Compartmentalization of Cyclic AMP Signalling

Biochemical Society Focused Meeting held at King's College, Cambridge, U.K., 29–30 March 2006. Organized by D. Cooper (Cambridge, U.K.), M. Houslay (Glasgow) and M. Zaccolo (Padua, Italy). Edited by D. Cooper.

Compartmentalized cAMP signalling: a personal perspective

J.D. Scott[1]

Howard Hughes Medical Institute, Vollum Institute, Oregon Health & Sciences University, MRB322, 3181 SW Sam Jackson Park Road, Portland, OR 97239, U.S.A.

Abstract

Scaffolding proteins create order out of chaos. Multifunctional binding proteins such as the AKAPs (A-kinase-anchoring proteins) oversee the dynamic organization of signalling events by clustering activator proteins with kinases, phosphatases and phosphodiesterases and directing them toward their downstream effectors. This article will focus on the role of AKAPs in the spatial and temporal control of cAMP signalling events.

Although the investigation of cAMP signalling has been underway for over 50 years, it was not until the early 1980s that the compartmentalized modulation of these events was appreciated. This concept developed from physiological experiments showing that stimulation of cAMP synthesis by different agonists produced distinct physiological outputs, even within the same cell. For example, adrenergic stimulation activated a particulate pool of PKA (protein kinase A or cAMP-dependent protein kinase) in cardiomyocytes, whereas stimulation with prostaglandin E_1 activated a cytosolic pool of the enzyme [1]. These observations were compatible with previous evidence that the PKA holoenzyme, which consists of two regulatory (R) and two catalytic (C) subunits, exists in two forms: a cytoplasmic type I PKA holoenzyme and a type II PKA holoenzyme that was considered to be particulate. This differential distribution of the PKA holoenzymes also implied that mechanisms were in place to segregate distinct cAMP signals. The development of fluorescent probes and advanced imaging techniques largely proved this hypothesis. For example, overexpressed rat olfactory cyclic nucleotide-gated channels, which have been modified to be sensitive to cAMP, or PKA subunits which have been engineered to function as FRET (fluorescent resonance energy transfer) reporters have been used to detect gradients of cAMP [2,3]. Clever use of these and other chemical biology tools have also conclusively shown the accumulation of cAMP within selected cellular microdomains [4].

The spatiotemporal control of cAMP signalling requires that PKA holoenzymes are tethered to specific subcellular locations (Figure 1). Vital evidence for how this occurs came initially from an observation that the type II PKA holoenzyme co-purifies with microtubules and the RII subunit binds to MAP2 (microtubule-associated protein 2) [5]. Hence, MAP2 proved to be the first AKAP (A-kinase-anchoring protein). A few years later, additional 'RII-binding proteins' were detected by an overlay technique that used a radioactive RII subunit to probe proteins immobilized on nitrocellulose membranes [6]. The RII overlay assay was used to show that RII dimerization was necessary for interaction with AKAPs. In subsequent studies, we demonstrated that each anchoring protein contained a reciprocal binding sequence of 14–18 amino acids that formed an amphipathic helix [7,8]. The RII overlay technique was also modified to screen phage cDNA libraries and, accordingly, several novel AKAPs were cloned [9]. One of these proteins, initially called Ht31 because it was isolated from a human thyroid cDNA library, but now also known as AKAP-Lbc, contained an 18-amino-acid sequence that can be used as a peptide disruptor of RII–AKAP interactions inside cells [10,11]. The utility of this peptide as a PKA-anchoring inhibitor was first shown when perfusion of the Ht31 peptide into cultured

Key words: A-kinase-anchoring protein (AKAP), cAMP cycling, compartmentalization, protein kinase A (PKA).

