MICROBIOLOGICAL REVIEWS, Sept. 1981, p. 462–480
0146-0749/81/030462-19$02.00/0

Vol. 45, No. 3

Adenosine 3′,5′-Phosphate in Fungi

MARTIN L. PALL

Program in Genetics and Cell Biology and Program in Biochemistry/Biophysics, Washington State University, Pullman, Washington 99164

GENERAL INTRODUCTION

Adenosine 3′,5′-phosphate (cyclic AMP) has been studied as an intracellular regulator in a wide variety of organisms, but the most intensive of these studies have been made in higher animals and in *Escherichia coli* and related gram-negative bacteria. Two paradigms of cyclic AMP action have arisen from studies of these two organism groups.

In *E. coli,* cyclic AMP levels are found to be depressed by glucose and other carbon sources, and cyclic AMP activates a variety of glucose-repressed functions, including catabolism of carbon sources. In animal cells, cyclic AMP is found to control a wide variety of often apparently unrelated functions, most commonly in a second messenger role. Attempts have been made to integrate these two observations by proposing cyclic AMP as a primitive signal for carbon starvation (65, 134), but the validity of this approach is uncertain.

These two paradigms of cyclic AMP action have dominated work on fungi, and studies of cyclic AMP function in fungi have concentrated on functions repressed by glucose as well as on diverse developmental and other specific responses reported to be influenced by cyclic AMP in higher animals. One important question facing researchers working with fungi is whether either of these two views of cyclic AMP function is appropriate to fungi or whether a different paradigm of cyclic AMP action should be developed for fungi and possibly for other lower eucaryotes.

Evidence has been obtained which links cyclic AMP to control of a variety of functions in fungi, including utilization of endogenous and exogenous carbon sources, conidiation (in *Neurospora crassa*), dimorphism in several fungi, the sexual process, and phototropism (in *Phycomyces*).

In this review of cyclic AMP in filamentous fungi and yeasts, I discuss the major progress that has been made in studies of the enzymes of cyclic AMP metabolism, the drugs and mutants influencing the cyclic AMP control system in fungi, and the many proposed but as yet unproven cyclic AMP functions in this group of organisms. A number of important lessons derived from studies of cyclic AMP in higher animals and in fungi may help in future studies of fungi; six criteria for cyclic AMP control are proposed to aid studies of function in fungi.

ADENYLATE CYCLASE

Adenylate cyclase (adenosine 5'-triphosphate [ATP] pyrophosphate lyase [cyclizing]; EC 4.6.1.1), the enzyme that synthesizes cyclic AMP from ATP-Me^{+2}, is thought to be the most important control point of cyclic AMP level in most organisms. It is controlled by various hormones in animal cells and by the sugar-phosphotransferase system in certain bacteria. It therefore seems likely that an understanding of the properties of fungal adenylate cyclase will be critical to the determination of the mode of regulation of endogenous cyclic AMP levels in fungi.

Extensive studies of adenylate cyclase in *Neurospora crassa* have been reported by Flawia and Torres (34–39) and by Scott (114); in *Saccharomyces cerevisiae* by Londesborough and co-workers (76, 78, 159, 160); in *Blastocladiella emersonii* by Gomes and co-workers (52, 53); in *Mucor rouxii* by Passeron and co-workers (86, 96); and in *Phycomyces blakesleeanus* by Cohen and co-workers (19). There are brief descriptions of the occurrence of enzyme activity in *Coprinus macrorhizus* (147), *Saccharomyces fragilis* (125), and *Aspergillus nidulans* (173).

Properties of Some Manganese-Dependent Enzymes

The *Neurospora* and *S. cerevisiae* enzymes are quite similar: both have a requirement for ATP-Mn^{+2}, a pH optimum of 5.5 to 6.0, and a K_m for ATP-Mn^{+2} of 0.5 to 1.5 mM, depending on conditions (34, 78). Neither enzyme is stimulated by fluoride or guanosine 5'-triphosphate, but both are stimulated by excess Mn^{+2}. The *Blastocladiella* enzyme shares all of these properties with the *Neurospora* and *Saccharomyces* enzymes except that its pH optimum is about 9 (52, 53). Adenylate cyclase of *M. rouxii* is also quite similar to these other fungal enzymes. It is dependent on ATP-Mn^{+2} and is not stimulated by guanosine 5'-triphosphate or fluoride (11, 96).

The fungal enzymes are thought to be membrane bound. The bulk of the activity is sedimentable and can be easily solubilized by detergents which solubilize membrane proteins, such as Lubrol PX or Triton X-100 (11, 34, 53, 159). In *Neurospora*, the properties of adenylate cyclase are influenced by the fatty acid composition of the membranes (114). Although the enzyme appears to be membrane bound, it can be solubilized considerably more easily than can the enzyme of animal cells (78, 114, 159).

The enzymes of the four fungi discussed above are manganese dependent, showing little or no activity with ATP-Mg^{+2} as the substrate (34, 35, 53, 96, 159, 160). Consequently, those enzymes differ substantially from a magnesium-dependent adenylate cyclase from *Phycomyces* sporangiophores (19) which is described below.

Certain other properties of the fungal enzymes have been described, including temperature dependence (76, 160), an apparent arginine residue at the active site of the *Saccharomyces* enzyme, and sigmoidal kinetics in the absence of excess Mn^{+2} in the *Neurospora* enzyme (35).

Hormonal Effects

Hormones are thought to be the main physiological effectors of animal adenylate cyclase; possible hormonal effects on the fungal enzymes have therefore been studied. Most hormones have little or no influence on any given fungal adenylate cyclase (19, 34, 53, 74). However, several hormones are reported to influence individual fungal adenylate cyclases. Glucagon and insulin have been reported to stimulate and inhibit, respectively, the adenylate cyclase activity of *N. crassa* (37–39) but not of *Saccharomyces* (74). Dopamine and epinephrine are reported to stimulate the adenylate cyclase of *Phycomyces* (19) but not of *N. crassa* (34) or *B. emersonii* (53). It is not known whether these hormones can influence cyclic AMP levels in vivo, and the physiological role of this type of hormone stimulation, if any, is undetermined.

Recently Liao and Thorner (74) reported that both purified and synthetic *S. cerevisiae* alpha mating factor inhibits *Saccharomyces* adenylate cyclase activity. They suggest that a decrease in cyclic AMP levels may be involved in part of the action of this pheromone.

Magnesium-Dependent Adenylate Cyclase

Recently, Cohen and co-workers (19) described an adenylate cyclase from *Phycomyces blakesleeanus* which uses ATP-Mg^{+2} as the substrate and is inhibited by Mn^{+2}. The activity is sedimentable and is stimulated by low levels of guanosine 5'-triphosphate. It is also stimulated by adenosine 5'-diphosphate, AMP, and blue light.

One question which must be raised is whether the fungal manganese-dependent activities are

an in vitro artifact. The animal enzyme has both a catalytic component and a guanine nucleotide-binding regulatory (G/F) component (for review, see reference 108). The G/F component is required in vitro for substantial magnesium-dependent activity, stimulation by guanine nucleotides or fluoride, and stimulation by hormones binding to hormone receptors. The animal catalytic component resembles most of the fungal enzymes (except the *Phycomyces* enzyme) in requiring manganese for high activity and not being stimulated by guanosine 5'-triphosphate or fluoride. It is clear that in living animal cells, the active enzyme requires both catalytic and G/F components because the cells do not respond to hormones with cyclic AMP increases. Mutants of cultured cells deficient in the G/F component are deficient in cyclic AMP (108). The fungal manganese-dependent enzymes therefore behave like the animal enzyme in which the G/F subunit has been lost or inactivated.

Recently, we found conditions in which *Neurospora* extracts show substantial magnesium-dependent, guanine nucleotide-stimulated, adenylate cyclase activity (G. Rosenberg and M. L. Pall, unpublished data). The magnesium-dependent activity has the same pH optimum as the manganese-dependent activity, and no activity is found in extracts of the *cr-1*, cyclic AMP-deficient mutant. We suggest that this magnesium-dependent activity represents the regulated form of the *Neurospora* enzyme.

Intracellular Distribution

As indicated above, adenylate cyclase in fungi appears to be a membrane-bound enzyme, although at least under some conditions, it is relatively easily solubilized. In animal cells and *E. coli*, most of the activity appears to be plasma membrane bound, and most investigators working with fungi have suggested a similar location in fungi, although Wheeler et al. (166) have reported that other membranes may have the bulk of the activity. The only studies which specifically implicate plasma membranes in fungi are those of Liao and Thorner (74), who studied adenylate cyclase activity in purified plasma membranes of *S. cerevisiae* using concanavalin A for such purification.

Using a different approach, Tu and Malhotra (139) histochemically stained for adenylate cyclase in electron microscopic studies of *P. blakesleeanus*. They found activity associated with plasma and outer mitochondrial and nuclear membranes. Cohen (15) also reported histochemical evidence of adenylate cyclase activity in the plasma membrane, mitochondria, and cytosol of *Phycomyces*. It should be noted that the methods involved in such histochemical studies have been criticized as being susceptible to artifacts (69, 72).

PHOSPHODIESTERASE

Cyclic AMP is removed from the intracellular pool by the action of phosphodiesterases and possibly also by efflux from the cell. The properties of various fungal phosphodiesterases are summarized in Table 1. Typically, they appear to require divalent cation, as indicated by their being stimulated by Mg^{+2} and Mn^{+2} and inhibited by chelators such as ethylenediaminetetraacetic acid. Many of the fungal enzymes are easily sedimentable, suggesting that they are membrane bound.

In many animal tissues, methylxanthines such as theophylline, caffeine, and 1-methyl-3-isobutylxanthine act as phosphodiesterase inhibitors and are used as drugs to raise endogenous cyclic AMP levels. In fungi, these agents are not very effective inhibitors of phosphodiesterase activity (Table 1). With the *Candida*, *Mucor*, and *Phycomyces* enzymes, little or no inhibition is seen. With most of the other fungal enzymes, high concentrations (ca. 10 mM) are required to obtain substantial inhibition. The difficulties of using these agents to raise endogenous cyclic AMP levels in fungi are discussed further in Phosphodiesterase Inhibitors.

Many animal cells are found to have two cyclic AMP phosphodiesterases (3, 165). They are a low-K_m enzyme (K_m, typically ca. 1 μM) and a high-K_m enzyme (K_m, typically 20 to 100 μM) (3). Based on the rates of hydrolysis at physiological concentrations of cyclic AMP (often 1 μM or less) it is thought that the low K_m enzyme plays the major role in cyclic AMP hydrolysis (3, 32). Several species of fungi have been reported to have low K_m and high K_m enzymes, notably *Candida albicans*, *N. crassa*, *P. blakesleeanus*, and *S. cerevisiae* (Table 1). It seems likely, for reasons similar to those pertaining to animal systems, that the low-K_m enzymes play the major role in fungal cyclic AMP hydrolysis. The *Neurospora* and *S. carlsbergensis* high-K_m enzymes are inhibited by physiological concentrations of other adenine nucleotides, such as ATP, and a high-K_m phosphodiesterase from *Aspergillus nidulans* was found, by using diverse substrates (100), to also have phosphomonoesterase activity.

