Is there a general paradigm of cyclic AMP action in eukaryotes?

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Summary

The cyclic AMP control system in eukaryotes has been highly conserved evolutionarily in four of its central properties. Such conservation suggests conservation of the regulatory function of cyclic AMP. Conservation is seen in the properties of adenylate cyclase, cyclic AMP-dependent protein kinase and, among diverse lower eukaryotes, the control of endogenous cyclic AMP levels. A conserved regulatory response to cyclic AMP is the stimulation of glycolysis and inhibition of gluconeogenesis. The control of glycolysis and gluconeogenesis is proposed to be evidence of general pattern of cyclic AMP action in many lower and higher eukaryotic cells.

Introduction

Cyclic AMP has been reported to influence an amazing variety of cellular properties in animal cells and in microorganisms. Any general pattern of action that can be discerned should be of great value in understanding the literature on this central regulatory molecule. The only proposed general paradigm of cyclic AMP action which has received wide attention is based on the role of this molecule as a mediator of carbon catabolite repression in bacteria, such as Escherichia coli. In E. coli, the availability of carbon components regulates cyclic AMP levels which, in turn, regulate a variety of functions which are said to be catabolite repres-sible. This has led to the suggestion that cyclic AMP may have a general role as a carbon starvation signal (1, 2).

The thrust of this paper is that: 1) the E. coli pattern of function appears not to be applicable to eukaryotes; 2) certain important properties of this regulatory system are conserved among diverse lower and higher eukaryotes; 3) a general pattern of cyclic AMP action can be seen in its action in several lower eukaryotes involving stimulation of glycolysis and inhibition of gluconeogenesis; 4) this lower eukaryotic pattern may also be relevant to cyclic AMP action in higher animal cells.

The possibility that cyclic AMP mediates carbon catabolite repression in eukaryotes (as in E. coli) has been tested recently using mutants of the fungus, Neurospora crassa, and the yeast, Saccharomyces cerevisiae. A glucose transport system which shows carbon catabolite repression in Neurospora shows normal control in mutants which have little or no adenylate cyclase or cyclic AMP (3). In mutants of yeast which have increased permeability to cyclic AMP, exogenous cyclic AMP fails to overcome carbon catabolite repression of galactokinase (4). It may be inferred that cyclic AMP does not act as a general mediator of carbon catabolite repression in these lower eukaryotes. This difference between E. coli and eukaryotes should not be surprising. The intracellular receptor for cyclic AMP in bacteria is a protein which regulates transcription, whereas in lower eukaryotes and in animals, it is a cyclic AMP-dependent protein kinase. It may well be that the evolutionary gap between prokaryotes and eukaryotes in this area, as in so many others, will remain enigmatic.
We have looked for evidence of evolutionarily conserved properties for the cyclic AMP control system in lower eukaryotes where perhaps more basic properties and functions may be more easily discerned than in higher animal cells.

**Four areas of eu karyotic conservation**

There are several areas of the cyclic AMP control system which show substantial conservation in eu karyotic evolution. Such conservation suggests that the cyclic AMP control function may have been substantially conserved, as well. Four of these areas are listed in Table 1.

Table 1. Areas of conservation of the cyclic AMP control system in eukaryotes.

1. Properties of protein kinase including regulatory subunit and catalytic specificity.
2. Separate catalytic and regulatory components of a guanine nucleotide-stimulated adenylate cyclase.
3. Similar control of endogenous cyclic AMP levels in diverse lower eukaryotes.
4. Stimulation of glycolysis and inhibition of gluconeogenesis by cyclic AMP.

One area of striking conservation is in the receptor for cyclic AMP, the cyclic AMP-dependent protein kinase. It was demonstrated some time ago that the catalytic specificity for different protein substrates of the yeast cyclic AMP-dependent protein kinase is strikingly similar to that of the animal enzyme (5). The regulatory subunits of these enzymes in lower eukaryotes are also quite similar to those of mammals (5–10). One strikingly similar set of comparisons of regulatory subunits are shown in Table 2.

Table 2. Properties of regulatory component of cyclic AMP-dependent protein kinase.

<table>
<thead>
<tr>
<th></th>
<th>Mammalian type 1</th>
<th>Neurospora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated molecular weight (SDS gels)</td>
<td>47 000</td>
<td>47 000</td>
</tr>
<tr>
<td>Aggregation state</td>
<td>Dimer</td>
<td>Dimer</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>5.35–5.57</td>
<td>5.4–5.5</td>
</tr>
<tr>
<td>Moles of cyclic AMP bound/subunit</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cyclic AMP binding sites with high affinity for 8 bromocyclic AMP</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Stokes radius</td>
<td>44Å</td>
<td>46Å</td>
</tr>
</tbody>
</table>

*Neurospora* data from Ref. 6.

It has also been recently shown that a second critical enzyme of the control system shows signs of conservation. The adenylate cyclase in *Neurospora*, yeast, and possibly *Dictyostelium* have separate catalytic and regulatory components (11–14). As in the animal enzyme (15), the regulatory components are required for high ATP-Mg\(^{2+}\)-dependent, guanine-nucleotide-stimulated activity. It has recently been reported that the regulatory component of the animal enzyme can interact with and regulate the catalytic component of the *Neurospora* enzyme (16).

A third area of conservation is in the control of endogenous cyclic AMP levels. Several diverse lower eukaryotes show similar cyclic AMP increases in treatment with uncouplers of oxidative phosphorylation or with certain pore-forming antibiotics (3, 17–19). These organisms are thought to be only very distantly related, *Neurospora* and yeast, for example are estimated to have diverged from a common origin about 800 million years ago (20) with *Mucor* and *Coprinus* being still more distantly related. The results suggest that as yet undetermined mechanisms which control endogenous cyclic AMP levels in these organisms have been conserved through much of the eukaryotic evolution.

A fourth area of conservation has direct implications on cyclic AMP function.

**Cyclic AMP stimulation of glycolysis and inhibition of gluconeogenesis**

It was first proposed about two and a half years ago that cyclic AMP may act to stimulate glycolysis in many lower eukaryotes (21). Since then, substantial new evidence has been obtained, not only for stimulation of glycolysis, but also for the inhibition of gluconeogenesis. The pattern of evidence available is diagrammed in Fig. 1. Both *in vitro* and *in vivo* effects of cyclic AMP are shown.

The two main storage carbohydrates in fungi are glycogen and trehalose. *In vitro* studies of the glycogen control system in *Neurospora*, *Coprinus* and in yeast (22–28) show that glycogenolysis is stimulated by cyclic AMP through its influence on protein phosphorylation. Studies in yeast also demonstrate that trehalose degradation is stimulated by cyclic AMP (28–31). Thus, cyclic AMP should provide an increase in hexose phosphates.