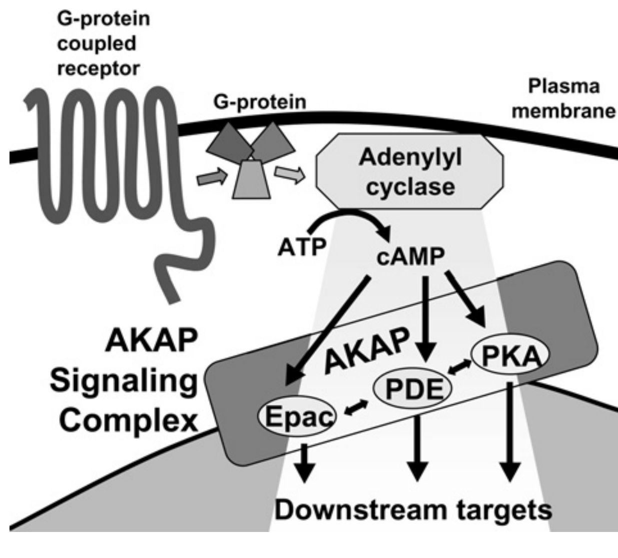
Abbreviations used: AC, adenylate cyclase; AKAP, A-kinase-anchoring protein; Epac, exchange protein directly activated by cAMP; ERK, extracellular-signal-regulated kinase; FRET, fluorescence resonance energy transfer; mAKAP, muscle-selective AKAP; MAP2, microtubule-associated protein 2; PDE, phosphodiesterase; PKA, protein kinase A or cAMP-dependent protein kinase; PKC, protein kinase C; RyR, ryanodine receptor.

[1]email scott@ohsu.edu

©2006 Biochemical Society

Figure 1 | AKAP signalling complex

Stimulation of G-protein-coupled receptors by a ligand such as a hormone catalyses the activation of the associated heterotrimeric G-protein which leads to activation of AC ('adenylyl cyclase') at the plasma membrane. ACs catalyse the synthesis of cAMP from ATP and the resultant cAMP gradient diffuses into the cell. The AKAP anchors PKA and may also tether other cAMP effectors such as Epac or PDE. Thus the AKAP signalling complex permits positioning of these enzymes near their intended targets to ensure the appropriate subcellular response.



hippocampal neurons disrupted the location of PKA in relation to a key substrate, the AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid)-type glutamate receptor. The functional ramifications of such an event were to decrease this ion channel's responsiveness to synaptic signals [12]. Later, with a steroyl group conjugated to render it cell-soluble, Ht31 was used to show that PKA anchoring participated in the control of mammalian sperm motility [13]. Similar Ht31 peptide-like derivatives have been used to establish that anchored pools of PKA participate in a range of cAMP signalling events [14,15]. Related work from other investigators has shown that peptides derived from dual-specificity anchoring proteins can disrupt AKAP interactions with the type RI subunit [16,17]. These and other biochemical studies have shown that all AKAPs bind the R subunit dimer through a common amphipathic α-helical motif, and that each anchoring protein is tethered to unique subcellular localizations through a defined targeting sequence.

Perhaps the most prominent biological role for AKAPs was discovered when it was shown that AKAP79 (the human orthologue of bovine AKAP75 and murine AKAP150) not only anchored PKA but also bound calcineurin, a calmodulin-dependent phosphatase {also called PP2B (protein phosphatase 2B) [18]}. Clustering of PKA with phosphatases provides a mechanism to control the forward and backward steps of the same phosphorylation event. The notion of multivalent AKAPs was consolidated when it was shown that AKAP79 also bound PKC (protein kinase C) [19]. Such a configuration is ideal for the integration of cAMP and calcium/phospholipid signals in the same subcellular environment. Subsequent studies have also shown that other anchoring proteins such as gravin/AKAP250 and AKAP-Lbc co-localize PKA with PKC, whereas AKAP220 and AKAP149 place PKA with the type 1 phosphatase PP1 (protein phosphatase 1) [20–23]. In fact, I would propose that most, if not all, AKAPs function to bring PKA together with protein phosphatases and a variety of other signal termination enzymes. These protein–protein interactions permit the formation of sophisticated signalling units that can respond to the ebb and flow of cAMP.

At any given time, the cellular concentration of cAMP represents a balance between the AC (adenylate cyclase) activity that produces this second messenger and the PDE (phosphodiesterase) activity that metabolizes it into AMP. There are nine membrane-bound AC isoforms and a distantly related cytoplasmic enzyme that responds to bicarbonate production [24,25] (Figure 1). Termination of cyclic nucleotide signalling is achieved through a superfamily of nine genes encoding over 70 different PDE isoenzymes with distinctive tissue expression patterns and unique subcellular locations [26,27]. For example, the cAMP-specific PDE4 family represents a group of 16 distinct isoforms that share a common catalytic core, but each has a unique N-terminal region that permits differential subcellular targeting [27]. In cardiomyocytes, we have shown that the muscle-selective AKAP, mAKAP, assembles a negative-feedback loop containing PKA and the PDE4D3 isoform [28]. PKA phosphorylation of Ser^{13} on PDE4D3 augments binding to mAKAP [29], whereas PKA phosphorylation of Ser^{54} enhances the catalytic efficiency of the enzyme to favour cAMP metabolism [30]. These effects are counterbalanced by ERKs (extracellular-signal-regulated kinases) that phosphorylate PDE4D3 on Ser^{579} to suppress PDE activity [31]. This latter phosphorylation event may be catalysed by ERK5, which is also a component of the mAKAP complex [32]. This configuration not only ensures bi-directional control of PDE4D3 activity, but has also been postulated to generate local fluctuations in cAMP and concomitant pulses of PKA activity.

