Cyclic AMP as a Second Messenger in Higher Plants

Status and Future Prospects

Sarah M. Assmann*

Department of Biology, Pennsylvania State University, 208 Mueller Lab, University Park, Pennsylvania 16802

Adenosine 3':5'-monophosphate, commonly known as cAMP, is a key second messenger in living organisms ranging from Dictyostelium to Homo sapiens. cAMP was first described as a regulator of glycogen breakdown in the liver and is now known to be a second messenger for a wide variety of cellular responses in animals. cAMP is produced from ATP by the action of adenylate cyclase and is hydrolyzed to AMP by the enzyme cAMP phosphodiesterase.

The question of whether cAMP is an authentic second messenger in higher plants has engaged plant scientists for decades. This article will highlight aspects of the controversies surrounding cAMP and suggest steps toward their possible resolution. Literature citations are largely restricted to the last 5 years, since earlier work has been summarized in several reviews (Lin, 1974; Amrhein, 1977; Brown and Newton, 1981; Francko, 1983; Verhey and Lomax, 1993).

CRITERIA FOR cAMP AS A REGULATOR OF PHYSIOLOGICAL PROCESSES

The criteria that cAMP should satisfy as a signaling metabolite in plants seem straightforward. The most inescapable criterion is that cAMP should be present in plants. Furthermore, the in vivo concentration of cAMP should be sufficient to elicit the biological response of interest. For example, if a response requires micromolar concentrations of exogenous cAMP for elicitation, then we might expect that if this response is not artifactual, we should find endogenous cAMP present at micromolar concentrations. cAMP concentration should change in response to the stimulus that elicits the biological response, unless mechanisms exist whereby sensitivity to a given cAMP concentration can be altered, as has been suggested for plant hormones (Trewavas, 1981, 1991). Enzymes of cAMP synthesis and cAMP catabolism should be present in plants, and target molecules of cAMP regulation should also be present. Although these criteria are clear enough, whether they have been satisfied in plants is still open to debate, as is described below.

IS cAMP PRESENT IN HIGHER PLANTS?

A major controversy in the field concerns the actual concentration of cAMP in higher plant tissues. Earlier estimates ranged from 1 to 1500 pmol/g fresh weight (summarized by Brown and Newton, 1981; Francko, 1983), or approximately 1 nM to 1.5 μM. Because technologies for measuring biological metabolites improved and expanded, many of these early estimates were subsequently questioned on procedural grounds. A particular problem was that some of the early assay techniques could not distinguish 2':3'-cAMP from the relevant isomer, 3':5'-cAMP (Brown and Newton, 1981). In 1989, Spiteri et al. published a landmark study using RIA techniques and found that after eliminating artifactual sources of cAMP and possible interfering substances, the cAMP levels in all of the plant tissues they tested were below the detection limit of 0.5 pmol/g fresh weight of tissue. This result might appear to eliminate the possibility of cAMP as a second messenger in higher plants, particularly given that typical levels in animals are 100 to 500 pmol/g fresh weight (Robison et al., 1971). On the other hand, the following points must be considered.

1. A detection limit of 0.5 pmol/g fresh weight corresponds roughly to a concentration of 500 pM. Biological signaling agents with efficacy in the picomolar range are well established; to give just one example, the nodulation factor that initiates nodulation responses in alfalfa roots is effective at picomolar concentrations (Ehrhardt et al., 1992).

2. Since the report of Spiteri et al. (1989), others have also employed the same assay techniques as that group and have in some cases detected significantly higher cAMP concentrations. Concentrations of 36 pmol cAMP/g fresh weight in Torenia stem segments (Ishioka and Tanimoto, 1990) and 5 pmol cAMP/g fresh weight in suspension-cultured cells of Phaseolus vulgaris (Bolwell, 1992) have been described. Gangwani et al. (1991) measured cAMP levels of 70 to 80 pmol/g fresh weight in axenic cultures of Lemma, as assayed by both HPLC and RIA. These data

---

1 Research in the author's laboratory on plant second messengers is supported by grants from the National Science Foundation (NSF), the National Aeronautics and Space Administration/NSF, and the U.S. Department of Agriculture.

* E-mail sma3@psuvm.psu.edu; fax 1-814-865-9131.

---

Abbreviations: Ca_{i}, cytosolic Ca^{2+} concentration; PI, phosphati-
dylinositol; PKA, protein kinase A; RIA, radioimmunoassay.
suggest that cAMP concentrations may vary significantly according to species and system. It would be helpful to now reexamine plant systems exhibiting biological responses to exogenous cAMP (see below) with regard to their in vivo cAMP concentrations.

