greater affinity (152). In minicells lacking DNA, the amount of CRP (about 1,200 molecules/haploid cell [13]) is comparable to the amount of CRP in the parent cell containing the chromosome, suggesting that most of the CRP is free in the cytoplasm (39). It was originally proposed that most CRP is bound nonspecifically to the chromosome (224).

The CRP-cAMP complex has different affinities for different promoters (143, 192), presumably because of differences in the DNA binding sequences of the promoters. The induction of the *lac*, *ara*, and *tna* (tryptophanase) operons responds differently to different concentrations of cAMP. This suggests that the CRP-cAMP complex is in equilibrium with dissociated CRP and cAMP. Increasing concentrations of cAMP would then increase the formation of the active CRP-cAMP complex, permitting initiation of operons with a lower affinity for the complex. It should be pointed out that there appears to be only a single cAMP binding protein in *E. coli* (28, 180), and there is no reason to think that different promoters are activated by different species of CRP.

In mammalian systems, cAMP activates several protein kinases (210). Protein kinase activities in *E. coli* and *S. typhimurium* have been reported. However, a cAMP effect could not be demonstrated on these activities (262).

cAMP and Catabolite Repression

The mechanism of catabolite repression of enzyme synthesis (148) is implicit in the model for the role of the CRP-cAMP regulatory complex in initiation of transcription (158, 176, 177). The induction of these operons responds to the intracellular concentration of cAMP, which is determined by the carbon source available to the cell (58). However, there is mounting evidence that additional factors are involved in this mode of regulation, i.e., that the CRP-cAMP regulatory complex is not solely responsible for catabolite repression (256).

The role of cAMP in mediation of catabolite repression was first questioned when the *alt-1* mutation was described (238). The original mutants were isolated as Ara^+ phenotypic revertants in a strain having a deletion of *cya*, the gene

for adenylate cyclase. The mutation did not map in the *crp* region or in any other region coding for known cAMP functions. When the *alt-1* mutation was introduced into strains with *cya* or *crp* mutations, 15 to 30% of wild-type levels of *lac* expression was observed. This expression was sensitive to catabolite repression. The *alt-1* mutation has since been shown to be a mutation in the gene coding for the sigma subunit of RNA polymerase and to alter the conformation of that enzyme significantly (248).

Several mutations suppressing deletions of adenylate cyclase (*cya*) have been isolated and described (26, 47, 244). These mutations map in the *crp* region and result in altered CRP function. The CRP appears to be in its active conformation in the absence of cAMP. In some of these strains, synthesis of β-galactosidase is still sensitive to catabolite repression (47, 244; J. G. Harman and W. J. Dobrogosz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, K95, p. 142; Harman, personal communication).

Another line of evidence suggesting that cAMP is not solely responsible for catabolite repression comes from studies of a factor found in the culture medium of wild-type *E. coli* growing in minimal glucose medium. This factor, termed CMF (catabolite modulator factor), causes catabolite repression of the *lac* and *gal* operons as well of tryptophanase. This repression is distinct from cAMP-mediated effects (47, 48, 258). CMF causes catabolite repression of the *lac* operon in strains having *cya* deletion mutations and mutations in *crp* that make the CRP independent of cAMP for activity. Catabolite repression by CMF is also observed in strains having a mutation in *lac*P making *lac* expression independent of cAMP. The factor has been characterized only to the extent that it is of low molecular weight, has no net charge, and is stable to acid, base, and heat.

Ullmann's group has devised a unique way to study catabolite repression under conditions of extreme nitrogen limitation or carbon limitation (47, 48). Cells grow with limiting nitrogen in a medium with urea and a small amount of urease present. Growth is a function of the amount of urease present which degrades the urea in the medium, making ammonium available to the cells. When glucose is not limiting, these conditions cause maximal repression of β-galactosidase induction. Conversely, cells can grow while limited for carbon in a medium with sucrose as the carbon source. The addition of invertase provides limiting amounts of glucose. These conditions provide maximal derepression (e.g., minimal catabolite repression) of β-galactosidase synthesis. In both situations, growth is linear rather than exponential. Under conditions of maximal derepression, induction of the *lac* operon requires cAMP in *cya* strains. Induction is stimulated by the addition of exogenous cAMP in wild-type strains. The addition of CMF causes inhibition of β-galactosidase induction (i.e., catabolite repression) under these conditions of extreme derepression. Ullmann's group has proposed tentatively that the CMF is a negative factor by contrast with cAMP, a positive factor in regulation of gene expression (47, 48).

Ullmann's group has isolated mutants defective in CRP function that still exhibit catabolite repression of the *lac*, *mal*, and *tna* operons (77). These mutants were isolated in a *rho-15*(Ts) *crp* background. All of the pseudorevertants were Lac^+, Mal^+, Ara^+, and Tna^+. Four were characterized further. All retained the *crp* allele and had no CRP function by several criteria. The induction of β-galactosidase, amylomaltase, and tryptophanase was reduced to a specific activity comparable to the wild-type level when cells were grown with glucose rather than succinate. The authors cited preliminary evidence that this mutation responsible for the pseudoreversion lies in the *rpoB* region and that the RNA polymerase is altered. Regardless of the mechanism, these experiments show that the CRP-cAMP complex need not be functional for catabolite repression to occur.

Factors other than cAMP can affect the activity of the CRP. For example, indole acetic acid and imidazole acetic acid can replace cAMP for expression of the arabinose operon (120, 121). Anderson et al. (12, 13) determined the level of CRP in cells by immunological means. They found that when cells were grown under some conditions, the CRP was not active when assayed in vitro for its ability to bind cAMP. They proposed that the CRP could exist in both active and inactive forms, depending on the growth conditions. This apparent inactivation of CRP activity could account, in part, for catabolite repression. A dialyzable factor in cell extracts of *E. coli* grown with glucose has been found to inhibit binding of cAMP to the CRP in vitro (43). The factor was not found in extracts of cells grown with succinate. This factor does not appear to mediate catabolite repression, at least of tryptophanase induction, as the factor was not found in cell extracts of cells induced for tryptophanase and growing with succinate after the addition of glucose (43).