Several interesting observations have been made about certain fungal cyclic AMP phosphodiesterases, such as the induction during sporulation in *B. emersonii* (27) and the in vitro stimulation of the *Phycomyces* enzyme by blue light (18). The *M. rouxii* enzyme is reported to be stimulated by an ATP-Mg^{+2} and cyclic AMP-

TABLE 1. *Fungal cyclic AMP phosphodiesterases*

Organism	Reference	Cyclic AMP K_m (μM)	pH optimum	Inhibition by methyl-xanthines	Stimulation by divalent cations	Inhibition by non-cyclic adenine nucleotides	Other features
A. niger	173	2,500	7.5	ND[a]	Yes; Mg^{+2}	ND	Intracellular
		18	3.5	ND	ND	ND	Extracellular
B. emersonii	27, 154	4.2	ND	Yes; 10 mM theophylline	Yes; Mg^{+2}, Mn^{+2}	ND	Induced during sporulation
C. albicans	56	4.2	ND	ND	ND	ND	No apparent cooperativity
		250	7.5	No	Yes; Mg^{+2}, Ca^{+2}	ND	No apparent cooperativity
C. macrorhizus	147	ND	8.5	Yes; theophylline, caffeine	Yes; Mg^{+2}, Mn^{+2}, Ca^{+2}	Yes	
M. rouxii	45, 96	1	ND	No	Yes; Mn^{+2}, Mg^{+2}	No	Apparent stimulation by cyclic AMP-dependent phosphorylation
N. crassa	115	4.8	ND	ND	ND	No	Cooperative
		57	ND	Yes; theophylline, caffeine	Inhibited by EDTA[b]	Yes	Partially sedimentable and cooperative
P. blakesleeanus	17, 18	3	ND	Slight	Yes; Mg^{+2}	ND	Stimulation by blue light
		12.5	ND	ND	ND	ND	Stimulation by blue light
S. carlsbergensis	122	220	8.5	ND	Yes; Mn^{+2}	Yes	No apparent cooperativity
S. cerevisiae	43, 73, 77	0.12	ND	Yes; theophylline, caffeine	Inhibited by EDTA[b]	ND	Probably membrane bound
		100–250	ND	ND	No	ND	Contains Zn^{+2}

[a] ND, Not determined.
[b] EDTA, Ethylenediaminetetraacetic acid.

dependent process, presumably cyclic AMP-dependent phosphorylation (45). Such control by phosphorylation may turn out to be an important aspect of the control of endogenous cyclic AMP levels in fungi.

ADENOSINE 3',5'-PHOSPHATE (CYCLIC AMP)-DEPENDENT PROTEIN KINASE AND CYCLIC AMP-BINDING PROTEINS

Protein Kinase Properties

In animal cells, the major and possibly sole receptors for cyclic AMP are cyclic AMP-dependent protein kinases (13, 48, 55). In contrast, in bacteria such as *E. coli*, the major and perhaps sole receptor for cyclic AMP is a cyclic AMP-binding protein devoid of protein kinase activity which acts directly to regulate genetic transcription (95, 103). One important area of research in fungi, consequently, has been to determine if fungal cyclic AMP receptors resemble either or both of the receptors of these other organisms. It is now clear that diverse fungal species possess cyclic AMP-dependent protein kinases with many properties quite similar to those of animal cells.

Studies have been reported on cyclic AMP-dependent protein kinases from *S. cerevisiae* (61, 127, 128), *N. crassa* (66, 101; J. M. Trevillyan and M. L. Pall, unpublished data), *B. emersonii* (68, 117), *M. rouxii* (86, 87) and *C. macrorhizus* (148). All fungal enzymes show increases in activities of 2- to 10-fold in the presence of low levels of cyclic AMP.

In all the studies, cyclic AMP-dependent protein kinase activity was found in the soluble

fraction of the extract. In *Mucor* (87) and *Blastocladiella* (68) preparations, little or no activity was found in low- or high-speed pellet fractions. Interesting observations have been made which suggest that a large fraction of the endogenous cyclic AMP in *B. emersonii* may be bound to the cyclic AMP-dependent protein kinase and that such bound cyclic AMP is resistant to phosphodiesterase degradation (117).

Assays of cyclic AMP-dependent protein kinase, of course, require the presence of a suitable protein substrate. Histones have been widely used as the substrate and probably histone H2B (also known as f2b) is the substrate of choice for the fungal enzyme (68, 101). Other good substrates include protamine (101, 117) and histone H2A (66). Casein and phosvitin are not useful substrates for fungal cyclic AMP-dependent protein kinase activity because they are poor substrates for this enzyme and because there are other cyclic nucleotide-independent kinases which are active in phosphorylating these substrates (51, 68, 87, 101, 128).

The *Blastocladiella* and *Neurospora* enzymes are inhibited by the mammalian protein kinase inhibitor (68, 101). *Neurospora* extracts are reported to have a protein kinase inhibitor which may be similar to the mammalian inhibitor (66).

It is useful to compare the properties of the fungal enzymes with those of the animal cyclic AMP-dependent protein kinases. Each molecule of the animal protein kinases is made up of two regulatory and two catalytic subunits (R_2C_2). The binding of cyclic AMP to the regulatory subunits activates the catalytic subunit. The activation is usually accompanied by dissociation as follows: $R_2C_2 \rightarrow R_2 + 2C$. The animal enzymes are found as two isozymes in most tissues designated type I and type II, differing in their regulatory but not in their catalytic subunits. The estimated molecular weights of the type I and type II regulatory subunits are 47,000 to 48,000 and 54,000 to 55,000, respectively.

To date, studies of the fungal cyclic AMP-dependent protein kinases have revealed only one enzyme in each species. Thus, the evolution of the two isozymes may postdate the evolutionary divergence of fungi and animals.

The sedimentation properties of the *Mucor* enzyme have been studied (86), and the sedimentation coefficient of the holoenzyme and apparent free catalytic and regulatory components are quite similar to those of their animal counterparts. Thus, the size of the fungal enzymes and its components are probably similar to those of the animal enzymes and their components. The *Mucor* enzyme differs, however, in being more resistant to dissociation of regulatory from catalytic components in the presence of cyclic AMP (86).

Several size measurements have been made of fungal regulatory (cyclic AMP-binding) subunits. These have been done either with purified subunit (61) or by analyzing the cyclic AMP-binding components of crude or partially purified enzyme using their binding activity. In some binding studies, the photoaffinity label, ^{32}P-8-azido cyclic AMP (57) was used to label the cyclic AMP-binding subunit (24, 41, 62, 90, 91; Trevillyan and Pall, unpublished data). Size studies of animal regulatory subunits have shown evidence of partial proteolysis, with some subunit fragments retaining both cyclic AMP binding activity and the ability to regulate the catalytic subunit (21, 164).

It seems clear that the fungal studies have also shown evidence of rapid partial proteolysis. Thus, in early studies of the yeast cyclic AMP-binding component, its molecular weight was estimated to be 14,000 to 28,000 (124, 128). More recent studies have indicated a molecular weight of 50,000 to 54,000, with smaller degradation products being observed in variable amounts when special care was not exercised to avoid proteolysis (24, 61). The *Neurospora* cyclic AMP-binding subunit has an estimated molecular weight of 48,000 (Trevillyan and Pall, unpublished data). It appears, consequently, that yeast, *Neurospora*, and mammalian regulatory subunits all have molecular weights of approximately 50,000.

Jaynes et al. (62) have recently reported the existence of plasma membrane-associated cyclic AMP binding proteins in yeasts which may differ from the soluble proteins described above. They found species of several molecular weights, the largest being about 58,000. A multiplicity of cyclic AMP-binding proteins were reported to occur in *Mucor* extracts (41, 90, 91). Two of the most intense bands had estimated molecular weights of 51,000 and 58,000. It is not clear from either the *Mucor* or yeast plasma membrane studies whether proteolysis occurs in the extracts, but incubation of *Mucor* spores in the presence of cycloheximide led to the disappearance of higher-molecular-weight binding proteins and the accumulation of lower-molecular-weight binding proteins (90), suggesting that in vivo, partial proteolysis may occur in *Mucor* spp.

In essentially all of the above cited studies on cyclic AMP binding and on the cyclic AMP-dependent protein kinase of fungi, the binding of labeled cyclic AMP or 8-azido cyclic AMP was tested for possible inhibition by other nucleotides. Whereas other cyclic nucleotides show presumably competitive inhibition of binding of labeled material, noncyclic nucleotides or nu-

cleosides show essentially no inhibition. The results show that the cyclic AMP-binding activity associated with these protein kinases is specific for cyclic nucleotides, and of the cyclic nucleotides, cyclic AMP itself has the highest apparent affinity.

Similarities of Fungal and Animal Cyclic AMP-dependent Protein Kinases

There are some striking similarities between animal and fungal cyclic AMP-dependent protein kinases which suggest strong evolutionary conservation of the properties of these enzymes. These similarities include the following.

(i) Protein substrate specificity. The fungal enzyme resembles that of the mammalian in having very high affinity for histone H2B (68, 101). When mixed histones are used as a substrate, the pattern of peptides phosphorylated by yeasts and animal enzymes are similar but not identical (127). The yeast enzyme also resembles the animal enzyme in being able to activate mammalian glycogen phosphorylase and to inhibit mammalian glycogen synthetase, presumably by phosphorylation of identical sites on these protein substrates (127).

(ii) Contact sites of regulatory and catalytic subunits. Mixtures of yeast regulatory subunits and animal catalytic subunits and apparently also of yeast catalytic and animal regulatory subunits show good cyclic AMP dependence (61, 128). These results strongly suggest that the contact points of the subunits have been highly conserved so that cyclic AMP stimulation occurs in such hybrid molecules.

(iii) Molecular weight of regulatory subunits. As indicated above, the molecular weights of the regulatory subunits all appear to be close to 50,000.

(iv) Dimer formation of regulatory subunits. Both the yeast and *Mucor* regulatory subunits appear to resemble the animal subunits in forming dimers (61, 86). It also seems likely but not certain that the *Mucor* holoenzyme has the form of R_2C_2 (86).

(v) Inhibition of catalytic activity by mammalian protein kinase inhibitor. Inhibition of catalytic activity has been reported for *Blastocladiella* and *Neurospora* (68, 101).

(vi) Displacement of bound cyclic AMP by hemin. Displacement of bound cyclic AMP by hemin, earlier reported for animal protein kinases, has also been found with the yeast regulatory subunit (61).

It is fascinating to this reviewer that, despite the proposed diversity of functions for cyclic AMP, the presumed receptor for it appears to have been so highly conserved.

Other Cyclic AMP-Binding Proteins

There are a number of reports of cyclic AMP-binding proteins which have not shown the proteins to be cyclic AMP-dependent protein kinases (41, 62, 90, 91, 124). It seems quite likely that some of this binding activity may be due to kinase regulatory subunits or to their degradation products. There are, however, examples of other proteins in fungi which have cyclic AMP-binding activity. Two enzymes with such binding are involved in glycolysis.

Glyceraldehyde 3-phosphate dehydrogenase of a yeast (*S. cerevisiae*) appears to have a low-affinity binding site for cyclic AMP, which when occupied, leads to inhibition of enzyme activity (8, 83, 105, 174). The estimated K_i is about 100 to 200 μM, which is much higher than the typical intracellular cyclic AMP levels in fungi (typically <1 μM). Consequently, it is not clear whether such inhibition has any physiological significance. However, the influence of high levels of cyclic AMP on reduced nicotinamide adenine dinucleotide oscillations in extracts of *Saccharomyces* spp. (11a) may be explainable by this inhibition.

