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REVIEW

Anchored cAMP signaling: Onward and upward – A short history of compartmentalized cAMP signal transduction

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Abstract

Intracellular signal transduction pathways require a high degree of spatial and temporal resolution in order to deliver the appropriate outputs. Specific signaling mediated by the ubiquitous second messenger cAMP and its effector, the cAMP-dependent protein kinase (PKA), is governed by the spatial organization of different pathway components by A-kinase anchoring proteins (AKAPs). This review discusses the history and future of anchored cAMP signaling pathways.

Keywords: cAMP-dependent protein kinase; PKA; AKAP; Phosphodiesterase; Signal transduction; Compartmentalization

Contents

Anchored cAMP signaling: how did we get here?

Intracellular signal transduction pathways require a high degree of spatial and temporal resolution in order to deliver the appropriate outputs. Specific signaling mediated by the ubiquitous second messenger cyclic adenosine 3′,5′-monophosphate (cAMP) and its effector, the cAMP-dependent protein kinase (PKA), is governed by the spatial organization of different pathway components by A-kinase anchoring proteins (AKAPs). This review discusses the history and future of anchored cAMP signaling pathways.

Early days

Protein phosphorylation is the principle means to reversibly modify protein function. Although work over the last 30 years has yielded great insights into the organization and function of the eukaryotic kinome

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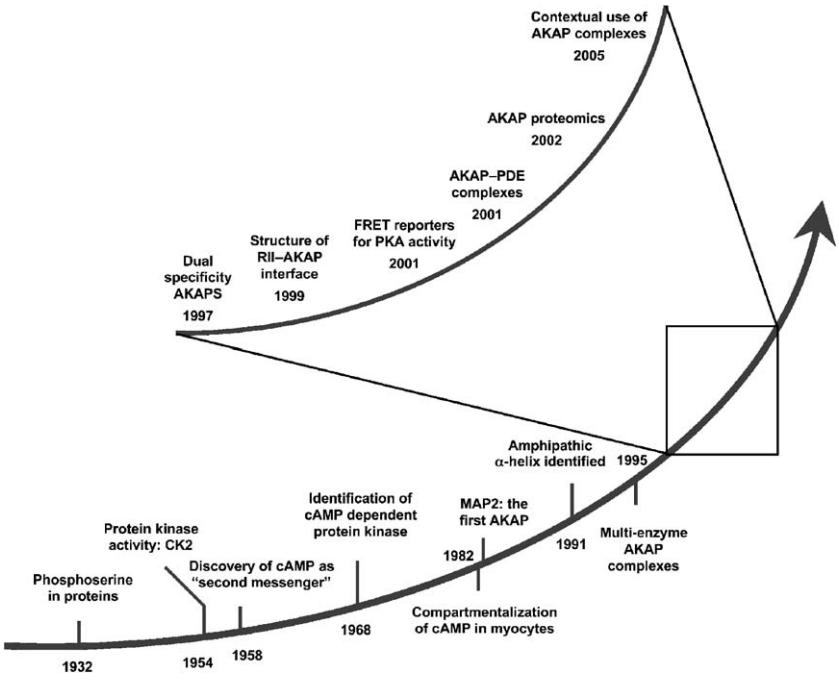

Fig. 1. A timeline for advancements in studies of compartmentalized cAMP signaling and AKAP function. See accompanying text for details and references.

(Manning et al., 2002), initial progress on protein phosphorylation was labored at the beginning of the 20th century. Although phosphoproteins were first reported in 1905, it was the identification of phosphoserine in proteins that laid the groundwork for future discoveries (Lipmann and Levene, 1932) (Fig. 1). In 1954, Burnett and Kennedy reported the discovery of an enzyme in rat liver mitochondrial preparations that catalyzed the transfer of phosphate from ATP to a protein substrate, casein (Burnett and Kennedy, 1954). This enzyme was later found to be casein kinase 2 (CK2) (Dobrowolska et al., 1999).