Another, less direct line of evidence suggesting that cAMP is not solely responsible for catabolite repression comes from the work of Wanner et al. (263). The specific activity of β-galactosidase in cells growing with different carbon sources was determined. An 18-fold variation in the specific activity of the enzyme over a 5.6-fold range

of growth rates was observed. This variation in specific activity could be reduced but not eliminated by the addition of 5 mM exogenous cAMP. This "residual variation" required the *lac* promoter region to be fully functional, indicating that the control was mediated at the level of the initiation of transcription. When induction of β-galactosidase was limited, not by the available catabolites, but by restricting the availability of a required amino acid, this variation in specific activity was not observed, arguing that the variation is due to limiting catabolites in these experiments. The authors concluded that cAMP alone cannot account for catabolite repression. Rather, the specific activity of β-galactosidase is determined by the "catabolic potential" of the cell (263), and cAMP is only one factor determining this potential. Catabolite repression is a consequence, in part, of the relationship between the cell's catabolic ability and the biosynthetic capacity in a given situation (166). This has been demonstrated with the *hut* operon in *Klebsiella aerogenes*. Induction of this operon is regulated both by the carbon source and the cell's requirement for nitrogen (153, 174, 200).

A comparison of the effect of cAMP on induction of tryptophanase and β-galactosidase in *E. coli* showed that some combinations of carbon sources inhibited induction of tryptophanase while stimulating induction of β-galactosidase. It was proposed that control elements other than the CRP-cAMP complex respond to the catabolic situation in the cell and that these hypothetical control elements could control some but not all catabolic operons (24). It should be pointed out that catabolite repression occurs in some microorganisms, such as *Bacillus megaterium*, that contain no cAMP (255). In *Pseudomonas aeruginosa*, catabolite repression occurs, but cAMP does not appear to be involved (191, 235). A similar situation occurs in *Rhizobium meliloti* (252). Presumably there are regulatory molecules in these bacteria that respond to the catabolic situation in the cell.

Catabolite Repression and Inducer Exclusion

Inhibition of enzyme induction by a second carbon source can be a consequence of inhibition of inducer uptake. This phenomenon, called inducer exclusion, was originally shown to be responsible, at least in part, for catabolite repression of the *lac* operon by glucose (149). Inducer exclusion is not limited to the *lac* operon. Glucose has been shown to inhibit transport of maltose, galactose, mannose, xylose, arabinose, and glycerol (157).

The mechanism(s) for inducer exclusion is not certain. Mutations in *ptsI* and *ptsH* of the phosphoenolpyruvate phosphotransferase system for carbohydrate transport (the PTS) and *crr*, a mutation that suppresses the effects of *pts* mutations on inducer exclusion, affect not only inducer exclusion but also cAMP synthesis (51, 183, 185, 215, 220). Recent genetic evidence argues that the two effects are not distinct (27). Some of the anomalies apparent in the evidence may be due to strain-specific differences (60).

Role for cAMP in Regulation of Termination of Transcription

A role for cAMP in termination of transcription has been proposed recently (256, 257). The authors examined the effect of the *rho-15*(Ts) mutation on the internal polarity of the *lac* operon in strains carrying mutations in the *lac* promoter and in *crp* making expression of the *lac* operon independent of cAMP. The interpretation of these experiments is ambiguous because the *rho-15*(Ts) mutation has pleiotropic effects (4), and termination of transcription of the *lac* operon requires L-factor coded by *nusA* (75). The intracistronic polarity observed in the *lac* operon can be readily accounted for by different rates of decay for the proximal and distal portions of the *lac* messenger RNA (115).

Regulation of cAMP Levels in *E. coli* and *S. typhimurium*

The intracellular concentration of cAMP can be regulated by three means: the nucleotide can be excreted; the nucleotide can be degraded by the enzyme cAMP phosphodiesterase; and the rates of synthesis can be controlled by inhibition (or activation) of adenylate cyclase and by varying the amount of adenylate cyclase in the cell.

Excretion of cAMP. cAMP is actively excreted into the medium. This has been demonstrated both in whole cells (78, 218) and in isolated membrane vesicles (72, 218). Isolated vesicles take up the nucleotide by what appears to be facilitated diffusion. Excretion of the nucleotide is energy dependent in vesicles. In whole cells, efflux of cAMP is stimulated by metabolizable sugars (218).

The physiological significance of excretion of cAMP is not certain. The nucleotide is excreted when cells grow in steady-state conditions at a rate proportional to the rate of synthesis (58). It is also excreted by washed cells when the carbon source is added to the growth medium (78; see reference 176 for a discussion of this point).

Degradation of cAMP. cAMP can be degraded by the enzyme cAMP phosphodiesterase. This enzyme has been partially purified and characterized from *Serratia marcescens* (170, 265) and from *E. coli* (167). The activity in crude cell extracts from *K. pneumoniae* (207) and from

K. aerogenes (31) has been characterized. In all cases, the enzyme has a K_m in the order of 0.1 to 0.5 mM, about two orders of magnitude greater than the intracellular concentration of the nucleotide. The specific activity of the enzyme in *E. coli* is not affected by the growth medium (26, 167). In *E. coli*, strains lacking the activity accumulate more cAMP than strains with the activity. In strains of *S. typhimurium* isogenic except for cAMP phosphodiesterase (8), the accumulation of cAMP in the culture is at least three times greater in the *cpd* strain than in the wild type. However, the intracellular concentrations of cAMP appear to be comparable in both strains (J. L. Botsford, unpublished data).

The significance of cAMP phosphodiesterase, then, is uncertain. There is no evidence that the enzyme is responsible for the differences in intracellular cAMP levels observed when bacteria grow with different carbon sources.

Regulation of adenylate cyclase activity. Studies of adenylate cyclase and of its regulation in procaryotes as well as in eucaryotes are fraught with problems. The enzyme is membrane associated in the enteric coliforms as it is in animal cells (101). The activity of adenylate cyclase is apparently regulated by interactions with transport proteins in enteric coliforms. This may be analogous to regulation of adenylate cyclase by interaction with hormone receptors in animal cells (88). Obviously, the organization of adenylate cyclase and of these transport functions is dependent on the integrity of the cellular membrane.