3. cAMP concentrations may vary significantly depending on the environmental conditions under which plants are grown. For example, several independent studies indicate that illumination significantly increases basal cAMP concentrations (see below; Brown and Newton, 1981; Brown et al., 1989), an effect that has been attributed in part to an increase in the $K_m$ of cAMP phosphodiesterase (Brown et al., 1989).

4. cAMP concentrations may be high only in localized areas of an organ or in particular cell types; thus, the effective cAMP concentration may be significantly higher than the “averaged” cAMP concentration (Trewavas and Gilroy, 1991). In the case of the secondary messenger Ca$^{2+}$, it is now evident that it is crucial to accurately quantitate cellular and even subcellular spatial gradients of this second messenger, and perhaps the same will prove true for cAMP in plants.

5. There may also be temporal variations in the cAMP response that make it important to “capture” the cAMP signal along an appropriate time course. In cultured P. vulgaris cells, for example, cAMP concentrations as measured by RIA increased from 5 to 18 pmol/g fresh weight upon elicitor treatment, but this increase was transient, returning to almost baseline within 1 h (Bolwell, 1992). The relative increase in cAMP concentration observed in this plant system is similar to that observed in some animal systems; e.g. glucagon typically induces a 3-fold increase in cAMP concentration under physiological conditions, although increases as large as 80-fold can be induced experimentally (Robison et al., 1971).

On one point there seems to be little argument, and that is that cAMP concentrations in plants are generally lower than in animals. Some plant systems may in fact have no cAMP; in other systems it may prove crucial to determine cAMP concentrations with fine enough spatial and temporal resolution that there is confidence in the biological significance of the results.

**DO THE LOW ENDOGENOUS CONCENTRATIONS OF PLANT cAMP ELICIT BIOLOGICAL RESPONSES?**

Given the low concentrations of cAMP in plants relative to animals, a second controversy concerns the significance, if any, of the wide variety of effects on plant systems of exogenously applied cAMP and cAMP analogs. Many of the effects reported have required millimolar concentrations of cAMP. These effects seem almost certain to be nonspecific, or to reflect the mimicking by cAMP of another, true regulator, a prime candidate for which would be the adenine-based cytokinins (Elliot and Murray, 1975; Giudici de Nicola et al., 1975). On the other hand, there are a few reports of nanomolar concentrations of exogenous cAMP stimulating responses. Ten nanomolar cAMP was sufficient to promote the elongation of self-incompatible pollen tubes in lily (Tezuka et al., 1993). Membrane-permeant, dibutyl cAMP at 100 nm and above stimulated adventitious budding in *Torenia* stem segments (Ishioka and Tanimoto, 1990). These concentrations are low enough that the resultant responses could in fact be regulated by endogenous cAMP in vivo, especially given points 1 to 5 cited above.

Responses to micromolar concentrations of cAMP have also been reported in a variety of systems over the past few years. These responses include stimulation of stomatal opening in *Vicia faba* (Curvetto et al., 1994), activation of Ca$^{2+}$ uptake in cultured carrot cells (Kurosaki and Nishi, 1993; Kurosaki et al., 1994), swelling of etiolated wheat protoplasts (Bossen et al., 1990), and stimulation of protein kinase activity in rice leaves (Komatsu and Hirano, 1993). Micromolar cAMP is still likely to be beyond the range of plausible in vivo concentrations. However, in some cases of exogenous cAMP application, the cAMP concentration that actually reaches the target cells may be lower than the applied concentration. For example, 50 nm to 5 $\mu$m exogenous cAMP was sufficient to increase outward K$^+$ currents in *V. faba* mesophyll protoplasts, but only if isobutylmethylxanthine, an inhibitor of cAMP phosphodiesterase, was also provided (Li et al., 1994). These results suggest that exogenously supplied cAMP may be rapidly metabolized by plant tissue, providing a possible explanation for why effective exogenous concentrations may be higher than apparent endogenous concentrations. In addition, cAMP has low membrane permeability, so experiments involving external application of cAMP rather than a membrane-permeant form of the molecule may require higher dosages. Because of the membrane permeability issue, experimenters applying cAMP externally should make every effort to quantify the concentration of cAMP actually reaching the target symplast.

