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Minireview

Compartmentalisation of phosphodiesterases and protein kinase A: opposites attract

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Abstract Understanding the molecular organisation of intracellular signalling pathways is a topic of considerable research interest. Since many signalling enzymes are widely distributed and have several substrates, a critical component in signal transduction is the control of specificity. This is achieved, in part by the assembly of multiprotein complexes where clusters of signalling enzymes create focal points to disseminate the intracellular action of many hormones. This is particularly true for the cAMP dependent protein kinase (PKA) that is localised throughout the cell via its association with A-kinase anchoring proteins (AKAPs). Recent data suggest that some AKAPs also interact with phosphodiesterases (PDEs). Compartmentalisation of PDEs not only provides an elegant means to control PKA activation by monitoring the local cAMP flux, but also serves to concentrate and segregate the action of these important regulatory enzymes.

1. Introduction

Environmental stimuli relay vital information throughout the cell via the mobilisation intracellular of signalling pathways. An important mediator of such signals is cyclic adenosine 3′,5′-monophosphate (cAMP) [1,2]. This ubiquitous second messenger of hormone action is generated by adenylyl cyclases at the plasma membrane. These enzymes are activated when agonists bind to their appropriate G-protein coupled receptors (GPCRs) and stimulate the heterotrimeric G-protein, G_s [3]. The newly synthesised cAMP diffuses throughout the cell to sites where it can access its effector proteins. A principle effector is the cAMP-dependent protein kinase holoenzyme (PKA), which, if unregulated, indiscriminately phosphorylates a myriad of substrates [4]. However, PKA activity is tightly regulated by the *R* subunits of the holoenzyme that keep the catalytic subunits in a dormant state [5,6]. In addition to PKA, there are other routes through which cAMP exerts its cellular effects (Fig. 1A). In certain cells, predominantly neuronal in origin, cAMP activates cyclic nucleotide gated ion channels (CNGCs) (Fig. 1A) [7]. More recently, EPACs proteins consisting of a cAMP-binding domain fused to a guanine nucleotide exchange factor (GEF) domain, have been identified as important intracellular receptors for cAMP [8]. This configuration permits cAMP to modulate guanine nucleotide exchange for the small molecular weight GTPase Rap1 [8].

Specificity of cAMP action is facilitated by the three dimensional organisation of signalling proteins within the cell [2,9,10]. Indeed, the notion of localised cAMP action was first proposed in the 1980s, when it was noticed that different G_s-coupled receptors could selectively activate the type I and type II PKA holoenzyme subtypes in cardiac myocytes [11,12]. This led to the idea that 'pools' of PKA could sample localised cAMP gradients [13–15]. The discovery of A-kinase anchoring proteins (AKAPs) has consolidated this hypothesis into the realms of a tangible model that can be experimentally accessed [16–20]. AKAPs not only serve to sequester PKA to distinct subcellular locations, but also to position specific enzymes to respond to increases in local camp concentrations. In parallel, the development of fluorescent probes that visualise cAMP gradients inside cells has contributed greatly to this PKA anchoring hypothesis [13–15,21–23].

In order to control the ebb and flow of cAMP, signal termination mechanisms need to be in place for cellular homeostasis. This critical regulatory process is catalysed by cAMP hydrolysing enzymes known as cyclic nucleotide phosphodiesterase (PDEs) [1,24–27]. The central importance of this gene family is underscored by the plethora PDE subtypes and splice variants that share the same catalytic function, namely hydrolysing cAMP and cGMP [1,23]. Indeed, there is a growing appreciation that PDEs can associate with other proteins, allowing them to be strategically anchored throughout the cell [25,28–30]. Tissue specific expression and compartmentalisation of these evolutionary conserved enzymes allows the tight control of cAMP gradients in all cell types and permits the integration of regulatory inputs from other signalling pathways [25]. Furthermore, the importance of PDEs in engineering compartmentalised cAMP gradients is clearly demonstrated by the therapeutic effectiveness of selective PDE inhibitors as drugs to combat a growing number of human diseases [25,28–32].