Recently, Brownlee and co-workers (8, 9) have measured binding of cyclic AMP to a higher-affinity binding site of glyceraldehyde 3-phosphate dehydrogenase of yeast (K_a, 1.3 to 10 μM, depending on conditions). The binding of cyclic AMP in this concentration range has no apparent effect on enzyme activity but is competitively inhibited by higher concentrations of nicotinamide adenine dinucleotide, AMP, adenosine 5'diphosphate, or ATP. It is suggested that the biological significance of this binding may be in the possible binding of large fractions of cyclic AMP in the intracellular pool by the glyceraldehyde 3-phosphate dehydrogenase. Some 10 to 20% of the soluble protein of the cell is glyceraldehyde 3-phosphate dehydrogenase, so the molar concentration of this enzyme in the cell is two orders of magnitude higher than that of cyclic AMP in a typical fungal cell. One possibility which is intriguing to the reviewer is that changes in the redox state of the cytoplasmic nicotinamide adenine dinucleotide-reduced nicotinamide adenine dinucleotide pool could change the amount of cyclic AMP bound through changes in the concentration of competing nicotinamide adenine dinucleotide. A cellular shift toward oxidizing conditions would increase the nicotinamide adenine dinucleotide concentration which, according to this hypothesis, would displace cyclic AMP from the enzyme, thus leading to a transient increase in available, thermodynamically active cyclic AMP. Such an increase would occur in the ab-

sence of an increase in total intracellular cyclic AMP. An additional consequence of such binding to glyceraldehyde 3-phosphate dehydrogenase is that, assuming that bound cyclic AMP is resistant to phosphodiesterase degradation, growth conditions which substantially alter the level of glyceraldehyde 3-phosphate dehydrogenase will substantially alter the total measured cyclic AMP pool even in the absence of changes in free cyclic AMP or changes in cyclic AMP synthesis or degradation.

Another glycolytic enzyme influenced by cyclic AMP is phosphofructokinase of *M. rouxii* (97), which was reported to be inhibited by cyclic AMP (K_i, 0.3 mM). Because of the high concentration of cyclic AMP required in vitro, it is difficult to evaluate the physiological significances of this inhibition, but, as pointed out by the investigators, cyclic AMP may not be homogeneously distributed in the cells, and the K_i in vivo may differ from that measured in vitro.

Finally, it was recently reported that cyclic AMP inhibited the plasma membrane ATPase of *Dendryphiella salina* (44) at very high concentrations (1 to 50 mM).

METHODS OF MANIPULATING CYCLIC AMP-DEPENDENT RESPONSES

In animal systems, much of the evidence for cyclic AMP involvement in the control of various processes is based on the use of diverse methods to manipulate cyclic AMP levels. These methods have included the use of hormones and agents like cholera toxin to stimulate adenylate cyclase activity; the use of phosphodiesterase inhibitors such as the methylxanthines, theophylline, caffeine, or 1-methyl-3-isobutylxanthine; the use of cyclic AMP analogs such as dibutyryl cyclic AMP; and more recently, the use of adenylate cyclase-deficient cell culture mutants.

Similar approaches have been used in certain experiments with fungi. In some cases, the drugs or mutants used should be more critically analyzed.

Mutants with Decreased or Increased Cyclic AMP Levels

The most clear-cut of the fungal mutants are the *cr-1* (*crisp*) mutants of *N. crassa*, which were first shown by Terenzi et al. (132) to have little or no adenylate cyclase activity. Several mutant alleles of the *cr-1* gene have been studied, and all of these show little or no adenylate cyclase activity and little or no cyclic AMP (33, 93, 107, 131, 132). The *cr-1* mutants were originally isolated as morphological mutants characterized by colonial growth, little or no formation of aerial hyphae, and early, even conidiation over the surface of the colony. All three of these characteristic features are phenotypically corrected by growth of the mutant in the presence of exogenous cyclic AMP (107, 131). The morphological aberrations are also partially reversed by the occurrence of frequent modifier mutations which occur in *cr-1* stocks (46, 133).

A second adenylate cyclase-deficient mutant of *Neurospora* is the *frost* mutant. This morphological mutant has lower, aberrant adenylate cyclase activity (114) and lower levels of cyclic AMP (93, 114). The adenylate cyclase deficiency is proposed to be an indirect consequence of a lesion in a structural gene for glucose-6-phosphate dehydrogenase (114).

In *Schizophyllum commune*, a mutant designated *bse* has elevated levels of cyclic AMP (112). The mutant has a characteristic aberrant morphology of its fruiting bodies which is also produced in the wild type by exogenous cyclic AMP (111, 112). The results suggest that the elevated cyclic AMP levels in the *bse* mutant are responsible for the aberrant fruiting body morphology.

In *C. macrorhizus*, variants have been isolated from wild-type strains which differ in their sexual fruiting properties and their reported levels of cyclic AMP (145–147). The strain differences appear to be multigenic (145). Strains constitutive or inducible for monocaryotic fruiting (*fis*^{c} or *fis*$^+$) were found to have higher levels of cyclic AMP than noninducible (*fis*$^-$) strains (146, 152).

Two mutants of *P. blakesleeanus* have been briefly described which appear to be changed in cyclic AMP responses. The *mad* strain is deficient in a transient depression in cyclic AMP levels produced in the wild type by blue light (16). A second mutant, designated C2, was stimulated in its growth rate on certain carbon sources by exogenous cyclic AMP (161).

It should be noted that in no case is there clear evidence that any of these fungal mutants are structural mutants in some specific enzyme involved in cyclic AMP metabolism. Consequently, one cannot assume that aberrations of the mutants necessarily reflect aberrations in the cyclic AMP control system. However, the evidence available to date is consistent with the *cr-1* mutants being structural mutants in adenylate cyclase.

Phosphodiesterase Inhibitors

Methylxanthines, the most commonly used phosphodiesterase inhibitors in animal systems, are relatively inefficient inhibitors of most fungal phosphodiesterases (see Phosphodiesterase). In many cases, very high concentrations (5 to 10

mM) are required to obtain even partial inhibition. High concentrations of such compounds may, of course, affect enzymes other than phosphodiesterases. In fungi, theophylline inhibits adenylate cyclase activity of *Neurospora* (116) and stimulates the cyclic AMP-dependent protein kinase of *M. rouxii* (87). It should not be surprising that methylxanthines such as theophylline influence enzymes other than phosphodiesterases. These compounds are, after all, purine bases and consequently may bind to binding sites for purine bases, nucleosides, or nucleotides.

Assumptions that methylxanthine effects are due to phosphodiesterase inhibition can lead to unwarranted suggestions of important cyclic AMP roles. For example, theophylline, caffeine, or both were reported to influence two oscillatory systems in *Neurospora*, a membrane potential oscillation (54) and the circadian rhythm in *Neurospora* (29) leading to the suggestion that cyclic AMP is involved in both oscillatory systems. However, subsequent studies revealed that both oscillations are normal in the *cr-1*, cyclic AMP-deficient mutant, showing that cyclic AMP has no required role in either process (30, 93).

Some in vivo studies have shown apparent effects of theophylline on cyclic AMP stability. Thus, theophylline is reported to increase cyclic AMP levels in solid-grown (but not liquid-grown) cultures of *Neurospora* (116) and to potentiate effects of exogenous cyclic AMP in *Neurospora* (107) and *Aspergillus* (171).

High levels of theophylline are not required for effective inhibition of phosphodiesterase in *C. macrorhizus*, for which 2 μM theophylline or caffeine are reported to give 100% inhibition of phosphodiesterase activity (147).

Exogenous Cyclic AMP and Its Analogs

Exogenous cyclic AMP has been used to try to trigger cyclic AMP-mediated responses in a wide variety of fungi (6, 12, 28, 31, 42, 71, 79, 102, 107, 111, 121, 131, 133, 136, 140, 146, 156, 167, 171). Typically, concentrations of 1 to 5 mM have been used, although concentrations an order of magnitude higher or lower have been used in some experiments. Similar concentrations of exogenous cyclic AMP are required for cyclic AMP-mediated effects in *E. coli* (for review, see references 95 and 103). Presumably, such high concentrations of exogenous cyclic AMP (on the order of 10^4 times the normal endogenous levels found in fungi) are required because of the low permeability of the cell to cyclic AMP and the rapid turnover of cyclic AMP in these cells.

In studies of exogenous cyclic AMP, it is important to demonstrate that any response is specific for cyclic AMP and is not produced by noncyclic adenine nucleotides, adenosine, or adenine. In some studies with fungi, such controls were not reported, and in others specificity was not found.

It may be questioned whether a hydrophilic, negatively charged compound like cyclic AMP enters fungal cells at all. However, experiments with the *cr-1* adenylate cyclase-deficient mutants of *Neurospora* strongly suggest that it does. The characteristic morphological defects of the mutant are largely corrected by exogenous cyclic AMP (33, 107, 131). It is difficult to interpret these results except by inferring that the exogenous cyclic AMP can enter the cell and bind to any cyclic AMP receptor, thus producing a response similar to the endogenous cyclic AMP normally present in the wild type. A similar inference can be drawn from the finding that exogenous cyclic AMP applied to wild type *S. commune* produces a phenocopy of the *bse* mutant which has elevated cyclic AMP levels (111, 112).

Although fungi generally require millimolar concentrations of exogenous cyclic AMP to produce a physiological response, one organism has been reported to respond to much lower concentrations. This organism is *C. macrorhizus*, in which 0.2 nM exogenous cyclic AMP induces sexual fruiting in a suitable strain of the organism (145). The exquisite sensitivity of this organism, about 10^6 times greater than that found in other organisms, suggests to this reviewer that an extracellular receptor must be involved.

The most commonly used cyclic AMP analog in animal systems is N^6,O^2-dibutyryl cyclic AMP. In several ascomycetous fungi, dibutyryl cyclic AMP has been used and found to be less active or no more active than cyclic AMP itself. These fungi include *N. crassa* (31, 107, 131), *S. cerevisiae* (28, 136), *Schizosaccharomyces pombe* (42), and *Aspergillus niger* (171). Thus, in ascomycetous fungi at least, dibutyryl cyclic AMP is not the analog of choice. It has been suggested that dibutyryl cyclic AMP is relatively inactive in these fungi, because it is not efficiently converted into N^6-monobutyryl cyclic AMP, which is the active analog in mammalian cells (107).

One fungus in which dibutyryl cyclic AMP is active is *Mucor* spp., in which dibutyryl cyclic AMP produces morphological changes similar to yeastlike growth (63, 70, 96, 98).

Because the *Neurospora cr-1* mutant responds specifically to exogenous cyclic AMP, this response has been used to screen for effective cyclic AMP analogs in that system (107).

The two effective analogs found, N^6-monobutyryl cyclic AMP and 8-bromocyclic AMP, should be investigated in other fungi.

MEASUREMENTS OF ENDOGENOUS CYCLIC AMP LEVELS: ARE THERE SPECIFIC CELLULAR PARAMETERS WHICH CONTROL CYCLIC AMP LEVELS?

Measurements of endogenous cyclic AMP levels in fungi are susceptible to two difficulties. Compounds other then cyclic AMP may have activity in the two most commonly used assays (10, 47, 59, 123), as has been reported in two studies of *Neurospora* (93, 131). In addition, rapid changes of cyclic AMP levels have been reported in many fungal species (15, 16, 92, 93, 135, 152, 155) and consequently, changes of cyclic AMP levels may occur during collection and extraction of the cultures to be studied.

Studies of exogenous cyclic AMP levels are discussed in Methods of Manipulating Cyclic AMP-Dependent Responses and in Proposed Functions for Cyclic AMP; consequently, this section concentrates on the issue raised in its title. The control of cyclic AMP levels in animal cells by hormones and neurotransmitters and by sugars in *E. coli* was a crucial element in our understanding of cyclic AMP function in those systems. By analogy, it can be argued that it is of great importance to determine what cellular parameter(s) controls cyclic AMP levels in fungi.