**ARE ENZYMES OF cAMP SYNTHESIS AND CATABOLISM AND cAMP TARGET PROTEINS PRESENT IN PLANTS?**

If the pathways of cAMP synthesis and degradation are similar in plants and animals, then one would expect to find adenylate cyclase and cAMP phosphodiesterase in plants. Within the last few years, adenylate cyclase activity has been reported from root extracts of *Medicago, Ricinus,* and *Pisum* (Carricarte et al., 1988; Lusini et al., 1991; Pacini et al., 1993). However, cloning of a plant gene encoding adenylate cyclase has yet to be accomplished, to my knowledge. Until plant adenylate cyclase is either cloned or the protein is purified sufficiently to allow microsequencing and complete enzymological characterization, the relevance of the adenylate cyclase activity reported in plants will be open to debate. Similarly, although phosphodiesterase activity that specifically hydrolyzes 3':5'-cAMP and is inhibited by methylxanthines has been reported (Brown et al., 1977; Brown and Newton, 1981; Francko, 1983), the enzyme has yet to be scrutinized with the detail now afforded by modern biochemical and molecular techniques.
Although cloning of the genes encoding enzymes of cAMP metabolism might seem to offer a clear answer to the question of whether cAMP is present in plants, the plot may thicken depending on the degree of sequence homology found (if, in fact, relevant sequences are found at all). This has proven to be the case in investigations of plant cAMP-dependent protein kinases. Kinases would be expected to be a major target for cAMP action based on analogy to animal systems that possess a cAMP-dependent protein kinase called PKA. Several groups have reported the cloning of genes with homology to PKA (Lawton et al., 1989; Biermann et al., 1990; Hayashida et al., 1993). However, DNA sequence homology is not sufficient to define protein function, and it has yet to be ascertained whether any of the gene products actually functions in vivo as a cAMP-regulated kinase. From a biochemical perspective, the picture is similarly murky. There are reports that addition of cAMP to plant extracts stimulates phosphorylation of specific proteins (e.g. Komatsu and Hirano, 1993). However, PKA has yet to be purified from plants. Polya and colleagues (1991) have reported the partial purification of a protein kinase from petunia, which phosphorylates Kemptide (LRRASLG), a synthetic substrate for PKA. It is interesting that the plant kinase is not stimulated by cAMP alone, but inhibition of the kinase by the regulatory (inhibitory) subunit of animal PKA is relieved by cAMP. Therefore, one might speculate that the catalytic subunit of this plant kinase is similar to PKA but that its regulation by cAMP in vivo is not mediated by a subunit of the same kinase, but rather by a separate plant enzyme. An alternative interpretation would be that cAMP is simply not a relevant regulator in vivo.

Addition of animal PKA to plant extracts can result in protein phosphorylation (Salimath and Marme, 1983; see also Li et al., 1994), including phosphorylation of Pr and Pfr (Wong et al., 1986), PEP carboxylase (Terada et al., 1990), and Suc phosphate synthase (Huber and Huber, 1991). Is animal PKA mimicking an effect of true plant PKA, or is the animal PKA merely able to phosphorylate a substrate that, in vivo, is phosphorylated by a different type of kinase? For example, the conductance of the seed-specific vacuolar water channel, α-tonoplast intrinsic protein, expressed in oocytes is apparently doubled when oocyte PKA is activated (Maurel et al., 1994). The α-tonoplast intrinsic protein channel is known to be phosphorylated in vivo; however, protein kinase activity found associated with the tonoplast to date is of the calcium-dependent protein kinase variety (Johnson and Chrispeels, 1992). Is there also a PKA-type kinase associated with the tonoplast, or is the Xenopus PKA simply mimicking an effect produced in vivo by a calcium-dependent protein kinase?

In addition to PKA, nucleotide-regulated channels are a major target of cAMP action in animals. Some of these channels are modulated by cAMP through PKA (e.g. Hosey et al., 1986), but others may be directly gated by cAMP (e.g. Delgado et al., 1991). In plants, the deduced amino acid sequence of an inward K⁺ channel appears to have a nucleotide-binding domain (Sentenac et al., 1992), and cAMP regulation of an outward K⁺ channel has been described (Li et al., 1994). Whether endogenous cAMP actually interacts with the channel peptides remains to be seen.

In a similar vein, transcription factors have been identified in plants (Katagiri et al., 1989; Ehrlich et al., 1992; Gubler and Jacobsen, 1992; Rushton et al., 1992) with significant homology to cAMP responsive element binding proteins, a class of animal transcription factors thought to be regulated by phosphorylation, including phosphorylation mediated by PKA (Montminy et al., 1990). However, whether these plant transcription factors are regulated by cAMP in vivo remains an open question.