A major breakthrough that catapulted protein phosphorylation into the forefront of biomedical research was the isolation of the cAMP-dependent PKA in 1968 in Krebs' laboratory (Walsh et al., 1968). This discovery was based on the pioneering work in Carl and Gerty Cori's lab during the 1940s on glycogen metabolism and the regulation of phosphorylase activity, as well as the later discovery by Rall and Sutherland of the second messenger cAMP as a key mediator of hormonal control of glycogen metabolism (Cori et al., 1939; Cori and Green, 1943; Green and Cori, 1943; Rall and Sutherland, 1958; Sutherland and Rall, 1958). The identification of PKA finally allowed a complete view of the hormone-mediated, cAMP-dependent signal transduction cascade from activation of adenyl cyclase to the downstream effect of glycogen breakdown. Later work from Gilman, Rodbell, Lefkowitz and many others filled in the pathway from cell surface hormone receptors to adenyl cyclase activation through the ''coupling proteins'' that we now know as the heterotrimeric G-proteins (Benovic et al., 1986; Lohse et al., 1990; Rodbell et al., 1971a, b; Ross and Gilman, 1977a, b). The regulation of cAMP signaling was shown to be even more complex with the characterization of the cAMP-metabolizing phosphodiesterase (PDE) enzymes by Beavo, Houslay, Conti and others (Beavo et al., 1971; Conti et al., 1984; Marchmont and Houslay, 1980; Soderling and Beavo, 2000). Also key to the understanding of protein phosphorylation as a reversible regulatory mechanism was the discovery and characterization of serine/threonine phosphatases that remove the phosphates from kinase substrates (Ingebritsen and Cohen, 1983; Khandelwal et al., 1976; Nimmo and Cohen, 1978).

Compartmentalized cAMP signaling

It was soon discovered that PKA could be activated by many hormone receptors in addition to those for epinephrine and glucagon. This led to the understanding that PKA phosphorylates multiple substrate proteins and set the foundation for our understanding of the many physiological roles of cAMP-PKA signaling (Greengard, 1978; Kuo and Greengard, 1969).

Biochemical analysis of PKA by Corbin and others (Corbin et al., 1975; Reimann et al., 1971) showed that the kinase exists as a holoenzyme consisting of two regulatory subunits (R) and two catalytic (C) subunits

that harbor the phosphotransferase activity. The regulatory subunits dimerize through an N-terminal dimerization domain and each R subunit binds one C subunit. Binding of cAMP to the regulatory subunits causes release of active catalytic subunits. There are two classes of PKA holoenzyme, type I and type II, which differ in their R subunit component (Corbin and Keely, 1977; Skalhegg and Tasken, 2000). A major step in understanding PKA function as well as general features of the kinase superfamily came with cloning of cDNAs encoding the catalytic subunit (Uhler et al., 1986) and subsequent structural characterization of the catalytic subunit bound to an inhibitor peptide by Susan Taylor and colleagues (Johnson et al., 2001; Sowadski et al., 1985). PKA has since become one of the main models for biophysical analysis of kinase properties. The kinase domain contains distinct, evolutionarily conserved hallmarks that can be identified across almost all subgroups of kinases (Taylor et al., 1993).

As more studies showed a role for cAMP and PKA in responses to hormones and neurotransmitters, it became clear that even in the same tissue, elevations in cAMP and PKA activity by different agonists led to different physiological outputs. These effects were especially prominent in cardiac tissue, where isoproterenol activation of β-adrenergic receptors leads to glycogen metabolism and contraction (mediated in part by phosphorylation of troponin I), but PGE_1, while activating PKA, does not induce phosphorylation of these substrates. Brunton and coworkers showed in 1983 that adrenergic stimulation selectively activated a pool of PKA associated with the particulate fraction of isolated cardiomyocytes, while PGE_1 predominantly activated cytosolic PKA (Buxton and Brunton, 1983). These data and others led to the concept of compartmentalization of PKA signaling inside cells such that different receptors are only able to access certain pools of PKA holoenzyme (Steinberg and Brunton, 2001).

The molecular basis for compartmentalization of cAMP signaling was the subject of much speculation during the early 1980s. There were many examples of cAMP-dependent signaling that could not be explained by the notion of random diffusion of cascade components and contemporary models of "collision coupling" between receptors and effectors. At the same time, cell biologists had determined that the interior of most cells was not an aqueous "sac" of protein and solutes, but instead was highly ordered by lattices of various cytoskeletal networks that were continuous with intracellular organelles and the plasma membrane. It was on this background that Vallee and co-workers demonstrated that type II PKA co-purifies with microtubules and is bound to the microtubule-associated protein MAP2 (Theurkauf and Vallee, 1982). Though it wasn't appreciated at the time, this was the first example of an AKAP.

