Regulation of ion channels by cAMP-dependent protein kinase and A-kinase anchoring proteins

Peter C Gray*, John D Scott† and William A Catterall‡

Subcellular targeting of the cAMP-dependent protein kinase is achieved, in part, through association with A-kinase anchoring proteins (AKAPs). Recent evidence suggests that specific AKAPs direct the kinase to submembrane sites to facilitate phosphorylation and modulation of a variety of ion channels. A new membrane-anchored AKAP targets cAMP-dependent protein kinase to calcium channels and enhances their regulation in multiple cell types.

Addresses
*‡Department of Pharmacology, University of Washington, Seattle, Washington 98195-7280, USA
‡e-mail: wcatt@u.washington.edu
†Howard Hughes Medical Institute, Vollum Institute, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, Oregon 97201-3098; e-mail: scott@ohsu.edu

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Abbreviations

AKAP	A-kinase anchoring protein
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
C	catalytic subunit
K_{Ca}	calcium-activated potassium channel
PKA	cAMP-dependent protein kinase
PKI	heat stable protein inhibitor of cAMP-dependent protein kinase
R	regulatory subunit
RI	type I regulatory subunit
RII	type II regulatory subunit
RGS	regulator of G protein signaling

Introduction

Multiple regulatory mechanisms ensure that signals transduced by the second messenger cAMP are precisely directed to specific sites inside the cell. Accumulation of cAMP at these sites permits the activation of cAMP-dependent protein kinase (PKA), which, in turn, controls cellular events by the phosphorylation of substrate proteins. Although the mechanisms of PKA activation are well understood, it is now clear that additional regulatory mechanisms are in place to control where the kinase becomes activated. This is particularly important in the central nervous system, where PKA phosphorylation has been implicated in the control of such diverse physiological processes as modulation of ion channels, transcription of neuronal genes, and long-term potentiation of synaptic transmission — a molecular model for learning and memory [1]. Therefore, selective activation of compartmentalized pools of PKA has emerged as an important mechanism to ensure that particular PKA substrates become rapidly and selectively phosphorylated in response to individual extracellular stimuli. At the molecular level, compartmentalization of PKA is maintained by A-kinase anchoring proteins (AKAPs) [2]. The AKAPs represent a family of functionally related proteins that bind the regulatory subunits of the kinase and serve as adapter proteins that maintain the PKA holoenzyme at specific subcellular locations. Although AKAP interactions with RII regulatory subunits of PKA have been extensively characterized, less is known about how AKAPs influence cAMP-responsive events or the functional consequences of PKA anchoring *in vivo*.

The purpose of this review is to briefly discuss our current understanding of the protein–protein interactions that govern PKA anchoring and to focus on recent studies that suggest that an important function of the AKAPs is to direct PKA to submembrane sites where it is available to phosphorylate and modulate the activity of ion channels.