**FUTURE PROSPECTS**

Given the current scenario of cAMP function in plants, it seems that the field might best be served by focusing the talents of several laboratories, with expertise in different areas (cAMP quantitation, adenylate cyclase and cAMP phosphodiesterase enzymology and molecular biology, cellular signaling), on one or two biological systems that show the most promise for a true in vivo role of cAMP. Ideally, these model systems should have shown responses to nanomolar or at least low micromolar concentrations of exogenous cAMP so that it is plausible that the response is not artifactual. The response should be one that can be assayed in vivo, not, for example, a phosphorylation effect occurring in a protein extract, where one can question the in vivo relevance of the results obtained. The system should be homogenous, so that uniform concentrations of cAMP can be uniformly and rapidly applied. The system should be one in which cytokinins can be similarly applied, to determine whether cAMP is merely a poor substitute for a cytokinin. The system should show a fast response to cAMP, so that problems associated with cAMP metabolism can be avoided and causal relationships can be more readily elucidated. The system should allow quantitation of endogenous cAMP levels by several different methods; in addition, it should be possible to sample the system for endogenous cAMP concentrations with a rapid enough time course that one can determine whether an elevation in endogenous cAMP temporally precedes the biological response. Finally, the system should be amenable to molecular and genetic analysis.

Of the systems described in the recent literature, it seems that two might qualify for further scrutiny given the criteria described above. The first is the pollen tube, where the stimulation of the growth of incompatible pollen tubes by as little as 10 nM exogenous cAMP has been reported (Tezuka et al., 1993). Molecular techniques are well developed for several plant species in which self-incompatibility is observed. In addition, single pollen tubes can be assayed in vivo for levels of second messengers such as Ca²⁺ (e.g. Pierson et al., 1994) that, by analogy with animal systems, are likely to be rapidly altered by cAMP.

The second system that holds promise for a definitive test of the role of cAMP in plants is the etiolated grass protoplast, where protoplast swelling may be cAMP regulated. In oat, red light or membrane-permeant dibutyl cAMP cause swelling of etiolated protoplasts (Kim et al., 1986; Chung et al., 1988). GA also stimulates swelling, and
it has been suggested that GA3 stimulates cAMP synthesis in grasses (Sim and Kim, 1987). Red light and GA similarly stimulate swelling of mesophyll protoplasts of etiolated wheat, and phytochrome was implicated in this response more than a decade ago (Blakely et al., 1983). More recently, Bossen et al. (1990) showed that dibutyl cAMP (30 μM) causes swelling of etiolated wheat leaf protoplasts comparable to that produced by red light. Additional data suggest that elevation of Ca2+ is involved in the swelling response (Bossen et al., 1988, 1990), as has been suggested for numerous phytochrome responses. Indeed, Shacklock and Trewavas (reported by Gilroy and Trewavas, 1994) showed preliminary confocal Ca2+-imaging data in which Ca2+ for numerous phytochrome responses. Indeed, Shacklock and Trewavas (1990) showed that dibutyl cAMP (30 μM) causes swelling of etiolated-wheat leaf protoplasts upon release of caged cAMP into the cytosol. After cAMP release, 13 out of 17 protoplasts also showed at least transient increases in volume; when both cAMP and EGTA were released in the cells, volume increases were not observed. Fallon et al. (1993) demonstrated that red light rapidly induced Ca2+-dependent phosphorylation of a 70-kD polypeptide in these cells, suggesting that a Ca2+-regulated protein kinase may operate downstream of Ca2++. Bossen et al. (1990) performed a detailed study of possible second messengers that might be involved in the swelling response. They found that agonists of the PI pathway partially mimicked red light in inducing swelling. Inhibitors of the PI pathway inhibited swelling, but again only partially. The conclusion may be that red light activates two pathways in these cells, one, the PI pathway, which releases Ca2+ from intracellular stores, and a second, the cAMP-dependent pathway, which may elevate Ca2+ by another mechanism, such as the opening of Ca2+ channels. Indeed, there is some information that suggests that elevated cAMP may open Ca2+ channels in plant cells (Kurosaki and Nishi, 1993; Kurosaki et al., 1994).

The protoplast system seems to be a good candidate for investigation of cAMP’s role in plants. The initial response (Ca2+ elevation) is observed within 1 min, and the physiological response of cell swelling can be measured within 30 min. The system is homogeneous and is amenable to both single-cell in vivo assays and large-scale in vitro assays. Particularly if the response can be replicated in a grass in its sequence specificity and DNA methylation sensitivity. Plant Cell Physiol 29: 855–860


Giudici de Nicola M, Amico V, Piattelli M (1975) Effects of light and kinetin on amaranthin synthesis induced by cAMP. Phytochemistry 14: 989–991

Received February 15, 1995; accepted March 15, 1995.

Copyright Clearance Center: 0032-0889/95/108/0885/05.

LITERATURE CITED


Giudici de Nicola M, Amico V, Piattelli M (1975) Effects of light and kinetin on amaranthin synthesis induced by cAMP. Phytochemistry 14: 989–991

ACKNOWLEDGMENT

I thank Dr. Simon Gilroy for helpful comments on the manuscript.