The activity of adenylate cyclase in broken cell extracts is much lower than in whole cells and is no longer sensitive to inhibition by glucose (1, 185, 186, 203). The activity of adenylate cyclase in whole cells can be measured in several different ways. Cells can be pulsed with radioactive adenosine. The resulting cAMP is separated by column chromatography, and the radioactivity is measured. The ATP pool must be measured to calculate the rate of cAMP synthesis. The technique does provide reliable results for initial rates of cAMP production (185a). A second technique is to treat cells with toluene to make them permeable to the radioactive adenosine triphosphate ATP (87). This technique has the disadvantage of making cells permeable to protons and destroys any chemiosmotic effects (188). Cells can be grown with radioactive phosphate, with the cAMP estimated after separation by thin-layer chromatography (61). The simplest method of measuring adenylate cyclase activity is to wash cells gently and rapidly by filtration, suspend the cells in prewarmed medium, and sample the culture for cAMP (cells and medium) at intervals. cAMP production is usually linear for at least 20 min. Rates of cAMP production are comparable to those observed with other techniques (26, 188). Apparently, the cell had adequate reserves of ATP to channel into cAMP production even when the cells are aerated without a carbon energy source. It should be pointed out that all of these techniques estimate adenylate cyclase activity from measurements of the net synthesis of the nucleotide. Presumably, a constant amount of the cAMP is degraded by cAMP phosphodiesterase.

It has been shown that sugars inhibit the net production of cAMP if the appropriate transport system for that sugar has been induced (189). The sugar need not be metabolized for the inhibition to occur. Sugars transported by a variety of mechanisms including the PTS mechanisms for glucose and mannitol (194, 208), proton symport mechanisms such as for lactose (236), and the facilitated diffusion of glycerol (141), can all inhibit cAMP production (51, 214, 215). Presumably, if transport of these different carbon sources by these various mechanisms regulates adenylate cyclase activity, several different regulatory mechanisms must be involved.

Two models for inhibition of adenylate cyclase activity concomitant with transport of PTS sugars have been proposed. Peterkofsky (182, 183, 185) has proposed that adenylate cyclase is activated in the absence of a PTS sugar. According to this model, adenylate cyclase is phosphorylated by the enzyme I of the PTS when there is no PTS sugar available. In the presence of a PTS sugar, the sugar rather than adenylate cyclase is phosphorylated, and adenylate cyclase is inactive. This model has been questioned on the basis of the isolation of a strain of *E. coli* having a deletion of *ptsI*. This mutant strain makes cAMP at rates comparable to those of the otherwise isogenic parent strain (272). This suggests that the PTS complex is not involved in the regulation of adenylate cyclase activity.

Saier et al. have proposed an alternative model (51, 215, 219) for regulation of adenylate cyclase involving activation of that enzyme by the *crr* gene product. According to this model, the *crr* gene product regulates activity of adenylate cyclase by an unknown allosteric mechanism. The model also proposes that the *crr* gene product mediates inducer exclusion. This model has been questioned by the isolation of a mutation in *crp* that suppresses the effect of the *crr* mutation (227).

Peterkofsky and Gazdar (188) have shown that inhibition of adenylate cyclase by transport of lactose and presumably of other sugars transported by proton symport mechanisms appears

to be due to the collapse of the proton electrochemical gradient. Although lactose does not inhibit adenylate cyclase activity when measured with the toluenized cell assay, this sugar does inhibit the activity in intact cells. Lactose has been shown to partially collapse the membrane potential in isolated membrane vesicles. Both lactose transport and adenylate cyclase are inhibited to a comparable extent by carbonyl cyanide-*m*-chlorophenyl hydrazone, a compound known to collapse the proton potential gradient completely. In a mutant of *E. coli* in which the *lac* permease is uncoupled with respect to proton symport, adenylate cyclase is not inhibited by lactose or by thiomethylgalactoside, a non-metabolizable analog of lactose. The authors suggest there are two different mechanisms affecting adenylate cyclase activity, one mediated by PTS transport and one mediated by the proton motive force developed within the cell.

The available evidence suggests that all of the various transport systems and adenylate cyclase are interdependent. Mutations in the PTS can affect transport of sugars not transported by PTS mechanisms. Mutations in the PTS can also affect adenylate cyclase activity. Mutations in non-PTS transport mechanisms influence adenylate cyclase activity. We have observed that sugars transported by mechanisms other than PTS mechanisms inhibit cAMP production very little in *E. coli* but do inhibit cAMP production in *S. typhimurium* and *K. pneumoniae* (25, 26, 84; Botsford, unpublished data). This suggests that it may not be correct to extrapolate results obtained with one enteric coliform to another. In *E. coli*, some of these interactions appear to differ from strain to strain (60).

Apparent repression of adenylate cyclase. Several authors have suggested that adenylate cyclase is a repressible enzyme (176, 189). This suggestion is supported by genetic and physiological evidence (26) and by direct measurements of adenylate cyclase activity in broken cell preparations (109, 151).

A speculative model has been proposed based on genetic and physiological experiments to account for the apparent repression of adenylate cyclase (26). The initial observations involved measurements of cAMP production in cells incubated in the absence of a carbon source. If cells had grown with a carbon source that does not inhibit adenylate cyclase (e.g., succinate), rates of cAMP production were much lower than in cells that had grown with a carbon source that does inhibit adenylate cyclase (e.g., glucose). In other words, adenylate cyclase appeared to be repressed when cells grew with succinate and to be derepressed when cells grew with glucose. Strains with mutations in *crp* were found to make cAMP at maximal levels regardless of the carbon source (26, 195). This suggested that the CRP could function as a negative repressor of adenylate cyclase. There is a physiological rationale for this. When cells grow with a carbon source that does not inhibit adenylate cyclase, cAMP is made in sufficient quantities for induction of alternative catabolic pathways. Only small amounts of adenylate cyclase are needed to supply the cAMP needed. However, when cells grow with a carbon source that does inhibit adenylate cyclase, very little cAMP is available to permit induction of alternative catabolic pathways. However, if the cell is maximally derepressed for adenylate cyclase, once the inhibitory carbon source is exhausted, the cell is capable of producing large amounts of cAMP.

cAMP Production in Cells Growing in Carbon-Limited Continuous Culture

Growth of cells in nutrient-limited chemostats provides a simple, reproducible steady-state experimental condition that is physiologically quite different from conditions in conventional batch culture (123). In chemostat cultures, the growth rate is determined, not by the composition of the growth medium, but by the dilution rate of fresh medium into the chamber. Cell numbers are set by the concentration of the limiting nutrient. It is not always possible to predict how synthesis of enzymes will be regulated when cells grow in chemostats from the situation observed in batch culture (44).