Overlapping modes of PKA regulation

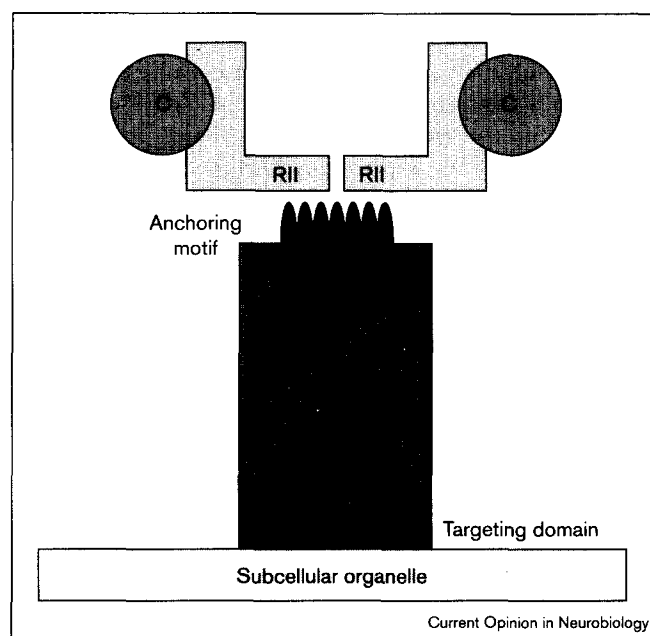
The PKA holoenzyme is a heterotetramer consisting of a regulatory (R) subunit dimer that maintains two catalytic (C) subunits in a dormant state [3,4]. The binding of cAMP to tandem sites in each R subunit alleviates autoinhibitory contacts in the holoenzyme and releases both C subunits. The active kinase is then free to phosphorylate substrates on serine or threonine residues that are presented in a consensus sequence context of Arg–Arg–X–Ser/Thr (where 'X' is any amino acid). Given the frequency of these sites in cellular proteins it is not surprising that overlapping regulatory processes are in place to restrict the action of the kinase. Cellular cAMP levels are fixed by a balance of adenylyl cyclase and phosphodiesterase activities, and signal-terminating mechanisms such as desensitization of adenylyl cyclase or localized activation of phosphodiesterases guarantee further reduction of the compartmentalized second messenger pools. Also, the R subunits are expressed in excess over C subunits favoring rapid reformation of the holoenzyme when cAMP levels return to the basal state and the ubiquitous heat-stable inhibitor PKI may well serve as a fail safe device that sequesters free C subunit and mediates export of the kinase from the nucleus. Other levels of regulation include the differential sensitivity of R subunit isoforms (RI and RII) to cAMP, which may partially explain the different subcellular locations of the type I and type II holoenzymes inside cells.

Properties of AKAPs

Subcellular fractionation studies first reported the differential compartmentalization of the type I and type II PKA holoenzymes whereas later reports identified the association of RII with the neuronal cytoskeleton and

microtubules. The first RII-binding proteins were identified as proteins that co-purified with RII after affinity chromatography on cAMP-Sepharose, including the microtubule-associated protein MAP2 and a component of the postsynaptic densities called AKAP79/150. Since then, numerous anchoring proteins have been identified or cloned by techniques that exploit the ability of AKAPs to bind RII when denatured and immobilized to nitrocellulose [2,5]. The assimilated information from these studies is presented in a model (Figure 1) that highlights the essential features of AKAPs. Each anchoring protein contains two classes of binding sites: a conserved 'anchoring motif', which binds the R subunit of PKA, and 'targeting domains', which direct the subcellular localization of the PKA/AKAP complex through association with structural proteins, membranes or cellular organelles.

Figure 1



Structural and functional domains of AKAPs. Each anchoring protein contains a conserved anchoring motif responsible for high affinity interaction with the R subunit and a unique targeting domain that tethers the PKA/AKAP complex to subcellular structures.

Anchoring motifs

Mapping experiments have established the determinants on RII required for interaction with AKAPs. Dimerization is a prerequisite for AKAP binding; however, distinct localization and dimerization determinants are contained within the first 45 residues of each RII protomer. These findings have been extended by the NMR structure of the RIIα 1–45 dimer [6]. Both RII protomers form an antiparallel four-helix bundle with the principal AKAP-binding determinants located within an α helix between residues 8–14, while a second helix between residues 28 and 42 maintains the dimerization contact. Structural analysis of an RII/AKAP peptide complex suggests that numerous sidechains within the AKAP binding region of RII contact the anchoring protein and may explain the high affinity of RII/AKAP interactions [6].

Although there is little sequence similarity between RII-binding regions on AKAPs, mutagenesis studies initially performed on the human thyroid anchoring protein Ht31 have demonstrated a requirement for intact secondary structure within the anchoring site. These findings were consolidated by the synthesis of peptides encompassing a predicted helical region in Ht31 that bind RII with nanomolar affinity [7,8]. The nanomolar affinity of these interactions has important consequences for the analysis of the intracellular localization of PKA as these reagents can be used to disrupt the intracellular location of PKA inside cells. Recently, dual function anchoring proteins, called dAKAPs, have been identified that also bind the type I regulatory subunit (RI) [9•,10•]. Binding involves a common anchoring site, although the affinity of the RI/AKAP interaction is approximately 1000-fold lower than that for RII [9•,10•,11]. Nevertheless, studies of RIIα knockout mice indicate that type I PKA anchoring may be important for the regulation of calcium channels under conditions where the intracellular concentration of RII is limiting [11].

