

Adenylyl cyclases and the interaction between calcium and cAMP signalling

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Adenylyl cyclase is the prototypical second messenger generator. Nearly all of the eight cloned adenylyl cyclases are regulated by one or other arm of the phospholipase C pathway. Functional and ultrastructural investigations have shown that adenylyl cyclases are intimately associated with sites of calcium ion entry into the cell. Oscillations in cellular cyclic AMP levels are predicted to arise because of feedback inhibition of adenylyl cyclase by Ca^{2+} . Such findings inextricably intertwine cellular signalling by cAMP and internal Ca^{2+} and extend the known regulatory modes available to cAMP.

THE concept of signalling by second messengers originated with the discovery of the role of cyclic AMP¹. The familiar cAMP-dependent protein kinase pathway elegantly accounts for the hormonal control of many cellular cascades including glyco-genolysis, lipolysis and catecholamine biosynthesis². In addition, the study of adenylyl cyclase has spawned the field of G-protein-mediated signal transduction and identified it as a widespread motif in numerous regulatory processes^{3,4}. But signalling mediated by cytosolic calcium ions ($[\text{Ca}^{2+}]_i$) has at least an equally vital role in many cellular events⁵. As with cAMP, there are also Ca^{2+} -initiated phosphorylation cascades⁶; however, Ca^{2+} also directly and, perhaps, more dynamically modifies numerous aspects of cellular function⁷. Ironically, although the study of adenylyl cyclase has fostered the importance of G proteins in many signal-transduction processes⁴, current studies suggest that regulation by factors other than G proteins, particularly $[\text{Ca}^{2+}]_i$, may be as important to many adenylyl cyclases.

Structure of adenylyl cyclases

The cloning of the first adenylyl cyclase revealed a quite unexpectedly complex structure⁸. This and all subsequently cloned adenylyl cyclases are large (1,080–1,248 amino acids) polypeptides that are predicted to cross the plasma membrane 12 times in 2 cassettes of 6 transmembrane-spanning domains, with each cassette followed by a large cytosolic domain^{9–19}. Although superficially resembling ion channels, these structures most resemble transporter molecules, such as the P-glycoprotein and the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel^{20,21}. The transmembrane domains are not highly conserved between adenylyl cyclases and share little or no homology with the highly conserved transmembrane domains of ion channels²². The complexity of adenylyl cyclase structure remains a tantalizing puzzle, which may yet reflect on the sensitivity of adenylyl cyclases to local $[\text{Ca}^{2+}]_i$ rises (see below). The two cytosolic regions include putative ATP-binding domains, which are quite homologous to each other⁸ and between different adenylyl cyclases (50–92%)^{23,24}. This internal homology in ATP-binding sites, along with results from the separate and combined expression of the two halves of adenylyl cyclase, has led to the suggestion that these two sites may interact cooperatively²⁵. Accumulating data indicate that non-overlapping regions exist on these enzymes, which allow separate interaction with α_s , α_i and $\beta\gamma$ subunits of G proteins^{26,27}, although the precise domains have not yet been defined. Interestingly, a peptide sequence that is conserved in all cyclases, spanning amino acids 425–444 in the first cytoplasmic loop, profoundly inhibits type V adenylyl cyclase activity²⁸. This could indicate that this sequence is an internal inhibitory domain, or that adenylyl cyclase may function as a dimer in the plasma membrane, as originally proposed, based on target size analysis²⁹. Another

proposed functional domain, the site of Ca^{2+} /calmodulin binding to type I adenylyl cyclase, a Ca^{2+} -stimulated species (see below), is believed to be in the first cytoplasmic loop, adjacent to the plasma membrane^{30,31}.