When cells grow in carbon-limited chemostats, adenylate cyclase should be uninhibited by transport of the carbon source, since the transport systems are not saturated under these conditions (92). However, rates of cAMP synthesis in a *cpd* (cAMP phosphodiesterase) strain of *S. typhimurium* were found to be lower than when cells grew in batch culture (25, 84). It has been proposed that, under these conditions, adenylate cyclase is repressed (25). Wright et al. (268) measured intracellular and extracellular concentrations of cAMP in *E. coli* growing in a chemostat culture limited by glucose or succinate. They found both the intracellular and extracellular levels of cAMP to be similar to those reported in cells grown in batch culture. However, the rates of synthesis of the nucleotide were much lower when cells grew in chemostats then when cells grew in batch culture. It has been proposed that when cells grow under these conditions, energy is limiting and the cells can-

not excrete cAMP readily (J. E. Leonard, C. Lee, A. Appleson, S. S. Dills, and M. H. Saier, Jr., *in* B. K. Ghosh, ed., *Membrane Structure in Bacterial Cells*, in press). Thus, cAMP does not accumulate in the culture medium in large amounts.

Guanosine Tetraphosphate and cAMP Metabolism

cAMP and guanosine 3′,5′-bis-pyrophosphate (ppGpp) are both unusual nucleotides involved in control of a number of activities (27, 63, 237). The intracellular levels of both nucleotides are generally inversely proportional to the growth rate. Both accumulate rapidly when cells are starved for glucose or when cells are shifted from glucose to a poorer carbon source (63, 64).

The accumulation of ppGpp is affected by mutations in the *relA* locus. Mutations in *relA* can also affect accumulation of cAMP (27). It appears that starvation for catabolites can enhance both ppGpp and cAMP synthesis. However, amino acid starvation, a condition that causes ppGpp to be made more rapidly, does not affect cAMP synthesis (26, 27).

Evidence from several sources suggests that ppGpp and cAMP influence transcription. Both cAMP and ppGpp, when added to cells made permeable to phosphorylated nucleotides by treatment with toluene, stimulate induction of β-galactosidase and tryptophanase. The effect is not limited to these two nucleotides. ppApp and pppApp also have stimulatory effects. The addition of ppGpp or ppApp does not compensate for a mutation in *cya*, indicating that the effect of either purine bis-pyrophosphate is independent of cAMP (276). It has been shown that ppGpp stimulate production of β-galactosidase in a refined system of in vitro-coupled transcription and translation (197, 240). It is not certain how ppGpp mediates its effects on these operons. There may be a ppGpp binding protein (199), or the nucleotide could act by altering the conformation of RNA polymerase (248).

Derepression of the *ilv* operon is severely impaired in *relA* strains. The addition of cAMP was found to permit one enzyme of this operon, acetohydroxy acid synthetase (coded by *ilvB*), to be derepressed in a strain having the *relA* allele (62). Apparently cAMP can replace ppGpp in this instance.

Strains of *E. coli* having the *relA* mutation are sensitive to inhibition of growth by serine. Mutants resistant to the inhibitory effects of serine can be isolated readily. Many of these resistant strains have mutations in *cya* or *crp* (42). At least one of these serine-resistant mutants in a *relA cya* background has a mutation in *crp* that results in enhanced threonine deaminase activity. The product of this reaction is made in excess by this mutant and is apparently responsible for the relief of inhibition by serine (42).

Other Functions of cAMP in *E. coli* and Other Enteric Coliform Bacteria

In addition to the well-known role of cAMP and CRP in the initiation of transcription of inducible catabolic operons (176, 177), the CRP-cAMP regulatory complex appears to affect many other functions. Many of these functions have no apparent catabolic role. Since the role of cAMP in most of these functions is not known in any detail, this work is summarized in a series of tables. The functions include lysogeny by bacteriophage (176), replication of plasmids (Table 1), a variety of envelope properties including regulation of synthesis of flagella, fimbriae, and pili (Table 2), regulation of a variety of enzymes associated with the membrane (Table 3), antibiotic susceptibility (Table 4), and several miscellaneous functions (Table 5).

The interaction between streptomycin and cAMP deserves special mention. Exogenous cAMP enhances streptomycin susceptibility (18). This fact has been used to select for *crp* mutations. Strains defective in CRP are more resistant to streptomycin in the presence of exogenous cAMP than are wild-type strains (18). Streptomycin-dependent mutants of *E. coli* show anomalous behavior with respect to cAMP metabolism. These mutants accumulate two to three times more cAMP than do wild-type cells and exhibit catabolite repression (193). The basis for this effect is not known. However, streptomycin has pleiotropic effects quite distinct from the effect of the antibiotic on ribosome function.

A priori, one would expect cAMP to affect susceptibility to tetracycline. This antibiotic is thought to be transported by the four-carbon dicarboxylic acid transport system (36). This transport system is dependent on cAMP for

TABLE 1. *cAMP and plasmids in enteric coliform bacteria*

Plasmid	Criterion[a]	Reference
ColE1, ColE2	A, B, C	114, 164
Cloacin DFB	A, C	260[b]
Rts-1	B	270

[a] Criteria for a role for cAMP include: (A) glucose effect noted; (B) *cya* or *crp* mutation affects the function; (C) exogenous cAMP affects the function; (D) cAMP levels measured, correlation between cAMP levels and function noted. Unless noted otherwise, all reports are for *E. coli*.

[b] In *Enterobacter cloacae*. All other plasmids are found in *E. coli*.

TABLE 2. *cAMP and envelope properties in enteric coliform bacteria*

Property	Criterion[a]	Reference
Morphology	B	125
Sensitivity to detergents	B	59, 125
Composition of proteins in outer membrane	A, B	15, 146, 153[b]
ompA gene product	—[c]	161
Fluidity of membrane	B, C	41, 250
Lambda receptor	B	52, 111, 215
T6/colicin K receptor	B	7
Flagella		
E. coli	A, B	53, 274
S. typhimurium	A, B[d]	122
Fimbriae		
E. coli	A	57
S. typhimurium	A	221
Pili	B	85

[a] As in Table 1.
[b] Synthesis of several proteins in the outer membrane appears to be negatively controlled by cAMP.
[c] Role of cAMP inferred from the sequence of the cloned gene.
[d] Synthesis of flagella in *E. coli* but not in *S. typhimurium* is repressed when cells grow with glucose.

TABLE 3. *cAMP and membrane-associated enzymes in enteric coliforms*

Enzyme	Criterion[a]	Reference
Adenosine triphosphatase	B	52
Formic dehydrogenlyase	B	178
Cytochromes	B	29
Glyoxalate shunt, isocitric lyase	A	245
Isocitric lyase	A	245
Succinic dehydrogenase	B	52
Nicotinamide adenine dinucleotide transhydrogenase	B	52

[a] As in Table 1.