The most extensive studies of this type have looked for possible lowering of cyclic AMP levels by the presence of sugars or other carbon sources in the medium, or conversely, the raising of levels by carbon starvation.

The most dramatic negative correlation between carbon source availability and measured endogenous cyclic AMP levels was reported in studies of *S. pombe* (110). However, in another study of the same species, the carbon source glucose was not found to depress cyclic AMP levels (42). In two species of *Saccharomyces*, glucose lowered cyclic AMP levels (125, 158), but in *Mucor*, *Neurospora*, and *Aspergillus*, little correlation was found between available exogenous carbon sources and measured cyclic AMP levels (92, 96, 176). It appears, consequently, that no general case can be made for lowering of cyclic AMP levels in fungi by glucose or other carbon sources.

Studies in my laboratory have shown a correlation between the electrical potential across the plasma membrane of *Neurospora* (and possibly other fungi) and cyclic AMP levels, with depolarizing treatments yielding cyclic AMP increases (92, 93, 135). Recently, a depolarizing treatment has been found (osmotic shock) which fails to produce a cyclic AMP increase (unpublished data). Consequently, the potential change may not be the direct cause of the cyclic AMP changes. What is clear from these studies is that cyclic AMP levels in fungi can change very rapidly, with a half-life of considerably less than a minute (my best estimate in *Neurospora* at 25°C would be ca. 15 s).

In one study, amino acid starvation produced a small increase in cyclic AMP levels (23).

PROPOSED FUNCTIONS FOR CYCLIC AMP

Criteria for Control by Cyclic AMP in Fungi

No single experimental approach is sufficient to infer cyclic AMP-mediated control of a specific response. Four criteria were proposed as tests of possible cyclic AMP involvement as a second messenger in hormonal action (reference 104, chapter 2). For fungi, if anything, such criteria should be more stringent than in hormonal control systems, because the possible roles of cyclic AMP in fungi are not necessarily constrained to second messenger functions.

Six criteria for cyclic AMP control of a specific function may be proposed: (i) correlation of endogenous cyclic AMP levels with proposed cyclic AMP-controlled function; (ii) correlation of the function with activation of presumptive cyclic AMP receptors (e.g., activation of cyclic AMP-dependent protein kinases); (iii) influence on that function by exogenous cyclic AMP or its analogs—this influence should be specific and not shared by noncyclic adenine nucleotides or adenosine; (iv) influence on that function by drugs which affect adenylate cyclase activity or phosphodiesterase activity and have been shown to produce changes in endogenous cyclic AMP levels; (v) changes of the function in mutants with increased or decreased cyclic AMP levels; and (vi) cyclic AMP control of the function in a cell-free, in vitro system.

Of these criteria, all are currently feasible in suitable fungal systems, and all except the second have been examined in some cases. However, only biochemically well-defined responses can be tested in any cell-free system; thus, it is not currently possible, for example, to look at developmental effects in vitro. Furthermore, in only a few fungal species have cyclic AMP mutants been isolated.

The activation of cyclic AMP-dependent protein kinases envisioned in the second criterion can probably be studied by the experimental approaches used in animal systems. These approaches entail measuring the titration of the

protein kinase regulatory subunit by endogenous cyclic AMP (25), determining protein kinase activation ratios (20, 94), or measuring apparent cyclic AMP-dependent phosphorylation in vivo.

Clearly, if all six criteria provide support for cyclic AMP stimulation or inhibition of a response, no one would hesitate to conclude that cyclic AMP has a regulatory role. If only four of the six criteria were clearly fulfilled, I propose that an inferred cyclic AMP role would probably be warranted.

In studies of possible cyclic AMP-controlled functions in fungi, three of these criteria at most have been investigated for any specific function. The fulfilling of particular criteria must be considered doubtful in many cases because of possible lack of specificity of drugs used or criticisms of studies of endogenous cyclic AMP levels. In general, proposed functions for cyclic AMP in fungi must be considered to be unproven.

Catabolism of Carbon Reserves

In many animal cells, cyclic AMP appears to have a role in stimulating the catabolism of stored carbon reserves such as glycogen and triglycerides. Consequently, one attractive hypothesis is that cyclic AMP may also stimulate such breakdown in fungi. In the case of glycogen degradation in animals, cyclic AMP-dependent phosphorylation activates (by phosphorylation) phosphorylase kinase, which in turn activates (by phosphorylation) glycogen phosphorylase (120).

N. crassa glycogen phosphorylase has been shown to occur in two different forms, designated a and b, which are catalytically active and relatively inactive, respectively (50, 129). The two forms appear to be interconvertible by the phosphorylation-dephosphorylation process. Under certain circumstances, cyclic AMP added to an enzyme extract was reported to stimulate the enzyme activity (129). In all of these properties, the *Neurospora* system resembles the animal system. *N. crassa* is reported to differ from the animal system in that cyclic AMP does not influence the activity of the fungal phosphorylase *a* phosphatase (130). It is not clear whether the fungal enzyme system resembles the animal system by having a phosphorylase kinase and cyclic AMP-dependent protein kinase involved in the cyclic AMP effect; nor is there evidence that in vivo fluctuations in cyclic AMP influence the activity of the system.

Glycogen phosphorylase from *C. macrorhizus* was also found to be activated by incubation in vitro with cyclic AMP and ATP (150). The activation required the presence of a protein fraction containing cyclic AMP-dependent protein kinase activity. Glycogen synthetase from *C. macrohizus* is inhibited by cyclic AMP (151).

The glycogen content in *Phycomyces* was lowered by exogenous cyclic AMP (140); no other nucleotides were tested for such effects. In *S. cerevisiae*, no simple correlation was found between glycogen degradation and endogenous cyclic AMP levels (40). It is possible, of course, that cyclic AMP may be one of several signals influencing glycogen metabolism.

In *S. cerevisiae*, the enzyme trehalase is found to be activated in vitro by a cyclic AMP and ATP-Me^{2+}-dependent process, presumably cyclic AMP-dependent phosphorylation (156, 157). The degradation of trehalose during incubation of stationary-phase yeast cells in fresh medium is correlated with cyclic AMP levels, suggesting a cyclic AMP role in trehalase activation under such conditions.

Mitochondrial Function in Yeast

In *E. coli*, repression by glucose and other catabolites is thought to be mediated, in part, by the lowering of cyclic AMP levels, and exogenous cyclic AMP can partially overcome the effect of glucose repression (for review, see references 95 and 103). In *S. cerevisiae*, mitochondrial synthesis is repressed by high glucose levels, raising the question of whether this glucose repression might be mediated by cyclic AMP. Fang and Butow (28) reported that exogenous cyclic AMP partially overcame glucose repression of mitochondrial respiration. These findings have been confirmed and extended in several other laboratories (5, 12, 71, 79, 121). This cyclic AMP effect extends to four mitochondrial enzymes (12, 79). The mitochondrion is reported to have a transport system for cyclic AMP (5) and mitochondrial transcription and translation (12) and is influenced by cyclic AMP. Ubiquinone-6-biosynthesis in isolated mitochondria is stimulated by cyclic AMP (119).

However, cyclic AMP shares the ability to overcome glucose repression of mitochondrial function with other nucleotides such as AMP, ATP, 2',3'-cyclic AMP, and guanosine 5'-monophosphate (28), so there may not be a specific cyclic AMP-mediated response. Furthermore, a recent report suggests that cyclic AMP may act, in part, by inhibiting the uptake of glucose into yeast cells (121), i.e., cyclic AMP may influence internal glucose levels. It appears to this reviewer that it will be necessary to investigate the above-mentioned in vitro cyclic AMP effects for their specificities and mechanisms in order to ascertain the role, if any, of cyclic AMP in mitochondriogenesis in yeasts.

Finally, it should be noted that glucose repres-

sion of the presumably glyoxysomal enzyme, isocitrate lyase in *Candida tropicalis*, is not reversed by exogenous cyclic AMP (88). Glucose repression of catalase induction in *Kloeckera* spp. is not overcome by either exogenous cyclic AMP or theophylline (175).

Utilization of Exogenous Carbon Sources

The possible role of cyclic AMP in activating utilization of exogenous carbon sources has also been studied. The evidence on this possible role in fungi is equivocal, with some evidence suggesting a positive role and other evidence indicating no role or a negative role. Some of the evidence is discussed in Are There Specific Cellular Parameters Which Control Cyclic AMP Levels?

The *cr-1* adenylate cyclase-deficient mutants of *N. crassa* are deficient in their growth on glycerol and several other carbon sources, and growth on these carbon sources is stimulated by exogenous cyclic AMP (73, 133). The mutant grows quite well on certain other carbon sources, such as glucose or acetate. These properties, then, provide strong support for the inference that the low cyclic AMP levels of these mutants are responsible for the lessened ability to grow on certain carbon sources. It is not clear whether these observations reflect changes of cyclic AMP levels within the normal physiological range, because the levels in the mutant are well below those found in the wild type (possibly zero). It is also not clear what steps involved in growth on these carbon sources are inhibited by the cyclic AMP deficiency.

Several (carbon) catabolite repression-insensitive mutants have been isolated in *Saccharomyces*, but no clear correlation has been found between such insensitivity and endogenous cyclic AMP levels (85, 109).

The influence of cyclic AMP on the activity of several catabolic enzymes has been probed. For example, exogenous cyclic AMP was reported to overcome glucose repression of α-glucosidase synthesis in *Saccharomyces* (167, 168), but exogenous cyclic AMP did not overcome glucose repression of β-glucosidase induction in *N. crassa* (26). Exogenous cyclic AMP was found to both repress and inhibit β-glucosidase in *Mucor racemosus*, but 5'-AMP, adenosine, and adenine had no effect (7). In the *Mucor* study, the effect of cyclic AMP was similar to that of glucose rather than antagonistic.

Finally, the glucose transport system II of *Neurospora*, which is repressed by glucose, is normally derepressed in the *cr-1* mutants (93), showing that elevated cyclic AMP levels are not required for such derepression.

Developmental Studies

The possible role of cyclic AMP in development and morphogenesis of fungi has attracted the attention of several laboratories. Such studies are among the most difficult, because development is such a complex and biochemically ill-defined process. Even when convincing evidence may be obtained for cyclic AMP involvement in a particular developmental phenomenon, it is difficult to determine if such involvement is a direct or indirect consequence of cyclic AMP controlling metabolism, cell surface structure, or some other parameter.

In several species of *Mucor*, cyclic AMP is postulated to produce yeastlike as opposed to mycelial growth morphology (11, 70, 96, 98, 126). The main evidence supporting this proposal is (i) that there is a correlation between measured endogenous cyclic AMP levels and yeastlike versus mycelial growth in *M. racemosus* (70, 98) and *M. rouxii* (96) and (ii) that exogenous dibutyryl cyclic AMP induces a transformation from mycelial to yeastlike growth in several *Mucor* species (70, 96). The lowering of the nicotinamide adenine dinucleotide-dependent glutamic dehydrogenase characteristic of normal yeastlike growth is also produced by dibutyryl cyclic AMP (99). Exogenous cyclic AMP added to swollen spores of *M. rouxii* is also reported to produce yeastlike growth (96), but cyclic AMP did not produce yeastlike morphology under other conditions or in other *Mucor* species investigated. Two questions arise in this context: does dibutyryl cyclic AMP act as a cyclic AMP analog (see Exogenous Cyclic AMP and Its Analogs) and are the morphological changes produced by dibutyryl cyclic AMP more complex than simply a transition to yeastlike growth (63)?