Multiple regulatory influences

A major surprise to emerge from the current cloning and expression of eight adenylyl cyclases is that most, if not all, adenylyl cyclases are multiply regulated. Conflicting views on universal mechanisms for the regulation of 'adenylyl cyclase' have dissipated with the realization that individual adenylyl cyclase species are uniquely regulated by a variety of influences; the traditional view that G_α subunits are the dominant regulatory influence on adenylyl cyclase is being superseded by the fact that protein kinase C (PKC), Ca^{2+} and $\beta\gamma$ subunits of G proteins can stimulate or inhibit particular adenylyl cyclases far more effectively than the G-protein α -subunits^{23,24}. For example, based on studies of cells transfected with various adenylyl cyclases, the following observations have been made: phorbol esters elicit double the stimulation of transfected type II adenylyl cyclase than does G_{sa} ^{32–34}; receptors operating through G_{sa} barely stimulate type I or type VIII adenylyl cyclase, whereas Ca^{2+} stimulates activity up to fourfold^{12,19}; type VI adenylyl cyclase can be at least as effectively inhibited by elevations in $[\text{Ca}^{2+}]_i$ as it can by G_i -linked receptors^{35,36}. Table 1 groups adenylyl cyclases by structural relatedness; the members of these groups share regulatory features. However, within these groupings, there is a range of responses; for instance, although both types II and VII are stimulated by PKC and G_s , type II is less susceptible to stimulation by G_s than by PKC^{33,34}, whereas the opposite is true for type VII³⁷. The concentration of Ca^{2+} that stimulates type I adenylyl cyclase is in the normal physiological range (0.1–1 μM), whereas stimulation of type III is by supra-normal Ca^{2+} concentrations^{12,38}. Indeed, of the eight adenylyl cyclases, no two are regulated in precisely the same manner. Thus, there is little redundancy in either the regulation or expression (see below) of the multiple isoforms so far detected (Table 1).

The stimulation by Ca^{2+} of types I, III and VIII adenylyl cyclases is mediated by calmodulin, which can be readily removed and added back to restore Ca^{2+} sensitivity^{10,12,23}. It is not clear how the Ca^{2+} sensitivity of the Ca^{2+} -inhibitable types V and VI is achieved. There is no recognizable Ca^{2+} -binding motif in their sequence. Dissociable calmodulin does not seem to mediate the effect^{12,35}, although it cannot be excluded that a Ca^{2+} -binding protein may be tightly bound, as in the case of calmodulin with phosphorylase kinase⁴⁰.

Localization of adenylyl cyclases in the CNS

Individual adenylyl cyclases have a remarkably discrete expression in the central nervous system (CNS), as determined by *in*

TABLE 1 Properties of cloned mammalian adenylyl cyclases grouped by structural relatedness

	Ca^{2+} effect	$\beta\gamma$ effect	G_s (stm)	PKC (stm)	Richest mRNA source
I	Stimulation	Inhibition	Mild	No	DG/HO
III	Stimulation	?	Yes	No	OE
VIII	Stimulation	?	Mild*	No	HO
II	No	Stimulation	Yes	Yes	CRB
IV	No	Stimulation	Yes	No	Rare
VII	No	?	Yes	Yes	CRB
V	Inhibition	No	Yes	No†	CN
VI	Inhibition	No	Yes	No	Heart

The regulatory susceptibilities of identified adenylyl cyclases summarized above, coupled with the distinct expression of each of these species in brain, provides opportunities for the generation of unique signalling patterns. Apart from the $\beta\gamma$ data, which necessarily come from *in vitro* reconstitution studies²⁶, most of the regulatory properties of adenylyl cyclases outlined derive from studies of cDNAs expressed in intact HEK 293 cells, which are subjected to either physiological elevation in $[\text{Ca}^{2+}]_i$, hormonal activation of G_s , or phorbol ester activation of PKC. Certain *in vitro* observations do not quite conform to these properties: for example, *, exogenous G_{sa} and Ca^{2+} stimulate type VIII activity synergistically¹⁹; and †, type V is potently activated *in vitro* by the non-phorbol-ester-sensitive, Ca^{2+} -independent isoform of PKC- ζ ³⁹. DG/HO, Dentate gyrus/hippocampus; OE, olfactory neuroepithelium; CRB, cerebellum; CN, caudate nucleus.

situ hybridization analysis. Although some brain regions express more than one cyclase (see Table 1), some isoforms are quite specific for particular brain regions, for example, the Ca^{2+} /calmodulin-stimulated type I occurs prominently in the dentate gyrus of the hippocampus and the granular layer of the cerebellum^{41,42}; the Ca^{2+} -inhibited type V occurs only in the striatum^{17,43}; the PKC-stimulated type VII is found only in the granular layer of the cerebellum⁴⁴ (Fig. 1); and the Ca^{2+} /calmodulin-stimulated type III is restricted to olfactory neuroepithelium¹⁰ (Table 1). However, with the exception of Ca^{2+} /calmodulin-stimulated forms, little is known about how the regulatory properties of particular species are exploited in discrete areas. The susceptibility of types I and VIII to stimulation by Ca^{2+} and their presence in hippocampus (Table 1) implicates