TABLE 4. *cAMP and antibiotic susceptibility in enteric coliforms*

Antibiotic	Criterion[a]	Reference
Ampicillin	B	125
Chloramphenicol	A	54,[b] 95
Fosfomycin	B	9, 112, 261
Mecillinam	B	16
Nalidixic acid	B	125
Penicillin	A	65
Streptomycin	A	18, 19, 89

[a] As in Table 1.
[b] Shown in vitro in a system of coupled transcription and translation, using a DNA template from phage P1 *cmc1r100*.

expression (144). However, strains of *E. coli* isogenic except for *cya* are equally susceptible to tetracycline in broth culture (Botsford, unpublished data).

TABLE 5. *Miscellaneous functions of cAMP in enteric coliform bacteria*

Function	Criterion[a]	Reference
Antipain lethality	B	241
Arylsulfate synthetase	A	3, 169
Asparaginase II, induction	B	212
Division in *div* mutants	C	37
Catalase and peroxidase regulation	A, C	90
Chemoreception	A, B	6
Extracellular hydrolyases	A, B	118
Extracellular lipase	A, B	265
Fatty acid degradation	C, D	179
Glutamate excretion	B	138
Glycogen synthesis	A, C	50
GMP reductase, regulation	B, C	21
Heat-stable enterotoxin production	A, C	155
Histidase regulation[b]	A, B, C, D	154, 174
Mannitol dehydrogenase[c]	B	136
Methyl glyoxal accumulation	C	2
Minicell formation	B	127
Phosphoenolpyruvate carboxylase synthesis	A, B, C	73
Prodigiosin production	A, B	265
Protoporphyrin IX production	A	196
Pyrimidine catabolism	A, B	82
Superoxide dismutase, catalase	A	90
Substrate-accelerated death	C	31, 32
Threonine dehydratase (biodegradative)	A, B, C, D	180
Tyramine oxidase, regulation	A, B	171
Ultraviolet light lethality	A, B, C	242
Uracil uptake	C	46
Xylose lethality	C	14

[a] As in Table 1.
[b] In *K. aerogenes*.
[c] Induction is not affected by glucose or exogenous cAMP in the wild type. Enzyme is not induced in *cya* or *crp* strains.

Guanosine 3′,5′-Phosphate in *E. coli*

cGMP is found in mammalian cells and may be involved in many regulatory functions (71, 163). Quite often its function is implied from studies of the effects of exogenous cGMP on some physiological function. However, cGMP can compete with cAMP for cyclic phosphodiesterase, and exogenous cGMP can increase the concentration of cAMP by inhibiting its breakdown. In mammalian cells, the concentration of cGMP is much lower than the concentration of cAMP. Under some circumstances, the amounts of cGMP increase or decrease, but the significance of these changes is not at all certain.

A similar situation with respect to cGMP is apparent in most procaryotes. The nucleotide has been found in many different bacteria and usually in concentrations at least an order of magnitude lower than those of cAMP.

cGMP has been found in *E. coli*. Bernlohr et al. (22), using a complex enzyme cycling assay, found cGMP to be present in concentrations as high as 30 nM in *E. coli*. With a more conventional radioimmune assay, steady-state intracellular concentrations of cGMP of 0.5 to 3 nM were found (40). In other experiments, these authors obtained synchronous division by starving cells for various nutrients. They found a peak of cGMP synthesis several hours after initiation of growth followed by a second peak about one generation later. These peaks corresponded to intracellular concentrations of 30 to 40 nM. They proposed that cGMP might regulate the cell cycle in *E. coli*.

Gonzalez and Peterkofsky (74), using a radioimmune assay, compared cGMP and cAMP levels. They detected no relationship between levels of cGMP and cAMP under a variety of conditions that profoundly altered cAMP levels.

The amount of cGMP in *E. coli* is very small. An intracellular concentration of 3 nM (40) is equivalent to 1.8 molecules of cGMP per cell, assuming that the cell has a volume of 10^{-15} ml (185). It has been proposed that cGMP in *E. coli* is physiological artifact (234). cGMP can be formed in vitro by adenylate cyclase (247). Mutations in *cya* and *crp* that affect cAMP synthesis can also affect cGMP synthesis (232–234). However, Macchia et al. (147) have purified guanylate cyclase from *E. coli*. The enzyme is not able to convert ATP to cAMP and, unlike adenylate cyclase, is a soluble, cytoplasmic enzyme. Although the specific activity of the purified enzyme is quite low, it is sufficient to account for the concentrations of cGMP found in these cells.

Very recently, evidence has been offered that cGMP is involved in regulation of the chemotactic response of *E. coli* (23). The authors showed a 75% increase in the intracellular concentration of cGMP when cells were presented with a compound that acts as a chemoattractant. Exposing cells to a chemorepellent caused a decrease in cGMP levels. The same effect was observed in strain CA8404, a strain having a deletion for adenylate cyclase. Exogenous cGMP made cells swim smoothly as they would in the presence of a chemoattractant. A mutant that tumbled incessantly was found to have reduced levels of cGMP. cGMP was found to increase the methylation of MCP, the methyl-accepting chemotaxis protein. The authors suggest that cGMP serves as a chemical mediator between the cellular components sensing attractants and repellents and the mechanism controlling the motion of the flagella. This is an attractive hypothesis. The small amounts of cGMP observed in these experiments, about 20 nM, could be highly localized in the region of attachment of the flagella. Once the appropriate response was elicited, the nucleotide could be excreted, and the cell would be prepared for the next chemotactic response. It has been reported that most of the cGMP in a culture of *E. coli* is in the extracellular fraction (233).

CYCLIC NUCLEOTIDES IN BACTERIA OTHER THAN *E. COLI*

Investigation of cyclic nucleotides in procaryotes has not been limited to *E. coli* and *S. typhimurium*. Initially, reports of these nucleotides in other bacteria were limited to observations of effects of the nucleotides added exogenously or preliminary reports of adenylate cyclase or cAMP phosphodiesterase activity. Table 6 provides a summary of reports of measurements of cAMP from various procaryotes. It should be noted that only the enteric coliforms appear to accumulate appreciable amounts of cAMP extracellularly.