In a related study, we used a modified FRET-based PKA activity assay to show that recruitment of PKA and PDE4D3 into the FRET reporter complex generated localized pulses of cAMP [32]. These pulses of cAMP are abolished without PDE present or in the presence of PDE4 inhibitors such as rolipram. Thus a variety of cAMP-responsive events, of differing durations and which occur at different thresholds of cAMP, could emanate from the same microdomain. This is particularly relevant in mAKAP signalling complexes where three functionally distinct cAMP dependent enzymes [PKA, PDE4D3 and Epac1 (exchange protein directly activated by cAMP 1)] reside (Figure 1). PKA is responsive to nanomolar cAMP levels and would become active early in a second-messenger response. However, PDE4D3 (K_m 1–4 μM) and Epac1 (K_d 4 μM) activities would only commence when cAMP accumulated to micromolar levels [32]. Conversely,

©2006 Biochemical Society

the inactivation of PDE4D3 and Epac1 would precede PKA holoenzyme reformation as cAMP levels decline.

Recently, pathophysiological changes in the composition of the mAKAP complex have been linked to certain forms of heart failure. RyRs (ryanodine receptors) are intracellular Ca^{2+}-release channels that are present in multiprotein signalling complexes at the sarcoplasmic reticulum in muscle cells to mediate excitation–contraction coupling. Upon β-adrenergic receptor activation, anchored PKA phosphorylates the RyR, sensitizing the channel to activation by increased Ca^{2+} levels. A body of work implies that chronic changes in the mAKAP–RyR1 complex, including a loss of anchored PDE4D3 and hyperactivation of the anchored PKA, correlate with the onset of 'leaky' channels found in certain models for exercise-induced cardiac arrhythmias and heart failure [33,34]. Whether these changes in the mAKAP complex can be detected routinely in patients with certain types of heart disease and whether this represents a viable therapeutic target for intervention remains to be seen. Nonetheless, these findings point towards a more active role for AKAPs in the synchronization of physiologically relevant signalling events. Furthermore, it suggests that AKAP signalling complexes might prove to be excellent targets for pharmacological manipulation of some of their associated enzymes. No doubt, future studies will explore the role of other AKAP signalling complexes in a variety of other disease states.

I thank Gretchen Deutschlander and Lorene Langeberg for critical reading of this manuscript. This work was supported in part by NIH (National Institutes of Health) grant DK54441.