The relationships between cyclic AMP and morphology in *Histoplasma capsulatum* may be opposite to that proposed for *Mucor* spp. (82). In *H. capsulatum*, cyclic AMP levels are found to be higher in mycelia than in yeast cells and exogenous dibutyryl cyclic AMP converts yeast cells to mycelia. The pattern in *Candida* appears to resemble that found in *Histoplasma* (99).

Another possible developmental influence of cyclic AMP is on conidiation and carotenoid biosynthesis in *N. crassa*, in which two types of evidence suggest a cyclic AMP role. The *cr-1* adenylate cyclase and cyclic AMP-deficient mutants tend to conidiate prematurely (131, 132), and conidiation is suppressed by exogenous cyclic AMP and its analogs both in *cr-1* mutants (107, 131) and in the wild type (58, 144). The levels of nicotinamide adenine dinucleotide gly-

cohydrolase, which tend to be coordinately regulated with conidiation in wild type *Neurospora*, is elevated in *cr-1* cultures and repressed by exogenous cyclic AMP (64). Exogenous cyclic AMP also lowers carotenoid synthesis (58, 144). No studies have yet been reported on whether abnormally low levels of cyclic AMP, such as those found in the *cr-1* mutants, stimulate carotenoid biosynthesis.

Two facts should be noted about the apparent effect of cyclic AMP on conidiation in *Neurospora*. Both cyclic AMP and carbon sources (142, 144) tend to repress conidiation so that unlike in *E. coli*, these agents act in parallel and not antagonistically. Secondly, conidiation responds to a variety of metabolic influences (143), so cyclic AMP may be acting here through an influence on metabolism rather than directly.

The formation of sexual fruiting bodies is another developmental control process which may be influenced by cyclic AMP. Evidence for this was first reported for *C. macrorhizus*, in which exogenous cyclic AMP stimulates such fruiting in certain strains and in which, when comparing genetic variants, cyclic AMP levels appear to be correlated with ability to fruit (145–147, 153). Cyclic AMP may also stimulate sexual fruiting in *S. commune* (113) and in other basidiomycetes (49). In *Neurospora*, exogenous cyclic AMP induces tyrosinase, an enzyme which tends to be produced during sexual fruiting and catalyzes the formation of the melanin pigment of the protoperithecia and perithecia (31). However, the *cr-1* cyclic AMP-deficient mutant does form melanin-pigmented protoperithecia (unpublished data), so cyclic AMP is not required for either tyrosinase synthesis or fruiting in *Neurospora*.

As mentioned above, it was recently found that the mating factor in *S. cerevisiae* inhibited adenylate cyclase from wild-type yeast strains but not from an α-factor-resistant mutant strain (74), suggesting a role of cyclic AMP in the yeast mating response. Somewhat surprisingly, the α mating factor inhibited adenylate cyclase from α and a/α strains as well as the a mating type strain, whereas only the latter strains normally respond physiologically to the mating factor.

Endogenous cyclic AMP levels are found to fluctuate during the cell cycle of *S. cerevisiae* (163). It should be noted, however, that although similar fluctuations occur during the cell cycle of mammalian cells, they are not required in mammalian cells for normal cell cycling (14). Changes in endogenous cyclic AMP levels have also been found during the life cycle and zoospore formation in *Blastocladiella* (27, 53, 118) and during spore germination in species of *Blastocladiella* (155) and *Mucor* (97) but not in those of *Neurospora* (106). Cyclic AMP levels in *S. cerevisiae* were found to fluctuate during sporulation (60, 162; but also see reference 40). Exogenous cyclic AMP was found to have some effect on glucose repression of sporulation in studies of one group (136–138) but not those of another (60).

Other Proposed Cyclic AMP Functions and a Unifying Hypothesis

Several other proposed cyclic AMP functions are listed in Table 2. In most of these cases, the major evidence supporting a role is from studies of the effects of exogenous cyclic AMP or of drugs purported to influence cyclic AMP levels.

The studies of a possible role of cyclic AMP in the phototropism of *P. blakesleeanus* by Cohen and co-workers (15–19) have involved several approaches. Cyclic AMP levels in *Phycomyces* were found to undergo a transient depression on exposure of the cells to blue light (15). Blue light was also found to activate both the phosphodiesterase (18) and the adenylate cyclase (19) of *Phycomyces*. The light effect on phosphodiesterase might be expected to produce the observed depression of cyclic AMP levels, but the effect on adenylate cyclase would yield the opposite response. A mutant strain with an aberrant phototropic response lacked the light-induced depression of cyclic AMP levels (16). Thus, several types of evidence suggest that

TABLE 2. *Miscellaneous suggested functions for cyclic AMP in fungi*

Organism	Possible function	Reference
A. niger	Stimulates citric acid production	1, 171
	Stimulates conidial aggregation	172
A. parasiticas	Influence on aflatoxin production	2
B. emersonii	Stimulates spore germination	81
Mucor, Neurospora, and *Schizophillum*	Influences morphology	63, 84, 111, 112, 116
P. blakesleeanus	Mediates phototropism	15–19
	Increases cell wall thickness	140
	Changes Ca^{2+} distribution	141
Saccharomyces and *Schizosaccharomyces*	Stimulates transport	42, 102

cyclic AMP may have a role in the light response.

The diversity of proposed functions for cyclic AMP in fungi confronts researchers with a bewildering array of complexities. I would like to propose a unifying hypothesis, which, although largely speculative at this point, may serve as a focus for future thought and experimentation. The hypothesis is that cyclic AMP in fungi acts to stimulate glycolysis and that many of its effects are produced through such stimulation. This hypothesis is consistent with the in vitro studies which showed that cyclic AMP stimulated breakdown of glycogen and trehalose (129, 150, 156, 157). It was originally suggested that stimulation of glycolysis may be responsible for its ability to stimulate citric acid accumulation in *A. niger* (171) and transport in *S. pombe* (42). In *Neurospora*, glycolytic activity is inversely correlated with conidiation (143), so the proposed effect on glycolysis may in turn produce the apparent cyclic AMP inhibition of conidiation (58, 144). Other proposed effects, such as those on spore germination in *B. emersonii* (81), may be indirect responses to a stimulation of glycolysis.

Whether or not this hypothesis is correct, it will be necessary in the future to examine common pathways of cyclic AMP action in analyzing its different influences.

ACKNOWLEDGMENTS

I am grateful to the investigators who sent me reprints of their work and to J. Paznokas, J. Trevillyan, and G. Rosenberg for helpful discussions.

This work was supported by Public Health Services grant GM 24368 from the National Institutes of Health.

LITERATURE CITED

1. **Alobaidi, Z. S., and D. R. Berry.** 1980. cAMP concentration, morphological differentiation and citric acid production in *Aspergillus niger*. Biotechnol. Lett. **2**:5–10.
2. **Applebaum, R. S., and R. L. Buchanan.** 1979. Intracellular concentration of cyclic AMP and cyclic GMP in an aflatoxigenic strain of *Aspergillus parasiticus*. J. Food Sci. **44**:116–117.
3. **Arch, J. R. S., and E. A. Newsholme.** 1976. Activities and some properties of adenylate cyclase and phosphodiesterase in muscle, liver, and nervous tissues from vertebrates and invertebrates in relation to the control of the concentration of adenosine 3':5'-cyclic monophosphate. Biochem. J. **158**:603–622.
4. **Atmar, V. T., G. O. Throneberry, and G. D. Kuehn.** 1976. Effects of adenine and cytokinins on growth and protein kinase activity of *Verticilium albo-atrum*. Mycopathololgy **59**: 171–174.
5. **Bandlow, W.** 1979. Control of mitochondrial gene expression in yeast by adenosine 3',5'-cyclic monophosphate. Hoppe Seyler's Z. Physiol. Chem. **360**:228–229.
6. **Bhattacharya, A., and A. Datta.** 1977. Effect of cyclic AMP on RNA and protein synthesis in *Candida albicans*. Biochem. Biophys. Res. Commun. **77**:1438–1444.
7. **Borgia, P., and P. S. Sypherd.** 1977. Control of β-glucosidase synthesis in *Mucor racemosus*. J. Bacteriol. **130**:812–817.
8. **Brownlee, A. G., D. R. Phillips, and G. M. Polya.** 1980. Purification and characterization of two high-affinity (adenosine 3',5'-monophosphate)-binding proteins from yeast. Identification as multiple forms of glyceraldehyde 3-phosphate dehydrogenase. Eur. J. Biochem. **109**:39–49.
9. **Brownlee, A. G., and G. M. Polya.** 1980. The ligand specificity of the (adenosine 3',5'-monophosphate)-binding site of yeast glyceraldehyde 3-phosphate dehydrogenase. Interaction with adenosine derivatives and pharmacologically active compounds. Eur. J. Biochem. **109**: 51–59.
10. **Cailla, H. L., M. S. Racine-Weisbuch, and M. A. Delaage.** 1973. Adenosine 3',5' cyclic monophosphate assay at 10^{-15} mole level. Anal. Biochem. **56**:394–407.
11. **Cantore, M. L., M. A. Galvagno, and S. Passeron.** 1980. Variations in the levels of cyclic adenosine 3',5'-monophosphate and in the activities of adenylate cyclase and cyclic adenosine 3',5'-monophosphate phosphodiesterase during aerobic morphogenesis in *Mucor rouxii*. Arch. Biochem. Biophys. **199**:312–320.

11a. **Chance, B., and B. Schoener.** 1964. Control of oscillations of the DPNH level in a cell-free extract of *Saccharomyces carlsbergensis* by 3',5' cyclic AMP. Biochem. Biophys. Res. Commun. **17**:416–423.

12. **Chandrasekaran, K., and J. Jayaraman.** 1978. Effect of cyclic AMP on the biogenesis of cytochrome oxidase in yeast. FEBS Lett. **87**: 52–54.
13. **Coffino, P., H. R. Bourne, P. A. Insel, K. L. Melmon, G. Johnson, and J. Vigne.** 1978. Studies of cyclic AMP function using mutant tissue culture cells. In Vitro **14**:140–145.
14. **Coffino, P., J. W. Gray, and G. M. Tomkins.** 1975. Cyclic AMP, a nonessential regulator of cell-cycle. Proc. Natl. Acad. Sci. U.S.A. **72**: 878–882.
15. **Cohen, R. J.** 1974. Cyclic AMP levels in *Phycomyces* in response to light. Nature (London) **251**:144–146.
16. **Cohen, R. J.** 1978. Aberrant cyclic nucleotide regulation in a behavioral mutant of *Phycomyces blakesleeanus*. Plant Sci. Lett. **13**:315–319.
17. **Cohen, R. J.** 1979. Adenosine 3',5'-cyclic monophosphate phosphodiesterase from *Phycomyces blakesleeanus*. Phytochemistry **18**:943–948.