them in the mammalian model of learning and memory, hippocampal long-term potentiation (LTP). A critical component of this LTP is glutamate (NMDA)-receptor-mediated Ca^{2+} entry⁴⁵, which is associated with an elevation of cAMP⁴⁶. Depending on the subdomain of the hippocampus, either type I or type VIII could be the adenylyl cyclase that responds to NMDA-mediated elevation in $[\text{Ca}^{2+}]_i$ ^{19,41,42}. Direct support for the involvement of type I in learning has been provided by its deletion in transgenic mice. These mice showed impaired hippocampal-dependent learning abilities and a 40–60% reduction in Ca^{2+} /calmodulin-stimulated adenylyl cyclase in various brain regions, including the hippocampus⁴⁷. A remarkable aspect of this experiment is that the animals survived to maturity in the putative absence of type I adenylyl cyclase. Presumably, their survival reflects compensatory mechanisms (most simply by upregulation of type VIII) during the development of their nervous system.

At the subcellular level, ultrastructural electron microscopic analysis reveals a striking concentration of adenylyl cyclase immunoreactivity in hippocampal postsynaptic densities of dendritic spines in the stratum radiatum, where synapses occur between Schaffer collateral and CA1 neurons⁴⁸. (Based on the presence of both types I and VIII messenger RNA in pyramidal neurons in the CA1 layers^{19,42}, this species should be a Ca^{2+} /calmodulin-stimulated adenylyl cyclase.) This observation places adenylyl cyclases precisely where they are most efficacious in the propagation of NMDA-mediated LTP (see above and refs 45, 46, 48). NMDA receptors are also postsynaptic in the Schaffer collateral commissural pathway associated with LTP in CA1 pyramidal neurons and are similarly concentrated in postsynaptic densities in dendritic spines^{49,50}. cAMP-dependent protein kinase anchoring proteins (AKAPs) tether cAMP-dependent protein kinase (PKA) regulatory subunits in high concentrations in these same dendritic spines^{51,52} (Fig. 2). Thus, it can be anticipated that Ca^{2+} , entering in response to glutamate opening of NMDA receptors, would readily activate Ca^{2+} /calmodulin stimulable adenylyl cyclases; the cAMP generated would need to diffuse only a short distance before activating the anchored PKA, greatly enhancing the downstream effects of the kinase. This organization of adenylyl cyclases prompts the speculation that specific targeting information is encoded within the enzyme's structure. The restricted localization of adenylyl cyclase is also consistent with recent data on the immobility of

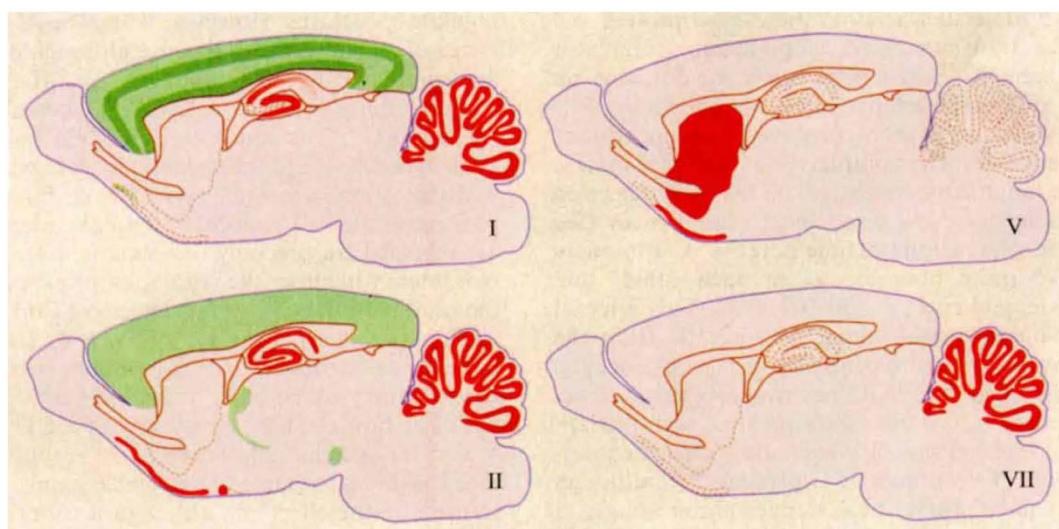
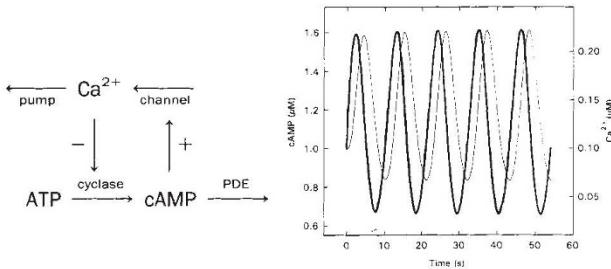