References

1 Buxton, I.L.O. and Brunton, L.L. (1983) J. Biol. Chem. **258**, 10233–10239
2 Adams, S.R., Harootunian, A.T., Buechler, Y.J., Taylor, S.S. and Tsien, R.Y. (1991) Nature (London) **349**, 694–697
3 Zaccolo, M., De Giorgi, F., Cho, C.Y., Feng, L., Knapp, T., Negulescu, P.A., Taylor, S.S., Tsien, R.Y. and Pozzan, T. (2000) Nat. Cell Biol. **2**, 25–29
4 Zaccolo, M. and Pozzan, T. (2002) Science **295**, 1711–1715
5 Theurkauf, W.E. and Vallee, R.B. (1982) J. Biol. Chem. **257**, 3284–3290
6 Lohmann, S.M., DeCamili, P., Enig, I. and Walter, U. (1984) Proc. Natl. Acad. Sci. U.S.A. **81**, 6723–6727
7 Scott, J.D., Stofko, R.E., McDonald, J.R., Comer, J.D., Vitalis, E.A. and Mangili, J.A. (1990) J. Biol. Chem. **265**, 21561–21566
8 Carr, D.W., Stofko-Hahn, R.E., Fraser, I.D.C., Bishop, S.M., Acott, T.S., Brennan, R.G. and Scott, J.D. (1991) J. Biol. Chem. **266**, 14188–14192
9 Carr, D.W. and Scott, J.D. (1992) Trends Biochem. Sci. **17**, 246–249
10 Carr, D.W., Stofko-Hahn, R.E., Fraser, I.D.C., Cone, R.D. and Scott, J.D. (1992) J. Biol. Chem. **24**, 16816–16823
11 Carr, D.W., Hausken, Z.E., Fraser, I.D., Stofko-Hahn, R.E. and Scott, J.D. (1992) J. Biol. Chem. **267**, 13376–13382
12 Rosenmund, C., Carr, D.W., Bergeson, S.E., Nilaver, G., Scott, J.D. and Westbrook, G.L. (1994) Nature (London) **368**, 853–856
13 Vijayaraghavan, S., Goueli, S.A., Davey, M.P. and Carr, D.W. (1997) J. Biol. Chem. **272**, 4747–4752
14 Burns-Hamuro, L.L., Ma, Y., Kammerer, S., Reineke, U., Self, C., Cook, C., Olson, G.L., Cantor, C.R., Braun, A. and Taylor, S.S. (2003) Proc. Natl. Acad. Sci. U.S.A. **100**, 4072–4077
15 Alto, N.M., Soderling, S.H., Hoshi, N., Langeberg, L.K., Fayos, R., Jennings, P.A. and Scott, J.D. (2003) Proc. Natl. Acad. Sci. U.S.A. **100**, 4445–4450
16 Huang, L.J., Durick, K., Weiner, J.A., Chun, J. and Taylor, S.S. (1997) J. Biol. Chem. **272**, 8057–8064
17 Huang, L.J., Durick, K., Weiner, J.A., Chun, J. and Taylor, S.S. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 11184–11189
18 Coghlan, V.M., Perrino, B.A., Howard, M., Langeberg, L.K., Hicks, J.B., Gallatin, W.M. and Scott, J.D. (1995) Science **267**, 108–112
19 Klauck, T.M., Faux, M.C., Labudda, K., Langeberg, L.K., Jaken, S. and Scott, J.D. (1996) Science **271**, 1589–1592
20 Nauert, J.B., Klauck, T.M., Langeberg, L.K. and Scott, J.D. (1997) Curr. Biol. **7**, 52–62
21 Carnegie, G.K., Smith, F.D., McConnachie, G., Langeberg, L.K. and Scott, J.D. (2004) Mol. Cell **15**, 889–899
22 Schillace, R.V. and Scott, J.D. (1999) Curr. Biol. **9**, 321–324
23 Steen, R.L., Martins, S.B., Tasken, K. and Collas, P. (2000) J. Cell Biol. **150**, 1251–1262
24 Cooper, D.M. (2003) Biochem. J. **375**, 517–529
25 Hanoune, J. and Defer, N. (2001) Annu. Rev. Pharmacol. Toxicol. **41**, 145–174
26 Maurice, D.H., Palmer, D., Tilley, D.G., Dunkerley, H.A., Netherton, S.J., Raymond, D.R., Elbatarny, H.S. and Jimmo, S.L. (2003) Mol. Pharmacol. **64**, 533–546
27 Houslay, M.D. and Adams, D.R. (2003) Biochem. J. **370**, 1–18
28 Dodge, K.L., Khouangsathiene, S., Kapiloff, M.S., Mouton, R., Hill, E.V., Houslay, M.D., Langeberg, L.K. and Scott, J.D. (2001) EMBO J. **20**, 1921–1930
29 Carlisle Michel, J.J., Dodge, K.L., Wong, W., Mayer, N.C., Langeberg, L.K. and Scott, J.D. (2004) Biochem. J. **381**, 587–592
30 Sette, C. and Conti, M. (1996) J. Biol. Chem. **271**, 16526–16534
31 Hoffmann, R., Baillie, G.S., MacKenzie, S.J., Yarwood, S.J. and Houslay, M.D. (1999) EMBO J. **18**, 893–903
32 Dodge-Kafka, K.L., Soughayer, J., Pare, G.C., Carlisle Michel, J.J., Langeberg, L.K., Kapiloff, M.S. and Scott, J.D. (2005) Nature (London) **437**, 574–578
33 Marx, S.O., Reiken, S., Hisamatsu, Y., Gaburjakova, M., Gaburjakova, J., Yang, Y.M., Rosemblit, N. and Marks, A.R. (2001) J. Cell Biol. **153**, 699–708.
34 Lehnart, S.E., Wehrens, X.H., Reiken, S., Warrier, S., Belevych, A.E., Harvey, R.D., Richter, W., Jin, S.L., Conti, M. and Marks, A.R. (2005) Cell **123**, 25–35

Received 16 March 2006

©2006 Biochemical Society