Recent studies with PDEs has led to the proposal of a more sophisticated model of compartmentalised cAMP signalling [9,23,33,34]. The concerted actions of adenylyl cyclases and phosphodiesterases create microdomains of cAMP. However,

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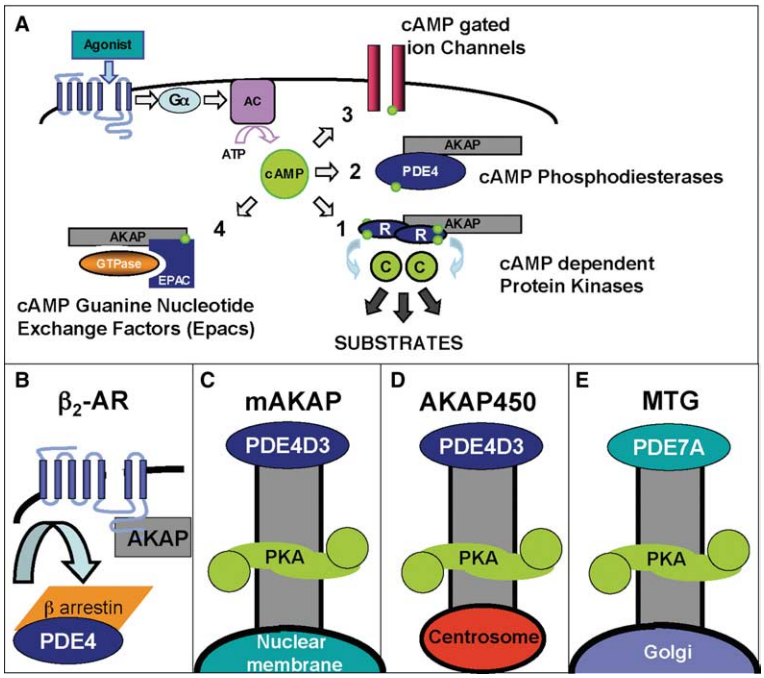

Fig. 1. The various actions of camp. (A) Schematic diagram showing the cAMP signalling pathway. All molecules in the pathway are indicated. The newly generated cAMP can be utilised by (1) the cAMP dependent protein kinase, (2) cAMP phosphodiesterases, (3) cyclic nucleotide gated ion channels and EPACs. (B) The ß andrenergic receptor signalling complex. The transient association of the ßarrestin/PDE4 complex and the association of AKAPs are indicated. (C) The mAKAP signalling complex of PKA and PDE4D3 at the nuclear membrane. (D) The AKAP450 signalling complex of PKA and PDE4D3 at the centrosomes and (E) the MTG–PKA–PDE7A complex at the golgi.

the predominant regulatory event is the hydrolysis of cAMP by compartmentalised phosphodiesterases. High PDE activity will reduce cellular cAMP levels and thus dampen the ability of anchored PKA become active whereas, low or reduced PDE activity will favour PKA activation [35]. Consequently, it would appear to make good signalling sense if PDEs were clustered with anchored pools of PKA [33,36,37]. Thus a signalling network, which brings enzymes that are activated by cAMP together with those that degrade cAMP, is an ingenious way to control both spatial and temporal PKA signalling [9]. AKAPs also provide an independent level of signalling specificity, as they are known to sequester other regulatory enzymes such as phosphatases, protein kinases and G protein effector proteins [10,38]. Such diverse signalling complexes foster cross-talk between signalling pathways and provides a mechanism to expand the range of processes influenced by cAMP. Here, we review the latest evidence for the compartmentalisation of PDEs with AKAPs and speculate on to the functional consequences of these protein–protein interactions.

2. cAMP phosphodiesterases

Dogma mandates that increased cAMP triggers PKA phosphorylation events [10]. However, there is increasing evidence that de-repression of signal termination mechanisms such as phosphodiesterase activity is just as important in controlling cellular function. Consequently over 40 different PDE species encoded by 11 different genes are capable of degrading cAMP [1,24,26–28,32,39]. Enzymes from the cAMP-specific phosphodiesterase 4 family (PDE4s) have attracted particular interest as they provide therapeutic targets in a number of disease states. Accordingly, selective PDE4 inhibitors can be used to treat asthma, chronic obstructive pulmonary disease and rheumatoid arthritis [25,28–32]. The PDE4 family are encoded by 4 genes (A, B, C and D) that generate approximately 20 different isoforms [25,31]. An emerging theme in PDE4 action is that individual isoforms appear to be restricted to defined intracellular microenvironments [25,28–30]. Compartmentalisation of PDE4 is mediated by their unique N terminal domains which provide the "postcode or zipcode" for cellular localisation. First demonstrated for PDE4A1 isoform, subcellular targeting often proceeds through protein–lipid or protein–protein interactions [40]. This isoform derives its targeting from a pair of adjacent tryptophan residues in the N-terminal region of PDE4A1, which form a bilayer insertion module called TAPAS-1 [41]. The insertion of the TAPAS-1 module is triggered upon an appropriate elevation in the free intracellular calcium concentration. TAPAS-1 also shows selectivity for interaction with phosphatidic acid, which appears to be due to the need for charge neutralisation, at the bilayer/aqueous interface. PDE4A4/5 for example, is associated with ruffles at the cell periphery by a proline-rich region, which enables binding to the SH3 domain found in various Src family tyrosyl protein kinases [42–44]; PDE4D3 interacts with a variety of proteins including the scaffold protein myomegalin that sequesters it to the golgi/centrosomal region [45] and PDE4A5 can form a complex with the immunophilin XAP2 [46].