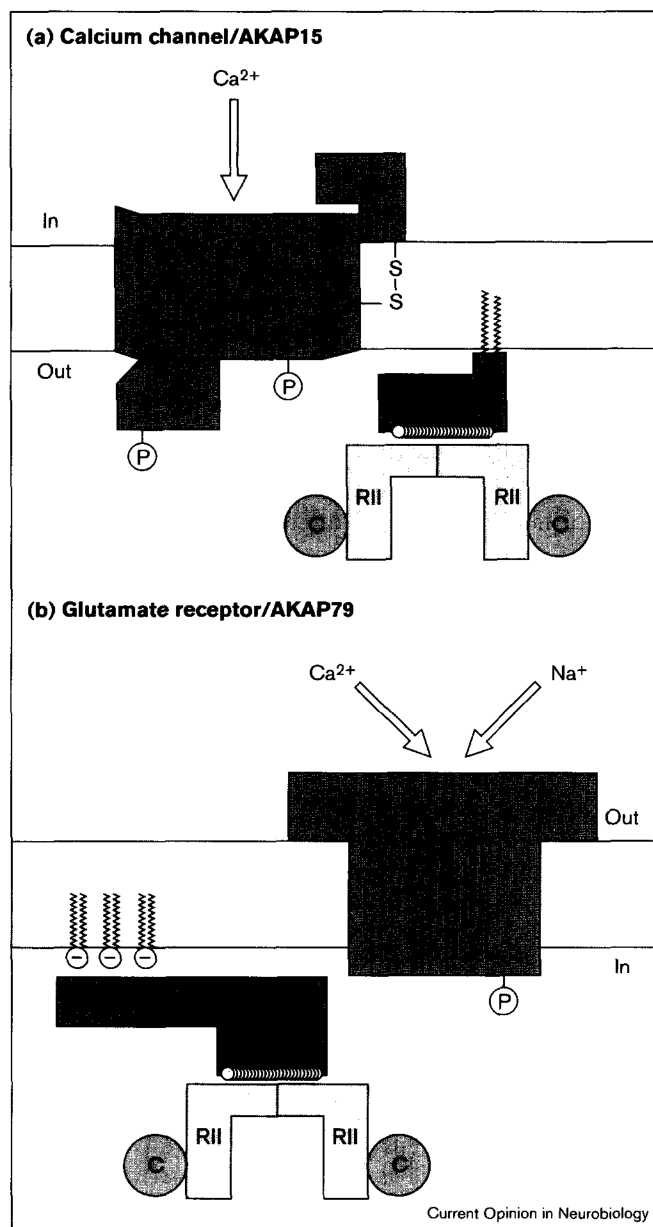
Targeting domains

The targeting domain is an essential feature of each AKAP as it confers specificity by tethering the anchored PKA complex to particular organelles. Although subcellular fractionation and immunochemical techniques have identified AKAPs localized to a variety of subcellular structures and organelles, only a few AKAP targeting sequences have been well characterized. Mitochondrial targeting of dAKAP-1 or its murine homolog sAKAP84 is mediated by an amino-terminal sequence motif [9•]. Dendritic targeting of AKAP75 or its human homolog AKAP79 involves two or more noncontiguous stretches of basic residues that bind acidic phospholipids to provide electrostatic interactions with the plasma membrane (Figure 2; [12,13•]). Myristoylation and dual palmitoylation signals facilitate the constitutive membrane attachment of the low molecular weight kinase anchoring protein AKAP15 (Figure 2; [14•,15•]), also called AKAP18 [16•], to the plasma membrane. Knowledge of these targeting sequences now allows the development of reagents that can redirect PKA to specific subcellular compartments.