18. **Cohen, R. J., and M. M. Atkinson.** 1978. Activation of *Phycomyces* adenosine 3',5'-monophosphate phosphodiesterase by blue light. Biochem. Biophys. Res. Commun. **83**:616–621.
19. **Cohen, R. J., J. L. Ness, and S. M. Whiddon.** 1980. Adenylate cyclase from *Phycomyces* sporangiophore. Phytochemistry **19**:1913–1918.
20. **Corbin, J. D., T. R. Soderling, and C. R. Park.** 1973. Regulation of adenosine 3',5'-monophosphate-dependent protein kinase I. Preliminary characterization of the adipose tissue enzyme in crude extracts. J. Biol. Chem. **248**:1813–1821.
21. **Corbin, J. D., P. H. Sugden, L. West, D. A. Flockhart, T. M. Lincoln, and D. McCarthy.** 1978. Studies on the properties and mode of action of the purified regulatory subunit of bovine heart adenosine 3',5'-monophosphate-dependent protein kinase. J. Biol. Chem. **253**:3997–4003.
22. **Dandekar, S., and V. V. Modi.** 1980. Involvement of cyclic AMP in carotenogenesis and cell differentiation in *Blakeslea trispora*. Biochim. Biophys. Acta **628**:398–406.
23. **De Carlo, R. R., and E. W. Somberg.** 1974. The effect of essential amino acid deprivation on macromolecular synthesis and nucleotide pool sizes in *Neurospora crassa*. Arch. Biochem. Biophys. **165**:201–212.
24. **Dery, C., S. Cooper, M. A. Savageau, and S. Scanlon.** 1979. Identification and characterization of the cAMP binding proteins of yeast by photoaffinity labeling. Biochem. Biophys. Res. Commun. **90**:933–939.
25. **Dufau, M. L., T. Tsuruhara, K. A. Horner, E. Podesta, and K. J. Catt.** 1977. Intermediate role of adenosine 3',5'-cyclic monophosphate and protein kinase during gonadotropin-induced steroidogenesis in testicular interstitial cells. Proc. Natl. Acad. Sci. U.S.A. **74**:3419–3423.
26. **Eberhart, B. M., and R. S. Beck.** 1973. Induction of β-glucosidases in *Neurospora crassa*. J. Bacteriol. **116**:295–303.
27. **Epstein, P. M., and P. M. Silverman.** 1978. Induction of cyclic AMP phosphodiesterase in *Blastocladiella emersonii* and its relation to cyclic AMP metabolism. J. Bacteriol. **135**:968–975.
28. **Fang, M., and R. A. Butow.** 1970. Nucleotide reversal of mitochondrial repression in *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **41**:1579–1583.
29. **Feldman, J. F.** 1975. Circadian periodicity in *Neurospora*: alteration by inhibitors of cyclic AMP phosphodiesterase. Science **190**:789–790.
30. **Feldman, J. F., G. Gardiner, and R. Denison.** 1979. Genetic analysis of the circadian clock of *Neurospora*, p. 57–66. *In* M. Suda, O. Hayishi, and H. Nakagawa (ed.), Biological rhythms and their control mechanism (The Naito Foundation, 1979). Elsevier/North Holland Biomedical Press, Amsterdam.
31. **Feldman, J. F., and J. P. Thayer.** 1974. Cyclic AMP-induced tyrosinase synthesis in *Neurospora crassa*. Biochem. Biophys. Res. Commun. **61**:977–981.
32. **Fell, D. A.** 1980. Theoretical analysis of the functioning of the high and low Km cyclic nucleotide phosphodiesterases in the regulation of the concentration of adenosine 3',5'-cyclic monophosphate in animal cells. J. Theor. Biol. **84**:361–386.
33. **Flawia, M. M., H. F. Terenzi, and H. N. Torres.** 1977. Characterization of *Neurospora crassa* mutant strains deficient in adenylate cyclase activity. Arch. Biochem. Biophys. **180**:334–342.
34. **Flawia, M. M., and H. N. Torres.** 1972. Adenylate cyclase activity in *Neurospora crassa*. I. General properties. J. Biol. Chem. **247**:6873–6879.
35. **Flawia, M. M., and H. N. Torres.** 1972. Adenylate cyclase activity in *Neurospora crassa*. II. Kinetics. J. Biol. Chem. **247**:6880–6883.
36. **Flawia, M. M., and H. N. Torres.** 1972. Adenylate cyclase activity in Lubrol-treated membranes from *Neurospora crassa*. Biochim. Biophys. Acta **289**:428–432.
37. **Flawia, M. M., and H. N. Torres.** 1972. Activation of membrane-bound adenylate cyclase by glucagon in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **69**:2870–2873.
38. **Flawia, M. M., and H. N. Torres.** 1973. Adenylate cyclase activity in *Neurospora crassa*. III. Modulation by glucagon and insulin. J. Biol. Chem. **248**:4517–4520.
39. **Flawia, M. M., and H. N. Torres.** 1973. Insulin inhibition of membrane-bound adenylate cyclase in *Neurospora crassa*. FEBS Lett. **30**:74–78.
40. **Fonzi, W. A., M. Shanley, and D. J. Opheim.** 1979. Relationship of glycolytic intermediates, glycolytic enzymes and ammonia to glycogen metabolism during sporulation in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **137**:285–294.
41. **Forte, J. W., and M. Orlowski.** 1980. Profile of cyclic adenosine 3',5'-monophosphate binding proteins during conversion of yeasts to hyphae in the fungus *Mucor*. Exp. Mycol. **4**:78–86.
42. **Foury, F., and A. Goffeau.** 1975. Stimulation of active uptake of nucleosides and amino acids by cyclic AMP in the yeast *Schizosaccharomyces pombe*. J. Biol. Chem. **250**:2354–2362.
43. **Fujimoto, M., A. Ichikawa, and K. Tomita.** 1974. Purification and properties of cyclic AMP phosphodiesterase from baker's yeast. Arch. Biochem. Biophys. **161**:54–63.
44. **Galpin, M. F. J., and D. H. Jennings.** 1980. A plasma-membrane ATPase from *Dendryphiella salina*: cation specificity and interaction with fusicoccin and cyclic AMP. Trans. Br. Mycol. Soc. **75**:37–46.
45. **Galvagno, M. A., S. Moreno, M. L. Cantore, and S. Passeron.** 1979. Cyclic adenosine 3',5'-monophosphate phosphodiesterase from *Mu-*

cor rouxii: regulation of enzyme activity by phosphorylation and dephosphorylation. Biochem. Biophys. Res. Commun. **89**:779–785.

46. **Garnjobst, L., and E. L. Tatum.** 1970. New crisp genes and crisp-modifiers in *Neurospora crassa*. Genetics **66**:281–290.
47. **Gilman, A. G.** 1970. A protein binding assay for adenosine 3',5'-cyclic monophosphate. Proc. Natl. Acad. Sci. U.S.A. **67**:305–312.
48. **Glass, D. B., and E. G. Krebs.** 1980. Protein phosphorylation catalyzed by cyclic AMP-dependent and cyclic GMP-dependent protein kinases. Annu. Rev. Pharmacol. Toxicol. **20**: 363–388.
49. **Gold, M. H., and T. M. Cheng.** 1979. Conditions for fruit body formation in white rot basidomycetes. Arch. Microbiol. **121**:37–42.
50. **Gold, M. H., R. J. Farrand, J. P. Livioni, and I. H. Segel.** 1974. *Neurospora crassa* glycogen phosphorylase: interconversion and kinetic properties of the "active" form. Arch. Biochem. Biophys. **161**:515–527.
51. **Gold, M. H., and I. H. Segel.** 1974. *Neurospora crassa* protein kinase. Purification, properties and kinetic mechanism. J. Biol. Chem. **249**: 2417–2423.
52. **Gomes, S. L., and J. C. C. Maia.** 1979. Differential effects of manganese ions on *Blastocladiella emersonii* adenylate cyclase. Biochim. Biophys. Acta **567**:257–264.
53. **Gomes, S. L., L. Mennucci, and J. C. C. Maia.** 1978. Adenylate cyclase activity and cyclic AMP metabolism during cytodifferentiation of *Blastocladiella emersonii*. Biochim. Biophys. Acta **541**:190–198.
54. **Gradmann, D., and C. L. Slayman.** 1975. Oscillations of an electrogenic pump in the plasma membrane of *Neurospora*. J. Membr. Biol. **22**: 181–212.
55. **Greengard, P.** 1976. Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. Nature (London) **260**:101–108.
56. **Gunasekaran, M., W. T. Hughes, and W. J. Maguire.** 1976. Cyclic 3',5' nucleotide phosphodiesterase of *Candida albicans*. Microbios Lett. **3**:47–53.
57. **Haley, B. E.** 1975. Photoaffinity labeling of adenosine 3',5'-cyclic monophosphate binding sites of human cell membranes. Biochemistry **14**: 3852–3857.
58. **Harding, R. W.** 1973. Inhibition of conidiation and photoinduced carotenoid biosynthesis by cyclic AMP. Neurospora Newsl. **20**:20–21.
59. **Harper, J. F., and G. Brooker.** 1975. Femtomole sensitive radioimmunoassay for cyclic AMP and cyclic GMP after 2'0 acetylation by acetic anhydride in aqueous solution. J. Cyclic Nucleotide Res. **1**:207–218.
60. **Hartig, A., and M. Breitenbach.** 1977. Intracellular cAMP in synchronous sporulation of *Saccharomyces cerevisiae*. FEMS Lett. **1**:79–82.
61. **Hixson, C. S., and E. G. Krebs.** 1980. Characterization of a cyclic AMP binding protein from baker's yeast: identification as a regulatory subunit of cyclic AMP-dependent protein kinase. J. Biol. Chem. **255**:2137–2145.
62. **Jaynes, P. K., J. P. McDonough, and H. R. Mahler.** 1980. Identification of cAMP binding protein associated with the plasma membrane of the yeast *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **94**:16–22.
63. **Jones, B. E., and J. D. Bulock.** 1977. Effect of N^6,O^2-dibutyryl adenosine 3',5'cyclic AMP on morphogenesis in mucorales. J. Gen. Microbiol. **103**:29–36.
64. **Jorge, J. A., and H. F. Terenzi.** 1980. An enzymatic alteration secondary to adenylate cyclase deficiency in the *cr-1 crisp* mutant of *Neurospora crassa*. NAD(P) glycohydrolase (EC-3.2.2.6) overproduction. Dev. Biol. **74**: 231–238.
65. **Jost, J. P., and H. V. Rickenberg.** 1971. Cyclic AMP. Annu. Rev. Biochem. **40**:741–773.
66. **Judewicz, N. D., G. C. Glikin, and H. N. Torres.** 1981. Protein kinase activities in *Neurospora crassa*. Arch. Biochem. Biophys. **206**: 87–97.
67. **Juliani, M. H., M. R. Brochetto, and J. C. C. Maia.** 1979. Changes in cyclic AMP binding and protein kinase activities during growth and differentiation of *Blastocladiella emersonii*. Cell Differ. **8**:421–430.
68. **Juliani, M. H., and J. C. C. Maia.** 1979. Cyclic AMP-dependent and independent kinases of *Blastocladiella emersonii*. Biochim. Biophys. Acta **567**:347–356.
69. **Kempen, H. J. M., J. J. H. H. M. de Pont, S. L. Bontins, and A. M. Stadhouder.** 1978. Cytochemical localization of adenylate cyclase: fact or artifact? J. Histochem. Cytochem. **26**: 298–312.
70. **Larsen, A. D., and P. S. Sypherd.** 1974. Cyclic adenosine 3',5'-monophosphate and morphogenesis in *Mucor racemosus*. J. Bacteriol. **117**: 432–438.
71. **Law, R. E., and A. J. Ferro.** 1977. Effect of S-adenosylmethionine and cyclic adenosine 3',5'-monophosphate on RNA synthesis during glucose derepression of *Saccharomyces cerevisiae*. FEBS Lett. **80**:153–156.
72. **Lemay, A., and L. Jarrett.** 1975. Pitfalls in the use of lead nitrate for histochemical demonstration of adenylate cyclase. J. Cell Biol. **65**: 39–50.
73. **LePage, R. W. F.** 1975. Altered cyclic AMP metabolism and pleiotropic catabolic deficiencies in mutants of the *crisp* gene complex of *Neurospora crassa*. Heredity **34**:293.
74. **Liao, H., and J. Thorner.** 1980. Yeast mating pheromone α factor inhibits adenylate cyclase. Proc. Natl. Acad. Sci. U.S.A. **77**:1898–1902.
75. **Londesborough, J.** 1977. Characterization of an adenosine 3',5'-cyclic monophosphate phosphodiesterase from baker's yeast. Biochem. J. **163**:467–476.
76. **Londesborough, J., and K. Varimo.** 1979. The temperature-dependence of adenylate cyclase from baker's yeast. Biochem. J. **181**:539–543.