These targeting motifs are analogous to the localisation signals found on AKAPs and type I phosphatase (PP1) targeting subunits [6,9,10,25,47]. The neuronal anchoring protein AKAP79/150 is tethered to the inner face of the plasma membrane by basic and hydrophobic motifs [48]; the amino terminal residues of AKAP18 are lipid modified to permit

membrane attachment [49,50] and gravin, also called AKAP250, is believed to be targeted to membranes via an amino terminal myristoyl group [51–53]. Likewise phosphatase targeting subunits tether the catalytic subunit of PP1 to membranes and cellular organelles [54]. Thus phosphodiesterases, kinases and phosphatases utilise common mechanisms to control their subcellular location.

3. A-kinase anchoring proteins

Anchoring proteins and targeting subunits provide a molecular framework that orients protein kinases and phosphatases towards selected substrates. Prototypic examples of these "signal-directing molecules" are AKAPs that sustain multi-component signalling complexes of the cAMP dependent protein kinase (PKA) and G proteins and other enzymes [55]. These protein–protein interactions not only focus PKA toward certain substrates, but also spatially segregate parallel signalling units. Each AKAP contains a conserved amphipathic helix that binds to the *R* subunit dimer with high affinity and targeting domains that direct the PKA–AKAP complex to specific subcellular compartments [19,56–60]. Another important role for AKAPs is to place PKA in the proximity of enzymes such as phosphatases that terminate signalling events [61–64].

A shared property of several AKAPs is to position enzymes in microenvironments where they can respond to upstream signals. Clearly, there are potential advantages of anchoring PKA in close proximity to primary transduction elements such as G-protein coupled receptors and the cAMP synthesis machinery. As will be discussed in the next section, two anchoring proteins, gravin/AKAP250 and AKAP79/150 maintain kinase complexes that bind to the ß$_2$-Adrenergic (ß2-AR) receptor [65,66]. However, other classes of G proteins have also been implicated in the channelling of signals through AKAP complexes, although not necessarily via cAMP dependant mechanisms [67]. Scar/WAVE-1 is a member of the Wiskott Aldrich syndrome family of scaffolding proteins that binds PKA, the Abl tyrosine kinase, and the Arp2/3 complex a group of seven proteins that control actin remodelling [68–71]. The dynamic assembly of this complex at sites of lamellapodial extension occurs in response to growth factor signals that activate low molecular weight GTPase Rac [69]. Consequently, Scar/WAVE may direct PKA and Abl toward cytoskeletal substrates, and synchronise cell movement by ensuring efficient transmission of Rac mediated signals to the actin remodelling machinery. Analogous AKAP signalling networks participate in the formation of actin stress fibres. AKAP-Lbc, a splice variant of the Lbc oncogene encodes a molecule that anchors PKA and functions as a Rho selective guanine nucleotide exchange factor [72]. Application of lysophosphatydic acid or selective expression of Gα12 enhances cellular AKAP-Lbc activation and leads to the formation of actin stress fibres in fibroblasts [72–75]. This provides an example where the spatial organisation of heterotrimeric and small molecular weight G proteins may involve interactions with the same AKAP. Finally, certain unconventional modes of signalling to PKA may also be governed by G protein recruitment to AKAP signalling complexes. For example, the testis specific anchoring protein, AKAP110, has been reported to interact with the heterotrimeric G protein subunit Gα13 that activates AKAP110-associated PKA via a cAMP-independent mechanism [76]. Each of these examples underscore the notion that AKAP signalling complexes can respond to G protein signalling events in a variety of manners.