FIG. 1 Comparison of the expression of a Ca^{2+} /calmodulin-stimulated (type I), a Ca^{2+} -inhibited (type V) and two PKC-stimulated (types II and VII) adenylyl cyclase mRNAs in rat brain. This schematic representation of the compiled results of *in situ* hybridization analysis^{17,41–44} underlines

the remarkable selective localization patterns of these quite distinctly regulated species. Such discrete expression hints strongly at as yet unknown local usefulness of these properties.

BOX 1 Stable oscillations in cAMP and Ca^{2+} arising from a negative-feedback control loop

We consider here a closed, negative-feedback loop between cAMP and Ca^{2+} that under certain conditions can result in stable oscillations in the concentrations of both intracellular messengers. The scheme shown below consists of elements that exist in a variety of cells. In the model, a rise in cAMP opens channels in the plasma membrane causing an increased influx of Ca^{2+} . The rise in Ca^{2+} decreases the rate of cAMP synthesis by inhibiting adenylyl cyclase.



This feedback system is described by the following equations: $\text{dx}/\text{dt} = aw - bx$; $\text{dy}/\text{dt} = c - dy$; $\text{dz}/\text{dt} = ey - fz$; $\text{dw}/\text{dt} = h - gw$; $c = c_m x^{N_1}/(x^{N_1} + K_1^{N_1})$; $g = g_m z^{N_2}/(z^{N_2} + K_2^{N_2})$, where x is the concentration of cAMP, y is the concentration of active channels, z is the concentration of free Ca^{2+} , w is the concentration of active cyclase, a is the rate constant of cAMP synthesis by adenylyl cyclase, b is the rate constant of cAMP hydrolysis by phosphodiesterase (PDE), c is the rate of conversion of inactive to active channels, c_m is the maximal rate of that conversion at saturating cAMP, d is the rate constant for conversion of active to inactive channels, e is the rate constant of Ca^{2+} influx, f is the rate constant of Ca^{2+} pumping or exchange, g is the rate constant for the conversion of active to inactive cyclase, g_m is the maximal rate constant for that conversion at saturating Ca^{2+} , h is the rate of reversal of Ca^{2+} inhibition of cyclase, K_1 is the concentration at which cAMP stimulation of channel activation is half-maximal, and K_2 is the concentration at which Ca^{2+} inhibition of cyclase is half-maximal. N_1 and N_2 are the Hill coefficients (an index of cooperativity) for those processes.

In examining the behaviour of the feedback system we have made the following simplifying assumptions: (1) concentrations in the cytosol are uniform; the effects of spatial gradients are ignored. (2) The precursors from which the four variable species are formed are in saturating supply and are taken to be constant. These include ATP, extracellular Ca^{2+} , inactive channels, and inactive cyclase. (3) The variable species are present at concentrations much less than the K_m values of the enzymes that cause them to decay. The rates of decay are therefore directly proportional to concentration. Relaxing these assumptions will change the quantitative behaviour, but not the ability of the system to oscillate.

In the simulation shown, the values of the parameters were as follows: $a = 2 \text{ s}^{-1}$, $b = 0.5 \text{ s}^{-1}$, $c_m = 0.45 \mu\text{M s}^{-1}$, $d = 1 \text{ s}^{-1}$, $e = 1 \text{ s}^{-1}$, $f = 0.5 \text{ s}^{-1}$, $g_m = 2 \text{ s}^{-1}$, $h = 0.11 \mu\text{M s}^{-1}$, $K_1 = 2 \mu\text{M}$, $K_2 = 0.2 \mu\text{M}$, $N_1 = 3$ and $N_2 = 3$. The initial concentrations of the four variables were: $x_0 = 1 \mu\text{M}$ (cAMP, thick line), $y_0 = 0.05 \mu\text{M}$, $z_0 = 0.1 \mu\text{M}$ (Ca^{2+} , thin line) and $w_0 = 0.5 \mu\text{M}$. At $t = 0$, active channel, Ca^{2+} and Ca^{2+} -regulated cyclase were all at steady-state (dy/dt , dz/dt , $\text{dw}/\text{dt} = 0$). The system was perturbed by a step change in the adenylyl cyclase rate constant, a , to the value of 2 s^{-1} ($\text{dx}/\text{dt} = 0.5 \mu\text{M s}^{-1}$ at $t = 0$). Such a perturbation, albeit not instantaneous, would occur upon G-protein stimulation.