Cyclic Nucleotides in Dimorphic Procaryotes

Arthrobacter crystallopoietes is one of several members of this genus which grow as rod-shaped cells during exponential growth and then become cocci in stationary phase. When glucose is the carbon source, bacteria of this species grow slowly and remain spherical. When succinate or certain amino acids provide a carbon source, these bacteria grow rapidly as rods. When the preferred carbon source is exhausted, cells change into the spherical form. Exogenous cAMP has been shown to inhibit this transition (80, 119). Measurements of cAMP levels, of adenylate cyclase activity, and of cAMP phospho-

TABLE 6. *Intracellular and extracellular levels of cAMP in various bacteria*

Organism	Intracellular	Extracellular	Reference
E. coli	0.5–10 μM	Variable	30, 58, 176
C. crescentus	0.1–1 μM	4–5 nM	230
R. japonicum	0.8–40 pmol/mg of protein	0.2–0.8 nM	140
S. hygroscopius	0–100 pmol	0–1 μM	66–68
P. aeruginosa	0.6–0.9 μM	0.008 μM	191

diesterase activity as the cells undergo the transition are consistent with a regulatory role of cAMP in the process (81). Finally, a mutant incapable of the transition into the rod morphology makes cAMP in reduced amounts (80). cAMP does not appear to influence catabolite repression in this bacterium (225). Presumably, the effect of cAMP on the transition is not simply an indirect consequence of an effect on carbohydrate metabolism.

Bacteria of the genus *Bacillus* undergo a familiar differentiation from vegetative cells to endospores. cAMP is not found in either the spores or vegetative cells of *B. megaterium* (229). Similarly, cAMP is not found in *B. licheniformis* (22), even though both adenylate cyclase activity and a cAMP phosphodiesterase activity have been detected in crude cell extracts (38). In *B. brevis*, if cAMP is present, it is in a concentration calculated to be less than 5×10^{-8} M (223). There are no reports of cAMP in *B. subtilis*, although small amounts of the nucleotide have been detected (206; H. V. Rickenberg, personal communication).

A role for cGMP in regulation of sporulation in *B. megaterium* has been investigated (221). cGMP levels in spores were found in concentrations calculated to be less than 1 molecule per spore. cGMP did increase as spores germinated and also during exponential growth. cGMP levels decreased during late exponential growth only to rise again in stationary phase. However, the concentration of cGMP was always quite small, equivalent to only a few molecules per cell. The authors concluded that it is very doubtful that cGMP is involved in differentiation in this bacterium.

Cook et al. (40) observed a sharp increase in cGMP levels in *B. licheniformis* undergoing synchronous division with a periodicity equivalent to one generation. They proposed that cGMP could be involved in some aspect of regulation of cellular division.

cAMP nonspecifically affects the induction of α-amylase in *B. subtilis*. AMP, adenosine diphosphate (ADP), and ATP were found to have a comparable effect (197). Substantial evidence against a role for cAMP in the induction of β-galactosidase in *B. megaterium* has been presented (273). A ribonuclease that is inhibited by cGMP has been found in *B. subtilis* (166) and in *B. brevis* (223). The K_i for cGMP for the enzyme from *B. brevis* was found to be 0.01 mM. The authors measured the cGMP levels in *B. brevis* and found the amount of cGMP to be less than 5×10^{-8} M. They concluded that the physiological significance of this inhibition by cGMP is doubtful.

In summary, the presence of cAMP in *Bacillus* has not been reported in the literature. cGMP has been found in bacteria of this genus, but in very small amounts. The physiological significance of the nucleotide is not apparent.

Caulobacter crescentus is stalked and divides by a process of unequal binary fission. When the stalked cell divides, the daughter cell, termed a swarmer, has a single flagellum. The swarmer cell eventually loses the flagellum, forms a stalk, and then divides.

Cyclic nucleotides appear to be involved in several aspects of growth and development of this bacterium. Both cAMP and cGMP have been isolated from the bacterium. There are distinct adenylate cyclase and guanylate cyclase activities and distinct cAMP and cGMP receptor proteins. Neither cyclic nucleotide appears to activate a protein kinase (230, 231).

cGMP and its derivatives (dibutyryl cGMP, etc.), when added exogenously, repress formation of the polar flagellum of the swarmer cell, pili, and several phage receptor sites. Exogenous cAMP reverses this effect of cGMP (128, 129). Mutants that no longer respond to cAMP in this manner have been isolated and characterized (130). It appears that the effects of cyclic nucleotides on morphogenesis are indirect. Events in the cell cycle appear to respond to the state of energy metabolism in the cell, and both cAMP and cGMP influence critical catabolic reactions. Intracellular cAMP levels vary no more than 25% during the cell cycle and in response to a variety of different carbon sources. cGMP levels vary nearly fivefold under the same conditions (128, 130).

Myxobacteria are a diverse group of procaryotes with a complex life cycle. Vegetative bacteria grow as individual cells, are motile by gliding, and obtain nutrients from lysed bacteria and yeasts. When nutrients become limiting, myxobacteria aggregate to form fruiting bodies, and some cells differentiate into resting structures called myxospores (113, 266). Nearly all the work on cyclic nucleotides in this group has been confined to one member, *Myxococcus xanthus*.

Exogenous cAMP and AMP stimulate fruiting body formation, as do some amino acids (33). Exogenous cGMP appears to inihibit fruiting body formation. However, since this nucleotide also acts as a chemoattractant, causing cells to aggregate together (96, 156), the physiological significance of this activity is not clear. cAMP has been found in vegetative cells (175). The intracellular levels of the cAMP decrease by half as vegetative cells grow from exponential phase to stationary phase (269). Finally, a protein that binds cAMP has been identified by affinity labeling with 8-azido-^{32}P-labeled cAMP and sodium dodecyl sulfate electrophoresis (172).

In bacteria of the genus *Streptomyces*, differentiation occurs with formation of reproductive spores. cAMP has been found in spores of *Streptomyces hygroscopius*. As spores germinate, intracellular cAMP levels increase nearly 100-fold (66–69).

cAMP has been reported to affect antibiotic production in *Streptomyces*. In *S. griseus*, cAMP and other nucleotides, including cGMP, ADP, AMP, and ATP, inhibit production of the antibiotic candicidin. The authors propose that the phosphate moiety of the nucleotide is responsible for the effect and that it is nonspecific (154). *S. hygroscopius* produces the antibiotic turimycin. Production of this antibiotic is inhibited by the addition of inorganic phosphate. The addition of phosphate causes the intracellular concentration of cAMP to rise dramatically and then to fall. Exogenous cAMP reverses the effect of added phosphate (66). In this bacterium, intracellular concentrations of cAMP and cGMP seem to be inversely related to antibiotic production (66, 202).