77. **Londesborough, J. C.** 1975. Soluble and membrane-bound cyclic AMP diesterase activity with a low michaelis constant in baker's yeast. FEBS Lett. **50**:283–287.
78. **Londesborough, J. C., and T. Nurminen.** 1972. A manganese-dependent adenyl cyclase in baker's yeast. Acta Chem. Scand. **26**:3396–3398.
79. **Mahler, H. R., and C. C. Lin.** 1978. Exogenous adenosine 3':5'-monophosphate can release yeast from catabolite repression. Biochem. Biophys. Res. Commun. **83**:1039–1047.
80. **Maia, J. C. C., and E. P. Camargo.** 1974. Cyclic AMP phosphodiesterase during growth and differentiation in *Blastocladiella emersonii*. Cell Differ. **3**:147–155.
81. **Maia, J. C. C., S. L. Gomes, and L. Mennucci.** 1979. Induction of germination in *Blastocladiella emersonii* by cyclic AMP and inhibitors of cyclic AMP phosphodiesterase. Arch. Biol. Med. Exp. **12**:407–410.
82. **Maresca, B., G. Medoff, D. Schlessinger, G. S. Kobayashi, and J. Medoff.** 1977. Regulation of dimorphism in the pathogenic fungus, *Histoplasma capsulatum*. Nature (London) **266**:447–448.
83. **Milne, J., and R. A. Cook.** 1974. The interaction of cyclic 3',5'-adenosine monophosphate with yeast glyceraldehyde-3-phosphate dehydrogenase. I. Equilibrium dialysis studies. Biochemistry **13**:4196–4199.
84. **Mishra, N. C.** 1976. The effect of cyclic AMP on the growth of *Neurospora crassa*. Naturwissenschaften **63**:485.
85. **Montinecourt, B. S., S.-C. Kuo, and J. O. Lampen.** 1973. *Saccharomyces* mutants with invertase formation resistant to repression by hexoses. J. Bacteriol. **114**:233–238.
86. **Moreno, S., and S. Passeron.** 1980. Further studies on cyclic adenosine 3',5'-monophosphate protein kinase from dimorphic fungus, *Mucor rouxii*. Arch. Biochem. Biophys. **199**: 321–330.
87. **Moreno, S., C. Paveto, and S. Passeron.** 1977. Multiple protein kinase activities in dimorphic fungus *Mucor rouxii*. Comparison with cyclic adenosine 3',5'-monophosphate binding protein. Arch. Biochem. Biophys. **180**:225–231.
88. **Nabeshima, S., M. Mishina, A. Tanaka, and S. Fukui.** 1977. Effect of various components on isocitrate lyase formation in *Candida tropicalis*. Agric. Biol. Chem. **41**:503–508.
89. **Niimi, M., K. Niimi, J. Tokunaga, and H. Nakayama.** 1980. Changes in cyclic nucleotide levels and dimorphic transition in *Candida albicans*. J. Bacteriol. **142**:1010–1014.
90. **Orlowski, M.** 1979. Changing pattern of cyclic AMP-binding proteins during germination of *Mucor racemosus* sporangiospores. Biochem. J. **182**:547–554.
91. **Orlowski, M.** 1980. Cyclic adenosine 3',5'-monophosphate and germination of sporangiospores from the fungus *Mucor*. Arch. Microbiol. **126**: 133–140.
92. **Pall, M. L.** 1977. Cyclic AMP and the plasma membrane potential in *Neurospora crassa*. J. Biol. Chem. **252**:7146–7150.
93. **Pall, M. L., J. M. Trevillyan, and N. Hinman.** 1981. Deficient cyclic AMP control in mutants of two genes of *Neurospora crassa*. Mol. Cell. Biol. **1**:1–8.
94. **Palmer, W. A., J. M. McPherson, and D. A. Walsh.** 1980. Critical control in the evaluation of cAMP-dependent protein kinase activity ratios as indices of hormonal action. J. Biol. Chem. **255**:2663–2666.
95. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 3',5'-monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**:527–551.
96. **Paveto, C., A. Epstein, and S. Passeron.** 1975. Studies of cyclic 3',5'-monophosphate levels, adenylate cyclase and phosphodiesterase activities in the dimorphic fungus, *Mucor rouxii*. Arch. Biochem. Biophys. **169**:449–457.
97. **Paveto, C., and S. Passeron.** 1977. Some kinetic properties of *Mucor rouxii* phosphofructokinase (EC-2.7.1.11). Effect of cyclic AMP. Arch. Biochem. Biophys. **178**:1–7.
98. **Paznokas, J. L., and P. S. Sypherd.** 1975. Respiratory capacity, cyclic adenosine 3',5'-monophosphate, and morphogenesis of *Mucor racemosus*. J. Bacteriol. **124**:134–139.
99. **Peters, J., and P. S. Sypherd.** 1979. Morphology-associated expression of nicotinamide adenine dinucleotide-dependent glutamate dehydrogenase in *Mucor racemosus*. J. Bacteriol. **137**:1134–1139.
100. **Polya, G. M., A. G. Brownlee, and M. J. Hynes.** 1975. Enzymology and genetic regulation of a cyclic nucleotide-binding phosphodiesterase-phosphomonoesterase from *Aspergillus nidulans*. J. Bacteriol. **124**:693–703.
101. **Powers, P. A., and M. L. Pall.** 1980. Cyclic AMP-dependent protein kinase of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **95**: 701–706.
102. **Ramos, E. H., L. C. de Bongioanni, M. C. Cuesta Casado, and A. O. M. Stoppani.** 1977. Some properties of L-(^{14}C)-leucine transport in *Saccharomyces ellipsoides*. Biochim. Biophys. Acta **467**:220–237.
103. **Rickenberg, H. V.** 1974. Cyclic AMP in prokaryotes. Annu. Rev. Microbiol. **28**:353–369.
104. **Robison, G. A., R. W. Butcher, and E. W. Sutherland.** 1971. Cyclic AMP. Academic Press, Inc., New York.
105. **Rock, M. G., and R. A. Cook.** 1974. The effect of cyclic 3',5'-adenosine monophosphate on yeast glyceraldehyde-3-phosphate dehydrogenase. II. Initial velocity kinetic studies. Biochemistry **13**:4200–4204.
106. **Rosenberg, G., and M. L. Pall.** 1978. Cyclic AMP and cyclic GMP in germinating conidia of *Neurospora crassa*. Arch. Microbiol. **118**: 87–90.
107. **Rosenberg, G., and M. L. Pall.** 1979. Properties of two cyclic nucleotide-deficient mutants of *Neurospora crassa*. J. Bacteriol. **137**:1140–1144.
108. **Ross, E. M., and A. G. Gilman.** 1980. Biochem-

ical properties of hormone-sensitive adenylate cyclase. Annu. Rev. Biochem. **49**:533–564.

109. **Schamhart, D. H. J., A. M. H. ten Berge, and K. W. van den Poll.** 1975. Isolation of a catabolite repression mutant of yeast as a revertant of a strain that is maltose negative in the respiratory-deficient state. J. Bacteriol. **121**: 747–752.
110. **Schlanderer, G., and H. Dellweg.** 1974. Cyclic AMP and catabolite repression in yeasts. In *Schizosaccharomyces pombe* glucose lowers both intracellular adenosine 3':5' monophosphate levels and the activity of catabolite-sensitive enzymes. Eur. J. Biochem. **49**:305–319.
111. **Schwalb, M. N.** 1974. Effect of adenosine 3',5'-cyclic monophosphate on morphogenesis in fruit bodies of *Schizophyllum commune*. Arch. Mikrobiol. **96**:17–20.
112. **Schwalb, M. N.** 1978. A developmental mutant affecting 3',5'-cyclic AMP metabolism in the basidiomycete, *Schizophyllum commune*. FEMS Lett. **3**:107–110.
113. **Schwalb, M. N.** 1978. Regulation of fruiting, p. 135–165. *In* M. N. Schwalb and P. G. Miles (ed.), Genetics and morphogenesis in the basidiomycetes. Academic Press, Inc., New York.
114. **Scott, W. A.** 1976. Adenosine 3',5'-cyclic monophosphate deficiency in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **73**:2995–2999.
115. **Scott, W. A., and B. Solomon.** 1973. Cyclic 3',5' AMP phosphodiesterase of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **53**: 1024–1030.
116. **Scott, W. A., and B. Solomon.** 1975. Adenosine 3',5'-cyclic monophosphate and morphology in *Neurospora crassa* drug-induced alterations. J. Bacteriol. **122**:454–463.
117. **Silverman, P. M.** 1978. Cyclic AMP binding proteins and cyclic AMP-dependent protein kinase from *Blastocladiella emersonii*. J. Bacteriol. **135**:976–980.
118. **Silverman, P. M., and P. M. Epstein.** 1975. Cyclic nucleotide metabolism coupled to cytodifferentiation of *Blastocladiella emersonii*. Proc. Natl. Acad. Sci. U.S.A. **72**:442–446.
119. **Sippel, C. J., R. R. Goewert, and F. N. Slachman.** 1979. The regulation of ubiquinone 6 biosynthesis by *Saccharomyces cerevisiae*. Fed. Proc. **38**:508.
120. **Soderling, T. R., and C. R. Park.** 1974. Recent advances in glycogen metabolism. Adv. Cyclic Nucleotide Res. **4**:283–333.
121. **Somasundaram, T., K. Chandrasekaran, J. Jayaraman, and C. Rajamanickam.** 1980. Role of cyclic AMP in mitochondriogenesis in yeast. Biochem. Biophys. Res. Commun. **92**: 655–661.
122. **Speziali, G. A. G., and R. Van Wijk.** 1971. Cyclic 3',5'-AMP phosphodiesterase of *Saccharomyces carlsbergensis*. Inhibition by adenosine 5'-triphosphate, inorganic pyrophosphate and inorganic polyphosphate. Biochim. Biophys. Acta **235**:466–472.
123. **Steiner, A. L., D. M. Kipnis, R. Utiger, and C. Parker.** 1969. Radioimmunoassay for the measurement of adenosine 3',5'-cyclic phosphate. Proc. Natl. Acad. Sci. U.S.A. **64**:367–373.
124. **Sy, J., and D. Richter.** 1972. Separation of a cyclic 3',5'-adenosine monophosphate binding protein from yeast. Biochemistry **11**:2784–2787.
125. **Sy, J., and D. Richter.** 1972. Content of cyclic 3':5'-adenosine monophosphate and adenylyl cyclase in yeast at various growth conditions. Biochemistry **11**:2788–2791.
126. **Sypherd, P. S., P. T. Borgia, and J. L. Paznokas.** 1978. Biochemistry of dimorphism in the fungus *Mucor*. Adv. Microb. Physiol. **18**: 68–104.
127. **Takai, Y., K. Sakai, Y. Morishita, H. Yamamura, and Y. Nishizuka.** 1974. Functional similarities of yeast and mammalian adenosine 3',5'-monophosphate dependent protein kinase. Biochem. Biophys. Res. Commun. **59**: 646–652.
128. **Takai, Y., H. Yamamura, and Y. Nishizuka.** 1974. Adenosine 3':5'-monophosphate-dependent protein kinase from yeast. J. Biol. Chem. **249**:530–535.
129. **Tellez-Iñon, M. T., and H. N. Torres.** 1970. Interconvertible forms of glycogen phosphorylase in *Neurospora crassa*. Proc. Natl. Acad. Sci. U.S.A. **66**:459–463.
130. **Tellez-Iñon, M. T., and H. N. Torres.** 1973. Regulation of glycogen phosphorylase *a* phosphatase in *Neurospora crassa*. Biochim. Biophys. Acta **297**:399–412.
131. **Terenzi, H. F., M. M. Flawia, M. T. Tellez-Iñon, and H. N. Torres.** 1976. The control of *Neurospora crassa* morphology by cyclic adenosine 3',5'-monophosphate and dibutyryl cyclic adenosine 3',5'-monophosphate. J. Bacteriol. **126**:91–99.
132. **Terenzi, H. F., M. M. Flawia, and H. N. Torres.** 1974. A *Neurospora crassa* morphological mutant showing reduced adenylate cyclase activity. Biochem. Biophys. Res. Commun. **58**:990–996.
133. **Terenzi, H. F., J. A. Jorge, J. E. Roselino, and R. H. Migliorini.** 1979. Adenylyl cyclase deficient *cr*-1 (Crisp) mutant of *Neurospora crassa*: cyclic AMP-dependent nutritional deficiencies. Arch. Microbiol. **123**:251–258.
134. **Tomkins, G. M.** 1975. The metabolic code. Science **189**:760–763.
135. **Trevillyan, J. M., and M. L. Pall.** 1979. Control of cyclic adenosine 3',5'-monophosphate levels by depolarizing agents in fungi. J. Bacteriol. **138**:397–403.
136. **Tsuboi, M., S. Kamisaka, and N. Yanagashima.** 1972. Effect of cyclic 3',5'-adenosine monophosphate on sporulation of *Saccharomyces cerevisiae*. Plant Cell Physiol. **13**:585–588.
137. **Tsuboi, M., and N. Yanagashima.** 1973. Effect of cyclic AMP, theophylline and caffeine on the glucose repression of sporulation in *Saccharomyces cerevisiae*. Arch. Microbiol. **93**:1–12.
138. **Tsuboi, M., and N. Yanagashima.** 1975. Com-