4. PDE4 and anchored PKA are involved in β_2-adrenergic receptor (β_2AR) desensitisation

A new facet of PDE4 signal termination was uncovered when it was shown that the β_2-adrenergic receptor (β_2AR) associated scaffold proteins, βarrestins, form stable complexes with the PDE4D5 isoform [77]. βarrestin1 and βarrestin2 are essential for the desensitisation of many G-protein coupled receptor (GPCR) types, including the prototypic β_2AR [77]. Upon agonist challenge, βarrestins translocate to the plasma membrane where they bind the phosphorylated β_2AR. This action physically uncouples signalling to G_s, which, in turn, suppresses adenylyl cyclase activity and the concomitant production of cAMP. Thus translocation of an active PDE4D5/βarrestin complex results in the localised degradation of the cAMP at its source of production (Fig. 1B) [77]. It has been shown that PDE4D5 interacts preferentially with βarrestin via its unique N-terminal region [46,78]. Overexpression of the catalytically inactive PDE4D5 displaces the endogenous enzyme from its anchor site, thereby preventing the suppression of cAMP synthesis [78]. These findings are intriguing as it has been know for some time that PKA phosphorylation of the β_2AR promotes a reprogramming of its coupling specificity from G_s to G_i, activating a mitogenic signalling cascade (MAP kinase) mediated by the tyrosine kinase Src [79]. Apparently, the βarrestin associated pool of PDE4D5 serves a dual role in β_2AR desensitisation; βarrestin uncouples the ability of the β_2AR to activate G_s, and the phosphodiesterase reduces cAMP levels to impair the ability of PKA to phosphorylate the receptor [78,80]. Together, these events efficiently reset β_2-adrenergic receptor responsive cAMP synthesis machinery for another round of agonist challenge (Fig. 1B).

Interestingly, PKA is brought within the vicinity of βarrestin via its association with AKAP79/150 or AKAP250 [81]. However, there are mechanistic differences in the action of both anchoring proteins as AKAP79 constitutively anchors PKA to the receptor whereas phosphorylated gravin binds the β_2AR in an agonist dependent manner [52]. Nonetheless, interaction between AKAPs and the β_2AR is, seemingly, essential for cAMP mediated phosphorylation of the β_2AR following agonist stimulation. Such exquisite orchestration of spatial and temporal signalling is clearly a consequence of AKAP-bound PKA and the dynamic targeting of PDE4s to the β_2AR [25]. These findings emphasise the diversity of downstream signalling that can be achieved through the co-localisation of PKA with cAMP-specific PDEs.

Recent gene targeting experiments in mice have further confirmed the role of PDE4D isoforms switching of β_2AR signalling from G_s to G_i in cardiac myocytes [82]. These authors have shown that stimulation of β_2AR profoundly activates a cAMP signalling pathway that controls heart function. This is mediated by PKA phosphorylation of proteins in the sarcoplasmic reticulum and muscle fibres, which trigger increased contractility and accelerated relaxation. Two AKAPs expressed in heart tissue seem to coordinate theses phosphorylation events [83]. AKAP18 binds and regulates L-type calcium

channels at the plasma membranes [49,84]. The heart muscle specific isoform of mAKAP, which is specifically induced under hypertrophic conditions and targets PKA, amongst other places, to sarcoplasmic reticulum where it phosphorylates the ryanodine receptor [85,86]. Intriguingly, mAKAP also binds to PDE4D3 [33]. The mAKAP/PKA/PDE4D3 complex is another tightly regulated signalling unit where the action of PDE activity under basal conditions reduces local cAMP levels, rendering the anchored PKA inactive (Fig. 1C). Following hormonal stimulation, cAMP levels rises and swamp PDE action, allowing PKA activation and phosphorylation of localised substrates, including PDE4D3 itself [33,37].

All PDE4 long isoforms share a common PKA site (Serine 53 using PDE4D3 nomenclature) in the upstream conserved region 1 (UCR1) [87]. PKA phosphorylation of this residue results in an activation of the enzyme [87]. Yet, PDE4D3 is unique in that it has an additional PKA site (Serine 13) within its unique N-terminal region [88,89]. Phosphorylation of this site increases the affinity of PDE4D3 for mAKAP, enhancing PDE recruitment and preventing its release from the locality [37]. These attributes allow a specialised negative feedback control where, upon elevation of cAMP levels, PKA phosphorylation promotes both activation and further recruitment of activated PDE4D3 to the mAKAP complex (Fig. 1C). The net effect of this is to lower localised cAMP levels, which facilitate the deactivation of AKAP bound PKA [9]. Since the type 2 phosphatase (PP2A) is also anchored to mAKAP it is possible that this enzyme functions to re-set the system by dephosphorylating PDE4D3 [86]. Variations on this theme occur at other sites within the cell. PDE4D3 also interacts with AKAP450 at the centrosome in Sertoli cells, which may generate a rapid feedback mechanism similar to the one identified for mAKAP. This may confer tight regulation of the phosphorylation status of centrosomal proteins involved in the regulation of microtubule stability (Fig. 1D) [10,36]. The identification of additional AKAP/PKA/PDE complexes will no doubt highlight the symbiotic relationship between cAMP phosphodiesterases and cAMP dependent protein kinases.