AKAPs and glutamate receptors

The first indication of effects of AKAPs on ion channels came from studies of synaptic transmission between cultured hippocampal neurons mediated by glutamate acting at fast AMPA/kainate-type glutamate receptors [17]. Phosphorylation by PKA is required to maintain the function of the AMPA/kainate type of glutamate receptors in hippocampal neurons [18,19]. A peptide containing the RII-binding domain of the human thyroid AKAP, Ht31 [8], disrupted PKA-mediated effects on the

Figure 2



Association of PKA and ion channels through the membrane anchoring of AKAPs. **(a)** The skeletal muscle calcium channel is illustrated as a complex of α1, α2δ, β, and γ subunits. α2 and δ subunits are linked by a disulfide bond. AKAP15 is anchored to the cell membrane by myristoyl and palmitoyl anchors and binds PKA through an amphipathic helix that interacts with the subunit interface of the RII regulatory subunit dimer. Close localization of PKA to the calcium channel enhances phosphorylation (P) of its α1 and β subunits. **(b)** The AMPA/kainate-type glutamate receptor is illustrated as a transmembrane ion channel. AKAP79 is anchored to the membrane via binding to acidic phospholipids and binds PKA through an amphipathic helix that interacts with the subunit interface of the RII regulatory subunit dimer. Close localization of PKA to the glutamate receptor enhances its phosphorylation (P).

basal glutamate receptor activity in these neurons. In whole-cell recordings, 88% of kainate-induced currents through glutamate receptors were retained after 25 min of intracellular dialysis with control medium containing ATP in the recording pipette. Inhibition of PKA with the peptide inhibitor PKI(5-24) increased run down of the kainate-induced current so that only 62% of the current was retained. Similarly, the anchoring inhibitor peptide Ht31 increased run down so that only 65% of the kainate-induced current remained at 25 min. Thus, phosphorylation by anchored PKA was responsible for retention of 23% of the glutamate receptor activity under the conditions of whole-cell voltage clamp, implicating AKAPs in the maintenance of glutamate receptor activity. AKAP79 [7] is the primary candidate for this effect on glutamate receptors as it is localized in the postsynaptic densities of hippocampal synapses (Figure 2; [13•]).

AKAP15 and skeletal muscle L-type calcium channels

In skeletal muscle transverse tubules, L-type calcium channels mediate increases in intracellular calcium through two distinct mechanisms [20]. A fast mechanism involving the direct coupling of the L-type calcium channel to ryanodine-sensitive calcium release channels in the sarcoplasmic reticulum is thought to provide the calcium required for contraction, whereas a slower mechanism in which calcium enters directly through the L-type calcium channel plays an important role in replenishing intracellular calcium stores and controlling contractile force. Skeletal muscle L-type calcium channels mediate slowly activated, long-lasting calcium currents that are stimulated by PKA following the application of β-adrenergic agonists [21,22]. Calcium channel activity is also increased in response to single long depolarizing pulses or brief high-frequency depolarizing stimuli that mimic trains of action potentials [23,24]. This stimulation-dependent enhancement of calcium currents requires PKA activity [23], leading to the hypothesis that voltage-activated, state-dependent phosphorylation rapidly regulates calcium channel activity.

Voltage-dependent potentiation of skeletal muscle calcium channels by PKA occurs within 50 ms and increases calcium currents approximately 20-fold at threshold test potentials [23]. In view of this rapid response time, it seemed that likely that PKA is concentrated near the channel. Consistent with this idea, the anchoring inhibitor peptide Ht31 essentially completely inhibits the PKA-dependent potentiation of calcium channels in skeletal muscle cells [25], indicating that anchored PKA is required for a 20-fold increase in calcium current. Surprisingly, similar results are obtained for skeletal muscle calcium channels expressed in a heterologous cell line [26], suggesting that AKAPs present in nonexcitable cells can also anchor PKA near expressed calcium channels. These results indicate that an AKAP-mediated association of PKA with the skeletal muscle calcium channel is required for rapid voltage-dependent potentiation.