Stable oscillations in a system of this type depend on finite time delays in the loop, and a certain degree of cooperativity in the processes regulated by the intracellular messengers. The delay in the activation of the channel could arise from the time it takes kinase A to phosphorylate the channel, or the time it takes cAMP or a hypothetical cAMP-binding protein to associate with the channel. The delay in the inhibition of cyclase might arise from similar processes or from the exchange of Ca^{2+} between intracellular buffers and the cytosol. Damped oscillations would arise if both channel activation by cAMP and adenylyl cyclase inhibition by Ca^{2+} are very rapid compared with the other processes in the loop. The necessary cooperativity could be cumulatively distributed between other steps in the loop, such as multiple phosphorylation events required for activation or inhibition, or multiple binding proteins associating with the channel or dimeric forms of the cyclase. Stimulation of PDE and inhibition of channel activity by Ca^{2+} are additional cooperative processes that would favour oscillations in a model of this type. □

G proteins⁵³, as postulated earlier in ref. 3, which suggests a high degree of organization of adenylyl cyclase regulatory complexes.

Regulation by local $[\text{Ca}^{2+}]_i$ rises

In excitable cells, it may seem inevitable that adenylyl cyclases are regulated by Ca^{2+} entry, given their localization in dendritic spines, which are major foci for Ca^{2+} entry. Indeed, Ca^{2+} entering via L-type channels causes a pronounced inhibition of the indigenous type V and VI adenylyl cyclases in chick myocytes⁵⁴. However, in non-excitable cells, the route by which intracellular $[\text{Ca}^{2+}]$ is elevated is also critical for inhibiting Ca^{2+} -sensitive adenylyl cyclases. Where the Ca^{2+} release and entry processes have been separately evaluated, it is clear that only Ca^{2+} entering the cell as a result of store depletion, and not Ca^{2+} released from intracellular stores, can regulate these enzymes⁵⁵. For instance,

the very substantial increase in $[\text{Ca}^{2+}]_i$ that can be elicited by ionomycin (in the absence of extracellular Ca^{2+}) is incapable of inhibiting type VI adenylyl cyclase, whereas the capacitative Ca^{2+} entry provoked by any mode of depletion of intracellular stores prominently inhibits activity⁵⁵. This implies either a coincidental or an organized colocalization of adenylyl cyclases with capacitative Ca^{2+} entry channels (I_{CRAC} s)⁵⁶ in these cells. Whether Ca^{2+} /calmodulin-stimulated adenylyl cyclases are similarly exclusively responsive to Ca^{2+} entry has not yet been determined, notwithstanding the morphological data of coexpression of Ca^{2+} entry sites and presumed Ca^{2+} -stimulated adenylyl cyclases. This apparent concentration of adenylyl cyclases in areas that are exposed to high $[\text{Ca}^{2+}]_i$ again prompts the suggestion that adenylyl cyclase sequences include domains that target them to Ca^{2+} entry sites.

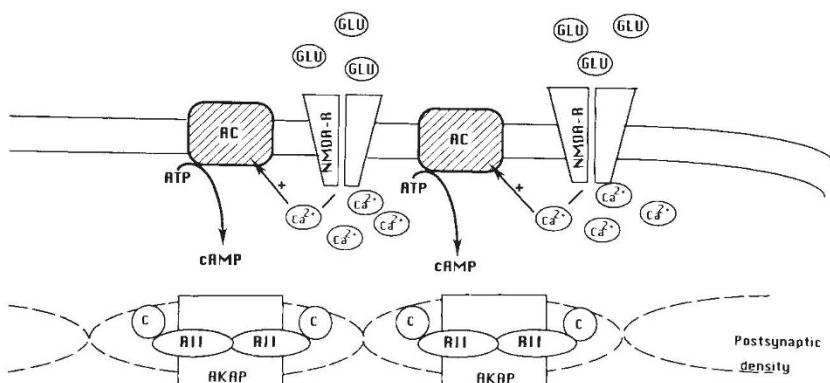


FIG. 2 Schematic representation of the concentration of regulatory elements present in hippocampal postsynaptic dendritic spines of the CA1 region, based on electron microscopic analysis of NMDA receptors (NMDA-R), adenylyl cyclase (AC) and cAMP-dependent protein kinase anchoring proteins (AKAP).