5. AKAP complexes and PDES as regulators of T cell signalling

It is known that cAMP has a potent anti-inflammatory action in T cells that blocks pro-inflammatory mediators [90,91]. Also, stimulation of the T cell receptor (TCR) leads to transient elevation in local cAMP concentrations and the recruitment of type I PKA holoenzyme into an ''immune synapse'' that forms around the T cell cap [92,93]. Taken together, these observations point to a tightly controlled cAMP signalling pathway in T cells, which must rely on the correct compartmentalisation of PDEs and PKA [90,91].

T cell receptor (TCR) engagement is a well-studied signalling process that involves the assembly of a multiprotein complex within cholesterol- and sphingolipid-rich lipid rafts. One consequence is activation of the tyrosine kinase Lck and its phosphorylation of ITAM domains within the TCR. In contrast, PKA is the upstream enzyme in a protein kinase cascade, formed in lipid rafts, that counterbalances this process by inhibiting Lck. Active PKA phosphorylates the C-terminal Src kinase, CSK which, in turn, phosphorylates and inhibits Lck [34,94,95]. Therefore, the ebb and flow of cAMP could have profound effects on the activity status of T cells. High cAMP would suppress T cells whereas low cAMP would favour their activation. Not surprisingly a role for T cell PDEs has been explored. Although these cells express all classes of PDE, the therapeutic utility of PDE4 selective inhibitors as anti-inflammatory agents has been most clearly shown in cell-based and animal models. Thus the interplay between anchored PKA and localised PDEs may be an important factor that contributes to the suppression of T cell function. This view is supported by a recent characterisation of PKA–AKAP–PDE interactions in Jurkat T-cells (Fig. 1E) [96]. These studies have shown that the cAMP-specific PDE7A isoform associates AKAP149 at the nuclear membrane, the nuclear anchoring protein AKAP95 and novel golgi associated AKAPs derived from the myeloid translocation gene (MTG) [96]. This is the first report of a PDE isoform other than PDE4D3 that can complex with AKAPs and is consistent with evidence that PDE7 is induced in T-cells stimulated with CD3/CD28 [97]. However, PDE7A knockout animals showed normal T functions such as CD3/CD28 mediated generation of IL2 [98]. This suggests that role anchored PKA–PDE7 complexes may be subtle or required for some compensatory process in T-cells (Fig. 1E). Nonetheless evidence that another PDE type interacts with AKAP-PKA complexes points to the utility of this as a mechanism to tightly control compartmentalised cAMP signalling.

As previously stated initiation of TCR signals occurs in lipid rafts and involves the recruitment of a variety of signalling proteins. It is well known that the co-receptor, CD28 augments these signalling events, albeit via an unknown mechanism [90]. However, recent data suggest that PDE4 isoforms play a crucial role in this process as PDE4A4, PDE4B2 and PDE4D1/2 have all been detected in lipid rafts. It would appear that their recruitment to lipid rafts involves association with βarrestin. CD-28 stimulated translocation of a βarrestin-PDE4 complex to lipid rafts should decrease the local cAMP concentrations. This will dampen PKA activity and prevent Csk mediated phosphorylation of Lck [34]. The net result would be sustained Lck activity and T prolonged cell activation. Although further testing of this model is ongoing it is tempting to speculate that the recruitment of PKA and PDEs to lipid rafts for their opposing roles in T cell function is consolidated by their association with the same AKAP.

6. Conclusion

As should be evident from this paper, the spatial and temporal regulation of cAMP gradients is greatly facilitated by the co-localisation of the PKA holoenzyme with phosphodiesterases in anchored signalling complexes. This configuration serves several purposes. First of all, the clustering of a tonically active signal termination enzymes serves to suppress local cAMP levels. This is important for cellular homeostasis as it keeps the cAMP dependent kinase in the dormant state. Secondly, the close proximity of PKA and PDEs in the mAKAP, AKAP450 or MTG complexes insures that fluctuations in cAMP levels are transient [9,25]. This creates a temporal mode to the regulation of PKA activation. Thirdly, the phosphodiesterase is the key regulatory enzyme in the complex. Any changes in its activity state will amplify fluctuations in the local concentrations of cAMP. Thus PKA–AKAP–PDE complexes

provide an efficient means to tightly regulate and focus cAMP action to very specific cellular microenvironments. This is exactly what you would want to have in order to be able to utilise a seemingly ubiquitous second messenger such as cAMP in a very specific manner. No doubt additional PKA–AKAP–PDE complexes will be discovered in the future.

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