Biochemical studies showed that AKAP15, a 15 kDa PKA-binding protein, co-purifies and co-immunoprecipi-

tates with the skeletal muscle calcium channel complex [14•,15•], and this is the only AKAP detected in membrane fractions containing skeletal muscle calcium channels [15•]. This AKAP is an 81-residue protein containing an amphipathic helix that binds PKA and targets it to calcium channels by membrane association through amino-terminal myristoyl and palmitoyl lipid anchors (Figure 2; [15•]). AKAP15 is co-localized with PKA and calcium channels in the triad junctions of skeletal muscle [15•]. Inhibition of AKAP15 with a competing peptide completely blocks PKA-dependent potentiation [15•], supporting an essential role for this novel AKAP in mediating the PKA anchoring required for potentiation of skeletal muscle calcium channels and regulation of contractile force (Figure 2).

AKAPs and cardiac L-type calcium channels

Like skeletal muscle calcium channels, the activity of cardiac L-type calcium channels is enhanced by PKA. Increases of three- to tenfold in peak calcium currents are observed in dissociated cardiac myocytes during stimulation of β-adrenergic receptors or treatment with PKA activators [27], and further increases in calcium current can be induced by voltage-dependent potentiation [28,29]. The anchoring inhibitor peptide Ht31 inhibits the PKA-dependent regulation of the calcium channel in acutely dissociated cardiac myocytes, indicating that this regulation requires an AKAP [30•]. Co-transfection of AKAP79 or AKAP15/18 with calcium channels can increase the PKA regulation of calcium currents by 18% to 38% in heterologous cells [16•,30•]. Because AKAP15/18 is expressed in heart and AKAP79 is not, AKAP15/18 is a strong candidate for anchoring the endogenous PKA involved in calcium channel regulation in cardiac myocytes [16•], as it is in skeletal muscle myocytes [14•,15•] (Figure 2).

AKAPs and calcium-activated potassium channels

AKAP-mediated PKA targeting has also been implicated in the regulation of smooth muscle calcium-activated potassium (K_{Ca}) channels [31]. K_{Ca} channel currents recorded from inside-out patches pulled from tracheal myocytes were augmented approximately threefold in response to ATP by an endogenous kinase activity. This kinase was blocked by the PKA inhibitor peptide PKI, implicating PKA as the endogenous kinase. The anchoring inhibitor peptide Ht31 blocked the stimulation caused by added ATP whereas the mutant proline-substituted peptide Ht31-P had no effect. These results are consistent with those observed for glutamate receptors and calcium channels and provide an additional example of ion channel regulation by anchored PKA.

Conclusions and perspectives

The anchoring of PKA near ion channels through interactions with AKAPs is an emerging theme in ion channel regulation. The targeting of PKA through AKAP protein domains that bind to acidic phospholipids or through lipid anchors that are inserted in the plasma membrane enhances ion channel regulation by increasing the proximity of enzyme and substrate. The results to date show that PKA anchored through interaction with AKAPs is required to prevent run down of the activity of glutamate receptors in synapses, to allow rapid potentiation of skeletal muscle calcium channels by PKA and depolarization, and to support PKA-dependent up-regulation of cardiac calcium channel activity. For the three systems studied to date, large (up to 20-fold) effects of AKAP anchoring are observed for the potentiation of skeletal muscle calcium channel activity on the millisecond timescale [15•,25,26], whereas smaller (18%–38%) increases in the activity of glutamate receptors and cardiac calcium channels have been observed as a result of slower phosphorylation processes [16•,17,30•]. This comparison suggests the hypothesis that PKA anchoring via AKAPs is most important for rapid regulatory processes on the millisecond timescale where diffusion of free kinase to its substrate would be prohibitively slow and variable, but that significant effects are also observed for slower regulatory processes on the second and minute timescale where local organization of the kinase and its substrate by AKAPs allows optimum regulation. Studies of further examples of AKAP-dependent regulation of ion channels will reveal whether this relationship holds for a wider range of experimental systems.

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