In 1984, Lohmann et al. reported the first iteration of the RII overlay technique, which is still widely used today and is fundamental to our studies of AKAPs and compartmentalized cAMP signaling. Here they probed nitrocellulose membranes containing protein samples from different sources with purified RII subunit and used an antibody-^{125}I-protein A complex to identify bound RII. Using this technique, they identified a number of RII-binding proteins from bovine brain and heart (Lohmann et al., 1984). Rubin and coworkers soon used this method to characterize a bovine brain protein, P75, known to co-purify with RII and subsequently to identify and clone this protein, as well as its rat ortholog P150 (now known as AKAP79 and AKAP150, respectively) (Bregman et al., 1989; Hirsch et al., 1992; Leiser et al., 1986).

Meanwhile, our lab was studying the molecular basis for RII subunit dimerization and discovered that the N-terminus of RII is responsible not only for dimerization, but also for interaction with other proteins that were becoming known as "anchoring proteins" or AKAPs (Carr et al., 1991; Scott et al., 1990). We then adapted the RII overlay technique to screen phage cDNA libraries for RII-binding proteins, and were able to identify and clone a number of novel AKAPs, including Ht31 (now known as AKAP-Lbc) (Carr et al., 1992). The RII-binding domain of Ht31 is an 18-amino-acid region that can be used as a peptide to disrupt RII interactions with most, if not all, AKAPs. The importance of AKAPs in cellular function was elegantly demonstrated by perfusion of this Ht31 peptide into cultured hippocampal neurons, where it uncoupled PKA from AMPA-type glutamate receptors, leading to time-dependent run-down of AMPA-mediated sodium currents and a decrease in post-synaptic AMPA receptor responsiveness (Rosenmund et al., 1994). The cloning of multiple AKAPs also allowed the comparison of their primary sequences as well as their subcellular distribution. From these studies, two important properties of AKAPs emerged. First, (almost) all AKAPs bind to RII through a well conserved amphipathic α-helical motif. Second, each AKAP has a unique distribution within a given cell type that is usually conferred by an identifiable targeting motif.

The modern age

In 1995, the field of anchored/compartmentalized cAMP signaling became significantly broader with the discovery of AKAP-mediated signaling complexes that contain multiple signal transduction enzymes. AKAP79, a well characterized AKAP at that point, was found to also bind to the phosphatase calcineurin/PP2B (Coghlan et al., 1995). These data were of great interest because for the first time an enzyme that opposes the action of

PKA by removing phosphate groups from substrates was molecularly linked in the same protein complex. Furthermore, AKAP79 also binds to a second kinase, PKC, which can act on an overlapping but distinct set of substrates (Klauck et al., 1996). This theme of AKAP anchoring of multiple signaling enzymes has become so prevalent that we now think of AKAPs as nodes of signal integration and signal distribution that act at distinct intracellular sites.

Originally it was thought that anchoring was limited to type II PKA holoenzyme. This was partially due to earlier biochemical data that suggested type II PKA was predominantly particulate (and therefore thought to be bound to insoluble cellular structures) and that type I PKA was cytosolic. This notion was furthered by the failure to identify binding proteins by overlay analysis using labeled RI subunit. Again, novel technical developments opened new avenues of exploration of anchored cAMP signaling. Taylor and co-workers used the yeast two-hybrid system to screen for proteins that interact with an oncoprotein formed by the fusion of the N-terminus of RIα and the C-terminal domain of the Ret receptor tyrosine kinase. Several of the identified interactors bound to the RI region of this protein, suggesting that they act as RI-anchoring proteins. These proteins also bound RII and were named D-AKAP-1 and -2 for "dual specificity" AKAP (Huang et al., 1997a, b). Several more RI-binding AKAPs have been identified in recent years (Gronholm et al., 2003; Li et al., 2001). It is currently unclear whether these AKAPs selectively bind one or the other R subunits in vivo, or whether, perhaps, there is a regulated switching between RI and RII binding that has distinct functional consequences. Although much was known about the interaction between the α-helical region on AKAPs and the N-terminal region of RII, it was 1999 before the first atomic structure of this binding interface was solved (Newlon et al., 1999). The NMR solution structure of the N-terminus of RII in complex with the Ht31 peptide revealed that the hydrophobic face of the amphipathic helix on the AKAP fits into a groove formed by the N-terminal dimerization domain of RII (Newlon et al., 1999).