Oscillations in cAMP

The Ca^{2+} sensitivity of five of the eight known adenylyl cyclases has profound ramifications for cells in which cAMP controls Ca^{2+} entry, or in which $[\text{Ca}^{2+}]_i$ oscillates; the Ca^{2+} sensitivity makes it possible that cAMP levels might also oscillate in harmony with $[\text{Ca}^{2+}]_i$ oscillations. Box 1 shows that cAMP accumulation can be pulsatile in a theoretical cell, in which cAMP generated by a Ca^{2+} -inhibitable adenylyl cyclase controls Ca^{2+} entry. (cAMP is known both directly to open cation channels that are permeable to Ca^{2+} (ref. 57) and indirectly, through PKA, to enhance Ca^{2+} entry by the phosphorylation of L-type Ca^{2+} channels⁵⁸ in cardiac myocytes; Ca^{2+} -inhibitable species of adenylyl cyclase are also the most prevalent in cardiac tissue^{12, 16, 54}.) As shown in Box 1, such a loop can give rise not only to oscillations in cAMP, but also in $[\text{Ca}^{2+}]_i$. It is important to note that oscillations can arise simply as a result of the very minimal negative feedback depicted, which is based on realistic estimates of the kinetic properties of the molecules involved. If, in addition, increases in $[\text{Ca}^{2+}]_i$ also stimulated phosphodiesterase (as occurs in many cell types⁵⁹), or if $[\text{Ca}^{2+}]_i$ oscillates independently of cAMP (as is widely encountered⁶⁰), the frequency of the oscillations would be increased. (Interestingly, this model is reminiscent of an earlier model⁶¹ with the same qualitative features, but which lacked the molecular insights on which this model is based.) Although current methodologies cannot detect cAMP with the temporal resolution of the anticipated oscillations in single cells, cAMP does oscillate in the synchronized preparation of frog ventricular strips as a function of the normal contraction/relaxation cycle⁶². Oscillations in extracellular cAMP have long been established in *Dictyostelium*⁶³, for which several mechanistic proposals have been advanced⁶⁴. In addition, $[\text{Ca}^{2+}]_i$ oscillations have been encountered in cells, such as GH3 cells, in which cAMP (generated by a Ca^{2+} -inhibitable adenylyl cyclase) controls Ca^{2+} entry⁶⁵. If cAMP levels do oscillate, cAMP could signal using a frequency-encoding mode (as has been proposed for Ca^{2+} oscillations) in addition to its familiar amplitude-encoded mode. Advantages of signalling by frequency rather than amplitude have already been suggested for Ca^{2+} , including: the fact that large, energetically wasteful transitions

in the signal are spared; insignificant fluctuations in signal can be ignored; cooperatively activated Ca^{2+} -binding proteins are well suited to detect spikes because they can evolve to respond only to peak concentrations; different effectors could respond to different frequencies of the signal⁶⁶; and, in addition, desensitization to a signal could be avoided and diffusion of the signal within the cell can be controlled. If cAMP concentrations do oscillate, such advantages greatly increase the signalling capability of cAMP. (At the experimental level, if oscillations in cAMP do occur, studies that examine only the effect of static concentration changes in cAMP may provide a very blinkered view of the signalling potential of cAMP.)

An assessment of the adenylyl cyclases reveals that, generally, no two forms are regulated in precisely the same manner and there is little redundancy in their tissue expression. At the cellular level, they can be discretely localized to respond rapidly and selectively to Ca^{2+} entering the cell. Thereby it appears as though evolution has put in place elaborate mechanisms for ensuring that the two major second messengers are tightly integrated, so that it is the summation of their interaction that controls cellular activity. Some time ago, in the absence of any molecular knowledge of the elements comprising cAMP- and Ca^{2+} -signalling pathways, the prescient proposal was advanced that the interaction between these two systems was central in controlling the activity of cellular processes^{61, 67}. Current molecular and topographical information strongly support and expand upon such theories. The challenge for the future is to determine the molecular basis for Ca^{2+} inhibitory of adenylyl cyclases, the physiological use of variously regulated and selectively expressed adenylyl cyclases, the elements in their structure that may target them to sites of Ca^{2+} entry, whether in fact cAMP concentrations oscillate and the regulatory implications of such oscillations. For now, current adenylyl cyclase cloning and expression has revealed new challenges and promises from this most familiar second messenger. □