Bacteria of the genus *Nocardia* do not form spores but are similar to streptomycetes in some respects. When *Nocardia salmonicolor* grows with acetate as the carbon source, the anaplerotic enzyme isocitrate lyase is induced. The addition of fumarate, but not of glucose, prevents this increase. This effect of fumarate appears to be mediated by catabolite repression. The addition of exogenous cAMP in millimolar quantities relieves the inhibition (264).

Nocardia can be easily grown in synchronous culture by diluting cells in stationary phase into fresh medium. Both the intracellular and the extracellular concentrations of cAMP oscillate during each generation, resulting in a threefold variation. The specific activity of adenylate cyclase measured in vitro also oscillates, and there is a good correlation between the specific activity of this enzyme and the resulting cAMP levels. Cyclic phosphodiesterase activity appears at the end of exponential growth. The specific activity of this enzyme oscillates out of phase with adenylate cyclase activity (135).

cAMP and Guanosine 3',5'-Phosphate in Nitrogen Fixation

Nitrogen fixation is a highly energy-intensive process (11). Since cAMP is involved in regulation of catabolism in enteric coliforms, it seems possible that the nucleotide might regulate nitrogen fixation in response to energy metabolism. cAMP has been shown to influence the synthesis of several enzymes involved in ammonia assimilation in *E. coli*, including glutamine synthetase (201). Presumably, these enzymes respond similarly in *K. pneumoniae*, an enteric coliform capable of nitrogen fixation. Glutamine synthetase plays a critical role in regulation of nitrogen fixation (145).

Many of the bacteria capable of nitrogen fixation are dimorphic. The rhizobia change to bacteroids once a nodule is established. *Azotobacter* spp. can form cysts, the clostridia sporulate, and the cyanobacteria are capable of many morphological variations. As we have already discussed, morphological variations in procaryotes frequently appear to correlate with changes in cyclic nucleotide levels.

Exogenous cAMP has been reported to stimulate derepression of nitrogenase in *Azotobacter vinelandii* (137). A possible role for cyclic nucleotides in the encystment of the bacterium has not been reported.

The occurrence of cAMP has been rigorously demonstrated in the cyanobacterium *Anabaena variabilis* (98). Preliminary evidence indicates that cAMP levels are lower when cells grow heterotrophically than when they grow autotrophically. cAMP levels increase when cells are starved for a fixed source of nitrogen. Exogenous cAMP alters the morphological development of *Nostoc mucorum*, another dimorphic cyanobacterium (124).

In *Rhizobium japonicum*, exogenous cAMP represses formation of three enzymes involved in ammonia fixation, including glutamine synthetase, glutamate synthase, and glutamate dehydrogenase. Enzyme levels are reduced two- to fivefold by 1 mM cAMP (259). This could affect the regulation of nitrogen fixation in these cells (145, 201).

R. japonicum has a catabolic hydrogenase (83). The activity of this hydrogenase in whole cells is inhibited by malate. Exogenous cAMP can overcome this inhibition. The effect requires de novo protein synthesis, indicating that synthesis of some sort of activator protein is involved. The effect of exogenous cAMP appears to be physiologically significant. Intracellular cAMP concentrations were found to be low when cells grew in malate. Conversely, when cells grew with glutamate, a carbon source that permits high levels of the hydrogenase, intracellular levels of cAMP were high. The authors proposed that cAMP levels vary in response to the availability of a readily oxidizable carbon source and that cAMP also regulates synthesis of a protein that activates the hydrogenase.

cGMP also appears to play a critical role in nitrogen metabolism in *R. japonicum* (139). cGMP added exogenously can completely inhibit the synthesis of the nitrogenase complex and partially inhibits synthesis of the hydroge-

nase activity as well as nitrate reductase, two other enzymes that require a great deal of reducing potential. Measurements of intracellular cGMP levels show that cGMP levels decrease 10-fold as the culture becomes microaerophilic and the nitrogenase complex is derepressed (139). It was suggested that cGMP levels respond to the activity of the electron transport system. By implication, cGMP levels could then regulate the synthesis of those enzymes dependent on the reducing power generated by electron transport.

Miscellaneous Reports of the Occurrence of Cyclic Nucleotides in Bacteria

The occurrence cAMP and cGMP has been reported in many bacteria. The following section is a brief survey of cyclic nucleotide metabolism in a variety of bacteria.

Alcaligenes eutrophicus. *A. eutrophicus* is a facultative chemoautotroph of some industrial significance. It grows autotrophically by the oxidation of hydrogen. The synthesis of the hydrogenase involved is stimulated by exogenous cAMP when cells grow heterotrophically. A cAMP receptor protein from the bacterium has been isolated and partially characterized (243).

Benekea (Vibrio) harveyi. The induction of the luminescent system in *B. harveyi* is sensitive to a glucose effect. Mutants with pleiotropic carbohydrate deficiencies have been isolated. These mutants are able to develop the luminescent system if provided with exogenous cAMP (253). A cAMP receptor protein activity has been detected in this bacterium. The protein is immunologically homologous with the CRP from *E. coli* (165).

cGMP added exogenously at levels as low as 28 μM inhibits the formation of the bioluminescent system (254). The physiological significance of this is uncertain. cGMP in millimolar quantities competes with cAMP for binding to the CRP in *E. coli* (17), but the concentration required for this effect is many orders of magnitude higher than the intracellular concentration of that nucleotide.

In contrast to the situation in *B. harveyi*, induction of the luminescent system in several species of *Photobacterium*, although sensitive to a glucose effect, does not respond to cAMP (165).

Bordetella. Nearly all adenylate cyclase activities in eucaryotes and procaryotes are membrane associated. However, the activity in the pathogenic bacterium *Bordetella pertussis* is soluble and is found in the periplasm (94). Appreciable amounts of the enzyme are excreted into the medium. The enzyme appears to have a single subunit with a molecular weight of 70,000. A protein factor can be isolated from rabbit erythrocytes or from beef liver catalase that stimulates the activity in whole cells 50- to 100-fold (95). The factor is thought to be calmodulin (267), a protein active in calcium binding (131). The authors (267) propose that this stimulation of the activity by calmodulin may contribute to the virulence of the bacterium. Adenylate cyclase activity in *Brevibacterium liquefaciens* also appears to be a soluble, cytoplasmic enzyme (96, 103).

Erwinia. Exogenous cGMP stimulates the synthesis of a pectin-degrading enzyme produced by *Erwinia carotovora* (99, 100). This activity appears to be sensitive to cAMP-mediated catabolite repression (251).