Functional compartmentalization of cAMP signaling was hypothesized to be due to spatially restricted access of PKA (and other effectors) to cAMP. However, this aspect was not fully appreciated until Marco Conti and Miles Houslay showed that compartmentalized cAMP PDEs are necessary to tailor individual cAMP responses. This concept was extended by work published from two groups in 2001. Almost simultaneously, Dodge et al. and Tasken et al. reported that PDE4D isoforms are associated with two distinct AKAP complexes (Dodge et al., 2001; Tasken et al., 2001). In cardiomyocytes, mAKAP anchors PKA to the perinuclear region in a complex that also contains PDE4D3 and components of the ERK5 MAP kinase cascade. PKA phosphorylation of PDE4D3 stimulates its activity, creating a highly efficient and localized mechanism for generating pulses of cAMP and PKA activity. Phosphorylation of PDE4D3 by ERKs inhibits its activity and this module allows crosstalk between cAMP and growth factor/cytokine signaling pathways (Dodge et al., 2001). Recently this complex expanded to include another cAMP-dependent enzyme, the Rap1 GTPase exchange factor (GEF), Epac (Dodge-Kafka et al., 2005). PDE4D3 also binds to centrosomally localized AKAP450 in testicular Sertoli cells, where it presumably controls pulsatile cAMP signaling in a similar manner, possibly in a cell cycle-dependent fashion (Tasken et al., 2001). Currently there are a number of labs investigating the role of AKAP-anchored PDEs in generating microdomains for specific cAMP signaling events, and this field is set to expand rapidly in the near future.

Building on early experiments that attempted to localize cAMP in cells and tissues immunocytochemically, Tsien and co-workers developed a method for monitoring cAMP production in living cells based on fluorescence resonance energy transfer (FRET) between fluorophore-labeled R and C subunits microinjected into cells (Adams et al., 1991). This technique was substantially improved with the advent and widespread use of genetically encoded fluorescent proteins. Zaccolo and co-workers developed an FRET-based reporter for cAMP in which CFP-R subunit and YFP-C subunit could be introduced into various cell types by transfection of corresponding cDNAs (Zaccolo et al., 2000). This reporter was successfully used to measure microdomains of cAMP production along sarcomeric Z lines in cardiomyocytes in response to adrenergic stimulation (Zaccolo and Pozzan, 2002). Zhang et al. modified this method by creating an FRET-based reporter for PKA activation (instead of cAMP production) based on phosphorylation of a consensus PKA site. Activation of PKA and phosphorylation of the reporter induces peptide binding to a phospho-serine/threonine-binding FHA domain. This association brings CFP and YFP moieties into close proximity, increasing the intramolecular FRET (Zhang et al., 2001, 2005). This reporter is now being used in multiple cell types to measure the dynamics of cAMP-PKA signaling (Dodge-Kafka et al., 2005; Zhang et al., 2005).

Proteomic techniques for analyzing protein complexes have recently been used to characterize AKAP complexes. WAVE-1, a neuronal AKAP involved in cytoskeletal remodeling, is found in a large protein complex containing WRP, Abi-1 and -2, Sra-1, Nap125 and HSPC300 (Eden et al., 2002; Soderling et al., 2002; Steffen et al., 2004). This complex translates growth factor receptor activation of Rac1 to Arp2/3-mediated changes in actin filament branching. Alternatively,

Danial et al. recently showed that WAVE-1 is also present on hepatocyte mitochondria where it binds BAD and hexokinase to regulate glucose metabolism (Danial et al., 2003). These data suggest the existence of tissue-specific AKAP complexes containing distinct sets of regulatory and effector proteins that coordinate different signaling networks in distinct cell types. Continuing modification of these techniques should improve our ability to identify dynamic changes in protein complexes by mass spectrometry (Blagoev et al., 2004; de Hoog and Mann, 2004).

AKAPs can also provide the cell with a variety of options for signaling. Hoshi et al. recently showed that the same complex can be used to achieve very different responses based on the input to the system (Hoshi et al., 2005). As mentioned previously, AMPA-type glutamate receptors are found in a complex of AKAP79 and its associated proteins, PKA, PKC and calcineurin/PP2B, in hippocampal and other CNS neurons. Here, glutamate receptors are basally phosphorylated by the anchored PKA and this phosphorylation is in favorable equilibrium with dephosphorylation by PP2B. Glutamate receptor activation causes increases in local Ca^{2+} concentrations such that PP2B is activated and the equilibrium is shifted to dephosphorylation and down-regulation of the channel. In sympathetic SCG neurons, KCNQ2 potassium channels are also coupled to AKAP79. Stimulation of muscarinic acetylcholine receptors in these neurons causes PKC activation, phosphorylation of KCNQ2 and inhibition of K^+ currents. This effect does not seem to involve PKA or PP2B, as siRNA knockdown of the AKAP can be rescued with a short fragment of AKAP79 that contains only the PKC-anchoring domain and the targeting domain (Hoshi et al., 2005). These studies demonstrate that while an AKAP may have multiple signaling enzymes associated with it, the signal output will depend on which of those enzymes are utilized following a given stimulus.