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1. Robison, G. A., Butcher, R. W. & Sutherland, E. W. *A. Rev. Biochem.* **37**, 149–174 (1968).
2. Walsh, D. A. & Van Patten, S. M. *FASEB J.* **8**, 1227–1236 (1994).
3. Rodbell, M. *Nature* **284**, 17–22 (1980).
4. Gilman, A. G. *A. Rev. Biochem.* **56**, 615–649 (1987).
5. Berridge, M. J. *Nature* **361**, 315–325 (1993).
6. Nishizuka, Y. *Nature* **334**, 661–665 (1988).
7. Penner, R. & Neher, E. *J. exp. Biol.* **139**, 329–345 (1988).
8. Krupinski, J. et al. *Science* **244**, 1558–1564 (1989).
9. Feinstein, P. G. et al. *Proc. natn. Acad. Sci. U.S.A.* **88**, 10173–10177 (1991).
10. Bakalyar, H. A. & Reed, R. R. *Science* **250**, 1403–1406 (1990).
11. Gao, B. & Gilman, A. G. *Proc. natn. Acad. Sci. U.S.A.* **88**, 10178–10182 (1991).
12. Yoshimura, M. & Cooper, D. M. F. *Proc. natn. Acad. Sci. U.S.A.* **89**, 6716–6720 (1992).
13. Premont, R. T., Chen, J., Ma, H., Ponnapalli, M. & Iyengar, R. *Proc. natn. Acad. Sci. U.S.A.* **89**, 9809–9813 (1992).
14. Ishikawa, Y. et al. *J. biol. Chem.* **267**, 13553–13557 (1992).
15. Katsushika, S. et al. *Proc. natn. Acad. Sci. U.S.A.* **89**, 8774–8778 (1992).
16. Krupinski, J., Lehman, T. C., Frankenfield, C. D., Zwaagstra, J. C. & Watson, P. A. *J. biol. Chem.* **267**, 24858–24862 (1992).
17. Glatt, C. E. & Snyder, S. H. *Nature* **361**, 536–538 (1993).
18. Wallach, J., Droste, M., Kluxen, F. W., Pfeuffer, T. & Frank, R. *FEBS Lett.* **338**, 257–263 (1994).
19. Cali, J. J., Zwaagstra, J. C., Mons, N., Cooper, D. M. F. & Krupinski, J. *J. biol. chem.* **269**, 12190–12195 (1994).
20. Chen, C.-J. et al. *Cell* **47**, 381–389 (1986).
21. Riordan, J. R. et al. *Science* **245**, 1066–1073 (1989).
22. Catterall, W. A. *Curr. Opin. Cell Biology* **6**, 607–615 (1994).
23. Tang, W. & Gilman, A. G. *Cell* **70**, 869–872 (1992).
24. Iyengar, R. *FASEB J.* **7**, 768–775 (1993).
25. Tang, W., Krupinski, J. & Gilman, A. G. *J. biol. Chem.* **266**, 8595–8603 (1991).
26. Taussig, R., Tang, W., Hepler, J. R. & Gilman, A. G. *J. biol. Chem.* **269**, 6093–6100 (1994).
27. Taussig, R., Quaraby, L. M. & Gilman, A. G. *J. biol. Chem.* **268**, 9–12 (1993).
28. Kawabe, J. et al. *J. biol. Chem.* **269**, 16554–16558 (1994).
29. Schlegel, W., Kempner, E. S. & Rodbell, M. *J. biol. Chem.* **254**, 5168–5176 (1979).
30. Vorherr, T. et al. *Biochemistry* **32**, 6081–6088 (1993).
31. Wu, Z., Wong, S. T. & Storm, D. R. *J. biol. Chem.* **268**, 23766–23768 (1993).
32. Jacobowitz, O., Chen, J., Premont, R. T. & Iyengar, R. *J. biol. Chem.* **268**, 3829–3832 (1993).
33. Yoshimura, M. & Cooper, D. M. F. *J. biol. Chem.* **268**, 4604–4607 (1993).
34. Lustig, K. D., Conklin, B. R., Herzmark, P., Taussig, R. & Bourne, H. R. *J. biol. Chem.* **268**, 13900–13905 (1993).