Mycoplasma. Despite the small size and minimal genetic potential, *Mycoplasma* spp. have a fully functional PTS for transport of several hexoses. In *Mycoplasma capricolus*, mutations in the PTS resulting in a nonfunctional enzyme II for glucose also result in an apparent loss of regulation of cAMP production (162). Normally these cells produce more cAMP when grown with glucose as the carbon source than when grown with fructose, in contrast to the situation in *E. coli*. However, the mutation that results in the loss of enzyme II activity results also in comparable amounts of cAMP being made with either carbon source. However, very little cAMP was detected in these experiments, about 3 to 20 pmol/mg (wet weight). Cells were harvested by centrifugation, and the cAMP in the pellet cells was measured. Some cAMP could have been lost by this procedure (see reference 176 for a discussion of measurements of intracellular cAMP). cAMP was measured by using a binding protein from ghost erythrocytes (93). No evidence was offered to verify that the compound measured by this assay procedure was in fact cAMP.

Mycobacterium smegmatis. The presence of cAMP in *M. smegmatis* has been demonstrated rigorously (132). The author used two methods to measure the nucleotide, an isotopic dilution technique and a protein binding assay (70). The criteria used to identify the compound assayed included (i) cochromatography of the compound with authentic cAMP with nine different solvent systems and (ii) degradation of the compound by cyclic phosphodiesterase. The product, AMP, was broken down in turn by *Crotalus atrox* venom nucleotidase to yield a compound identified as adenosine; a phosphate analysis of the product showed a 1:1 ratio between phosphate and adenosine. Only small amounts of the nucleotide were found, 4.5 pmol/

mg (wet weight). A cAMP phosphodiesterase activity from this bacterium has been described (133). No function for this nucleotide in *M. smegmatis* has been shown (173).

Neisseria gonorrhoeae. There are two reports concerning cAMP in *N. gonorrhoeae*. One report maintains that the nucleotide is not present; the second maintains that it is. The authors of the first report (160) found no evidence for cAMP in five strains of *N. gonorrhoeae* and in several other *Neisseria* species. They found no cAMP with a protein binding assay, nor did they observe adenylate cyclase or cAMP phosphodiesterase activity. Exogenous cAMP, dibutyryl cAMP, and cGMP had no effect on growth of any of the strains. As a control, these authors performed all of their analytical techniques with *E. coli* and found cAMP as well as adenylate cyclase and cyclic phosphodiesterase activities in amounts comparable to those reported in the literature. In the second report (159), cAMP was detected in two laboratory strains *N. gonorrhoeae*. These authors grew the cells in a medium with a much lower concentration of glucose than was used in the experiments described in the first report. When the cells grew in the higher concentration of glucose, no cAMP could be detected. However, these authors did not offer any evidence that the substance they assayed was actually cAMP.

Pseudomonas aeruginosa. cAMP, adenylate cyclase, and cyclic phosphodiesterase have been found in *P. aeruginosa* (235). The intracellular levels of the nucleotide were lower than those found in *E. coli* (191). The nucleotide does not accumulate in the growth medium even after long incubations. The intracellular concentration of the nucleotide is not markedly affected by the carbon source used by the cells. Furthermore, exogenous cAMP does not reverse catabolite repression of glucose 6-phosphate dehydrogenase caused by the addition of succinate. cAMP does not appear to influence catabolite repression of histidase in either *P. aeruginosa* or *P. putida* (191).

Vibrio cholerae. Mutants lacking adenylate cyclase have been isolated in *V. cholerae* (275). These mutants have a phenotype very similar to that of *cya* strains of *E. coli*. They have pleiotropic defects in carbohydrate utilization, they are not motile, and they lack some types of fimbriae. They are abnormally sensitive to salts and grow at 37°C only in isotonic media, indicating defects in the cell envelope. They also have altered cell morphology and are more spherical than wild-type cells.

UNUSUAL CYCLIC NUCLEOTIDES

Cyclic pyrimidines including 3′,5′-uridine and -cytidine monophosphates have been found in cultures of *Corynebacterium murisepticum* and *Micrococcus* spp. (104). Cyclic deoxyadenosine monophosphate has also been isolated from *C. murisepticum* (105, 107). In all cases, cAMP was found in much greater quantities. No functions for these nucleotides in the bacteria have been established.

BACTERIA IN WHICH cAMP IS NOT FOUND

cAMP has not been detected in several bacteria despite intensive searchs for the nucleotide. As already discussed, cAMP has not been detected in several species of *Bacillus*. In addition, cAMP has not been detected in *Bacteroides fragilis*, an obligately anaerobic bacterium (102, 235). The nucleotide has not been detected in *Lactobacillus plantarum* (213), and it does not appear to be involved in induction of either L-arabinose isomerase or β-galactosidase in this bacterium (91).

CONCLUDING REMARKS

Many intriguing questions remain concerning cyclic nucleotides in procaryotes. For example, what is the role of cAMP in oxidative bacteria such as *Pseudomonas* and *Rhizobium*? The nucleotide is found but does not appear to be involved in catabolite repression. Does cAMP act through a receptor protein at the level of transcription in all procaryotes, or could there be cAMP-activated protein kinases? Perhaps cAMP acts in a totally unprecedented fashion in some procaryotes.

A better understanding of cyclic nucleotides could be of utilitarian as well as heuristic value. These nucleotides appear to be involved in production of some antibiotics and in regulation of nitrogen fixation.

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A. TOTAL NO. COPIES (Net Press Run)	17,793	18,011
B. PAID CIRCULATION 1. SALES THROUGH DEALERS AND CARRIERS, STREET VENDORS AND COUNTER SALES (Member)	12,140	13,140
2. MAIL SUBSCRIPTION (Nonmember)	3,403	3,593
C. TOTAL PAID CIRCULATION (Sum of 10B1 and 10B2)	15,543	16,683
D. FREE DISTRIBUTION BY MAIL, CARRIER OR OTHER MEANS SAMPLES, COMPLIMENTARY, AND OTHER FREE COPIES	100	100
E. TOTAL DISTRIBUTION (Sum of C and D)	15,643	16,783
F. COPIES NOT DISTRIBUTED 1. OFFICE USE, LEFT OVER, UNACCOUNTED, SPOILED AFTER PRINTING	2,150	1,228
2. RETURN FROM NEWS AGENTS	---	---
G. TOTAL (Sum of E, F1 and 2 - should equal net press run shown in A)	17,793	18,011

11. I certify that the statements made by me above are correct and complete
SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER
Walter G. Peter III, Managing Editor

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(See instruction on reverse)