Where are we going?

The experiments discussed above are leading the field into a new era of research on anchored and compartmentalized signaling pathways where both temporal and spatial aspects of a given signaling cascade can be measured simultaneously and in real time. One example of where we would like to proceed with these types of analysis is with AKAP-Lbc. Originally identified as Ht31, AKAP-Lbc is a large, multifunctional anchoring protein that binds PKA and contains a DH-PH module that functions as a GEF for the small GTPase RhoA. Activation of Gα12-coupled G protein-coupled receptors (GPCRs) increases AKAP-Lbc-mediated GTP loading of RhoA and leads to changes in cytoskeletal architecture and other Rho-dependent processes (Diviani et al., 2001). One of these events is the activation of protein kinase D (PKD) through a PKC-dependent mechanism (Yuan et al., 2001). Our recent data shows that AKAP-Lbc also binds to PKD as well as its upstream activator PKCη. Association of these two kinases with AKAP-Lbc enhances activation of PKD through PKC-dependent signals. Furthermore, AKAP-Lbc provides a point for signal integration in which PKA phosphorylation of the PKD-binding region causes release of PKD from the scaffold (Fig. 2). Although the mechanism is not clear, PKD release

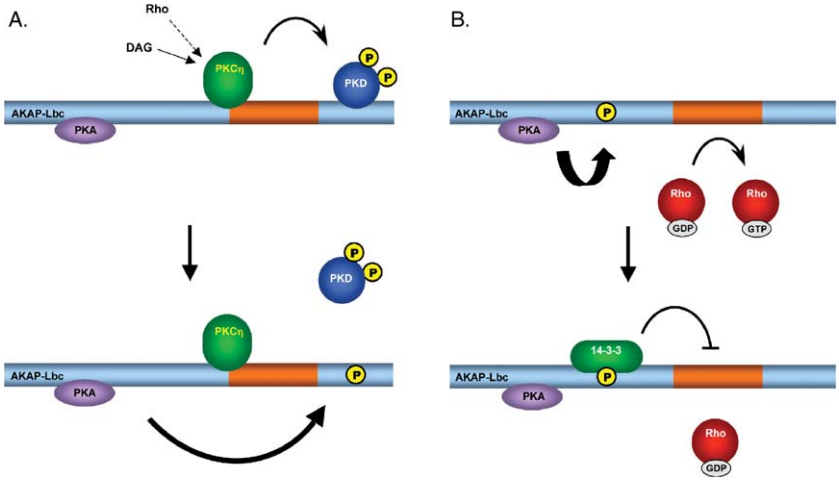

Fig. 2. AKAP-Lbc is a multifunctional AKAP and RhoGEF: (A) AKAP-Lbc binds multiple kinases and provides a platform for activation of protein kinase D (PKD). Signaling to PKC through Rho and diacylglycerol (DAG) leads to activation of PKD. PKA phosphorylation of AKAP-Lbc near the PKD-binding site dissociates PKD from the scaffold which may allow enhanced access to its upstream activating kinase, PKCη. (B) PKA also phosphorylates AKAP-Lbc in the central domain, creating a 14-3-3-binding site. 14-3-3 binding to AKAP-Lbc suppresses its RhoGEF activity.

from the complex facilitates its phosphorylation and activation by PKCη (Carnegie et al., 2004).

AKAP-Lbc is also phosphorylated by the anchored PKA on a central serine residue that forms a phospho-dependent binding site for 14-3-3 proteins. 14-3-3 proteins are small, dimeric modules that bind to specific phosphorylated serine- or threonine-containing motifs (Dougherty and Morrison, 2004; Muslin et al., 1996). Association of 14-3-3 with AKAP-Lbc inhibits its RhoGEF activity (Diviani et al., 2004; Jin et al., 2004). This inhibition may feedback into the PKD activation pathway to suppress further Rho-mediated PKD activation. We are now beginning to investigate these signaling events using real-time FRET analysis with different components of the AKAP-Lbc complex during different agonist stimulations. Coupled with FRET-based reporter assays for PKA, PKC and PKD activation, these imaging experiments will give us quantitative spatial and temporal information about signaling through the AKAP-Lbc complex. It is also interesting, given what we know about other AKAP complexes, to speculate that phosphatase or PDE activity may be associated with the AKAP-Lbc complex. These enzymes are good candidates for regulators of AKAP-Lbc phosphorylation and protein–protein interactions within the complex.

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