35. Boyajian, C. L., Garritsen, A. & Cooper, D. M. F. *J. biol. Chem.* **266**, 4995–5003 (1991).
36. DeBernardi, M. A., Seki, T. & Brooker, G. *Proc. natn. Acad. Sci. U.S.A.* **88**, 9257–9261 (1991).
37. Watson, P. A., Krupinski, J., Kempinski, A. M. & Frankenfield, C. D. *J. biol. Chem.* **269**, 28893–28898 (1994).
38. Choi, E.-J., Xia, Z. & Storm, D. R. *Biochemistry* **31**, 6492–6498 (1992).
39. Kawabe, J. et al. *J. biol. Chem.* **269**, 16554–16558 (1994).
40. Klee, C. B., Crouch, T. B. & Richman, P. G. *A. Rev. Biochem.* **49**, 489–515 (1980).
41. Xia, Z., Refsdal, C. D., Merchant, K. M., Dorsa, D. M. & Storm, D. R. *Neuron* **6**, 431–443 (1991).
42. Mons, N., Yoshimura, M. & Cooper, D. M. F. *Synapse* **14**, 51–59 (1993).
43. Mons, N. & Cooper, D. M. F. *Molec. Brain Res.* **22**, 236–244 (1994).
44. Hellevuo, K. et al. *J. biol. Chem.* **270** (in the press).
45. Bliss, T. V. P. & Collingridge, G. L. *Nature* **361**, 31–39 (1993).
46. Chetkovich, D. M., Gray, R., Johnston, D. & Sweatt, J. D. *Proc. natn. Acad. Sci. U.S.A.* **88**, 6467–6471 (1991).
47. Wu, Z.-L. et al. *Proc. natn. Acad. Sci. U.S.A.* **92**, 220–224 (1995).
48. Cooper, D. M. F. & Mons, N. *J. Neurochem.* **64**, 79 (1995).
49. Petralia, R. S., Yokotani, N. & Wenthold, R. J. *J. Neurosci.* **14**, 667–696 (1994).
50. Siegel, S. J. *Proc. natn. Acad. Sci. U.S.A.* **91**, 564–568 (1994).
51. Giantz, S. B., Ama, J. A. & Rubin, C. S. *Molec. Biol. Cell* **3**, 1215–1228 (1992).
52. Carr, D. W., Stoffko-Hahn, R. E., Fraser, I. D. C., Cone, R. D. & Scott, J. D. *Proc. natn. Acad. Sci. U.S.A.* **89**, 16816–16823 (1992).
53. Neubig, R. R. *FASEB J.* **8**, 939–947 (1994).
54. Yu, H. J., Ma, H. & Green, R. D. *Molec. Pharmac.* **44**, 689–693 (1993).
55. Chiono, M., Mahey, R., Tate, G. & Cooper, D. M. F. *J. biol. Chem.* **270**, 1149–1155 (1995).
56. Hoth, R. & Penner, R. *Nature* **355**, 353–356 (1992).
57. Yau, K.-W. *Proc. natn. Acad. Sci. U.S.A.* **91**, 3481–3483 (1994).
58. Trautwein, W. & Heschler, J. *A. Rev. Physiol.* **52**, 257–274 (1990).
59. Wu, Z., Sharma, R. K. & Wang, J. H. *Adv. Sec. Mgr. Phosphoprot. Res.* **25**, 29–43 (1992).
60. Cuthbertson, K. S. R. & Cobbold, P. H. *Cell Calcium* **12**, 2/3 (1991).
61. Rapp, P. E. & Berridge, M. J. *J. theor. Biol.* **68**, 497–525 (1977).
62. Brooker, G. *Science* **182**, 933–934 (1973).
63. Roos, W., Scheidegger, C. & Gerisch, G. *Nature* **266**, 259–261 (1977).
64. Monk, P. B. & Othmer, H. G. *Phil. Trans. R. Soc. B* **323**, 185–224 (1989).
65. Schlegel, W. et al. *Nature* **329**, 719–721 (1987).
66. Meyer, T. & Stryer, L. *Proc. natn. Acad. Sci. U.S.A.* **85**, 5051–5055 (1988).
67. Rasmussen, H. *Ann. N. Y. Acad. Sci.* **356**, 346–353 (1980).

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