parative studies on sporulation-promotion action of cyclic AMP, theophylline and caffeine in *Saccharomyces cerevisiae*. Arch. Microbiol. **105**:83–86.

139. **Tu, J. C., and S. K. Malhotra.** 1973. Histochemical localization of adenyl cyclase in the fungus *Phycomyces blakesleeanus*. J. Histochem. Cytochem. **21**:1041–1046.
140. **Tu, J. C., and S. K. Malhotra.** 1977. The significance of cAMP induced alterations in the cellular structure of *Phycomyces*. Can. J. Microbiol. **23**:378–388.
141. **Tu, J. C., S. K. Malhotra, B. L. Gupta, and T. A. Hall.** 1979. Cyclic AMP induced distribution of calcium in *Phycomyces*. Microbios. Lett. **8**:103–112.
142. **Turian, G.** 1973. Induction of conidium formation in *Neurospora* by lifting of catabolite repression. J. Gen. Microbiol. **79**:347–350.
143. **Turian, G., and D. E. Bianchi.** 1972. Conidiation in *Neurospora*. Bot. Rev. **38**:119–157.
144. **Turian, G., and E. Khandjian.** 1973. Action biphasique de l'AMP-cyclique sur la conidiogenese de *Neurospora* et la carotenogenese en general. Ber. Schweiz. Bot. Ges. **83**:345–350.
145. **Uno, I., and T. Ishikawa.** 1971. Chemical and genetical control of induction of monokaryotic fruiting bodies in *Coprinus macrorhizus*. Mol. Gen. Genet. **113**:228–239.
146. **Uno, I., and T. Ishikawa.** 1973. Purification and identification of the fruiting-inducing substances in *Coprinus macrorhizus*. J. Bacteriol. **113**:1240–1248.
147. **Uno, I., and T. Ishikawa.** 1973. Metabolism of adenosine 3',5'-cyclic monophosphate and induction of fruiting bodies in *Coprinus macrorhizus*. J. Bacteriol. **113**:1249–1255.
148. **Uno, I., and T. Ishikawa.** 1974. Presence of multiple protein kinase activities in *Coprinus macrorhizus*. Biochim. Biophys. Acta **334**: 354–360.
149. **Uno, I., and T. Ishikawa.** 1975. Subcellular localization of adenylate cyclase (EC-4.6.1.1) during fruiting body formation in *Coprinus macrorhizus*. J. Gen. Appl. Microbiol. **21**:335–343.
150. **Uno, I., and T. Ishikawa.** 1976. Effect of cyclic AMP on glycogen phosphorylation in *Coprinus macrorhizus*. Biochim. Biophys. Acta **452**: 112–120.
151. **Uno, I., and T. Ishikawa.** 1978. Effect of cyclic AMP on glycogen synthetase (EC-2.4.1.11) in *Coprinus macrorhizus*. J. Gen. Appl. Microbiol. **24**:193–197.
152. **Uno, I., and T. Ishikawa.** 1981. Control of adenosine 3',5'-monophosphate level and protein phosphorylation by depolarizing agents in *Coprinus macrorhizus*. Biochim. Biophys. Acta **672**:108–113.
153. **Uno, I., M. Yamaguchi, and T. Ishikawa.** 1974. The effect of light on fruiting body formation and cyclic AMP metabolism in *Coprinus macrorhizus*. Proc. Natl. Acad. Sci. U.S.A. **71**:479–483.
154. **Vale, M. R., S. L. Gomes, and J. C. C. Maia.** 1975. Independent cAMP and cGMP phosphodiesterases in *Blastocladiella emersonii*. FEBS Lett. **56**:332–336.
155. **Vale, V. L., S. L. Gomes, and J. C. C. Maia.** 1976. Transient cyclic AMP accumulation in germinating zoospores in *Blastocladiella emersonii*. FEBS Lett. **67**:189–192.
156. **van der Platt, J. V., and P. van Solingen.** 1974. Cyclic 3',5' adenosine monophosphate stimulates trehalose degradation in baker's yeast. Biochem. Biophys. Res. Commun. **56**: 580–587.
157. **van Solingen, P., and J. B. van der Platt.** 1975. Partial purification of the protein system controlling the breakdown of trehalose in baker's yeast. Biochem. Biophys. Res. Commun. **62**:553–560.
158. **van Wijk, R., and T. M. Konijn.** 1971. Cyclic 3',5'-AMP in *Saccharomyces carlsbergensis* under various conditions of catabolite repression. FEBS Lett. **13**:184–186.
159. **Varimo, K., and J. Londesborough.** 1976. Solubilization and other studies on adenylate cyclase of baker's yeast. Biochem. J. **159**:363–370.
160. **Varimo, K., and J. Londesborough.** 1979. Evidence for essential arginine in yeast adenylate cyclase. FEBS Lett. **106**:153–156.
161. **Viswanath-Reddy, M., and R. J. Cohen.** 1979. Strain C2 and cyclic AMP. Phycomyces **3**:23–24.
162. **Watson, D. C., and D. R. Berry.** 1977. Fluctuation in the levels of intracellular and extracellular cAMP during sporulation in *Saccharomyces cerevisiae*. FEMS Lett. **1**:83–86.
163. **Watson, D. C., and D. R. Berry.** 1977. Fluctuations in cAMP levels during the cell cycle of *Saccharomyces cerevisiae*. FEMS Lett. **1**:175–178.
164. **Weber, W., and H. Hilz.** 1978. Adenosine-3',5'-monophosphate-binding protein from bovine kidney. Isolation by affinity chromatography and limited proteolysis of the regulatory subunits of protein kinase II. Eur. J. Biochem. **83**: 215–225.
165. **Wells, J. N., and J. G. Hardman.** 1977. Cyclic nucleotide phosphodiesterases. Adv. Cyclic Nucleotide Res. **8**:119–143.
166. **Wheeler, G. E., A. Schibeci, R. M. Epand, J. B. M. Rattray, and D. B. Kidby.** 1974. Subcellular localization and some properties of adenylate cyclase activity in the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta. **372**:15–22.
167. **Wiseman, A., and T.-K. Lim.** 1975. Removal of glucose repression of α-glucosidase synthesis in yeast protoplasts by adenosine 3',5'-cyclic monophosphate addition and its prevention by chloramphenicol. Biochem. Soc. Trans. **2**:932.
168. **Wiseman, A., and T.-K. Lim.** 1977. Adenosine 3',5'-monophosphate in lifting of glucose repression of α-glucosidase by fluoride in yeast. Biochem. Soc. Trans. **5**:970.
169. **Wiseman, A., T.-K. Lim, and L. J. F. Woods.**

1978. Partial repression of cytochrome P-450 by cyclic AMP in protoplasts of *Saccharomyces cerevisiae*. Biochem. Soc. Trans. **6**:397–400.

170. **Wiseman, A., T.-K. Lim, and L. F. J. Woods.** 1978. Regulation of the biosynthesis of cytochrome P-450 in brewer's yeast. Role of cyclic AMP. Biochim. Biophys. Acta **544**:615–623.
171. **Wold, W. S. M., and I. Suzuki.** 1973. Cyclic AMP and citric acid accumulation by *Aspergillus niger*. Biochem. Biophys. Res. Commun. **50**:237–244.
172. **Wold, W. S. M., and I. Suzuki.** 1973. Promotion of conidia aggregation in *Aspergillus niger* by cyclic AMP and 5'GMP. Biochem. Biophys. Res. Commun. **55**:824–830.
173. **Wold, W. S. M., and I. Suzuki.** 1974. Demonstration in *Aspergillus niger* of adenyl cyclase, a cyclic adenosine 3',5'-monophosphate binding protein and studies on intracellular and extracellular phosphodiesterases. Can. J. Microbiol. **20**:1567–1576.
174. **Yang, S. T., and W. C. Deal, Jr.** 1969. Metabolic control and structure of glycolytic enzymes. VI. Competitive inhibition of yeast glyceraldehyde 3-phosphate dehydrogenase by cyclic adenosine monophosphate, adenosine triphosphate and other adenine-containing compounds. Biochemistry **8**:2806–2813.
175. **Yasuhara, S., S. Kawamoto, A. Tanaka, M. Osumi, and S. Fukui.** 1976. Induction of catalase activity in a methanol utilizing yeast *Kloeckera* SP 2201. Agric. Biol. Chem. **40**: 1771–1780.
176. **Zonneveld, B. J. M.** 1976. The effects of glucose and manganese on adenosine 3',5'-monophosphate levels during growth and differentiation of *Aspergillus nidulans*. Arch. Microbiol. **108**: 41–44.
177. **Zonneveld, B. J. M.** 1980. Protease levels in relation to cAMP and a reserve polymer during growth and sexual differentiation of *Aspergillus nidulans*. Exp. Mycol. **4**:140–146.