

Adenylyl Cyclase-AKAP Complexes: The Next Dimension in cAMP Signaling

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Abbreviations: AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; AR, adrenergic receptor; cAMP, cyclic adenosine 3'-5' monophosphate; CaN, calcineurin; CAM, calmodulin; EPAC, exchange protein activated by cAMP; FRET, fluorescence resonance energy transfer; GPCR, G protein-coupled receptor; PAM, protein activator of myc; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C; PP1, protein phosphatase 1; RGS, regulator of G protein signaling.

Abstract

The formation of multiprotein complexes is a repeated theme in biology ranging from the regulation of the ERK and cAMP signaling pathways to the formation of postsynaptic density complexes or tight junctions. A-kinase anchoring proteins (AKAPs) are well known for their ability to scaffold protein kinase A and components upstream and downstream of cAMP production, including G protein-coupled receptors, cAMP-dependent Rap-exchange factors, and phosphodiesterases. Recently, specific adenylyl cyclase (AC) isoforms have also been identified as components of AKAP complexes, namely AKAP79, Yotiao, and mAKAP. In this review, we summarize recent evidence for AC-AKAP complexes and requirements for compartmentalization of cAMP signaling. The ability of AKAPs to assemble intricate feedback loops to control spatio-temporal aspects of cAMP signaling adds yet another dimension to the classical cAMP pathway.

The generation of cAMP and subsequent activation of protein kinase A (PKA) is one of the best-understood signal transduction pathways. However it remains unclear how the soluble second messenger cAMP achieves any type of subcellular or molecular specificity. PKA phosphorylates a broad range of substrates but somehow manages to mediate precise phosphorylation events at specific sites within the cell. For example, stimulation of β 1 adrenergic receptors (AR), β 2AR, or prostaglandin E1 receptors have clearly distinguishable effects on cardiac myocytes, despite each being coupled to adenylyl cyclase (AC) (Buxton and Brunton, 1983; Steinberg and Brunton, 2001). The follicle-stimulating hormone and luteinizing hormone also appear to use the same intracellular intermediates but activate different sets of genes in granulosa cells (Conti, 2002). Measurements of cAMP using cyclic nucleotide gated channels (CNG or HCN) or FRET reporters based on PKA or EPAC have provided direct evidence for limited cAMP diffusion throughout the cell (reviewed in (Berrera et al., 2008; Fischmeister et al., 2006). Where cAMP is produced is also critical, as cAMP generated at the plasma membrane versus cytosol can have opposite effects on endothelial barrier function (Sayner et al., 2006). However, until recently it was not clear how a restricted pool of cAMP generated by AC was specifically targeted to a select subset of effectors to give rise to distinct physiological outcomes. This problem is solved by tethering AC to complexes containing cAMP effectors and downstream targets. This review will focus on recent evidence that signalosomes formed by A-kinase anchoring proteins (AKAPs) help to coordinate cAMP synthesis and downstream signaling by assembling AC-AKAP complexes.

Cyclic AMP Synthesis: Mammalian Adenylyl Cyclase Isoforms

In higher eukaryotes, two basic families of adenylyl cyclase exist: the G protein-regulated transmembrane adenylyl cyclase isoforms and a soluble adenylyl cyclase. The latter AC is regulated by bicarbonate and calcium and is insensitive to forskolin or activated G α s (Kamenetsky et al., 2006). The topology of transmembrane ACs consists of a variable intracellular N-terminus (NT) and two large cytoplasmic domains separated by two membrane spanning domains (6 TM's each) (Sadana and Dessauer, 2009). The transmembrane class of ACs is generally considered the target of most hormone-sensitive cAMP control.

Regulation of AC isoforms - All nine membrane-bound AC isoforms are activated by GTP-bound G α s and the plant diterpene, forskolin, with the exception of AC9. The only other feature shared by all isoforms is the inhibition by adenosine analogs known as P-site inhibitors (Dessauer et al., 1999). Additional regulation among isoforms differs widely as shown in Figure 1 (reviewed in (Sadana and Dessauer, 2009)). Therefore, one emerging question is how all of the regulation of AC is coordinated? Additionally, how does a particular AC isoform generate a pool of cAMP that is positioned near appropriate downstream effectors, given that most cells contain multiple AC isoforms? Possibilities include 1) localization of ACs, AC regulators, and downstream effectors of cAMP to specific regions of the plasma membrane (i.e. lipid rafts) and 2) formation of higher order complexes to facilitate interactions and provide specificity.

Lipid raft localization of ACs – Compartmentalization of signaling can be

achieved by localization of select ACs and other signaling molecules in lipid rafts. These highly dynamic structures are rich in cholesterol and sphingolipids, a subset of which also contains the protein caveolin. Lipid rafts are increasingly appreciated for their role in organizing a wide-range of signal-transduction cascades. For example, a growing number of G protein coupled receptors (GPCRs), ion channels, and receptor tyrosine kinases are localized to lipid rafts (Insel and Patel, 2009). All of the calcium sensitive ACs (AC 1, 3, 5, 6, and 8) but not the Ca^{2+} insensitive ACs (AC 2, 4, 7, and 9) are also localized to lipid raft structures, independent of caveolin expression (reviewed in (Cooper and Crossthwaite, 2006)). Destruction of lipid rafts by extraction of cholesterol disrupts regulation of AC6 and AC8 by capacitative calcium entry (Fagan et al., 2000; Smith et al., 2002), suggesting these structures are required for at least some forms of regulation. In addition, AC6 shows differential coupling to GPCRs that co-segregate with AC6 in lipid rafts, enhancing β AR and prostacyclin receptor signaling, but not prostaglandin EP2 or adenosine receptors upon overexpression of AC6 (Bundey and Insel, 2006; Liu et al., 2008). In addition to GPCRs, many other components of the cAMP pathway including G proteins, PDEs, phosphatases, PKC, PKA, and cyclic nucleotide gated channels can be found within lipid rafts.

Complexes of AC with G proteins, GPCRs, and effectors - Although lipid raft localization can, in part, explain the selective coupling of GPCRs to AC, the differential ability of ACs to regulate downstream pathways such as PKA, ERK, cell doubling times, or cAMP-mediated cytoskeletal re-organization clearly requires additional mechanisms to generate specificity (Gros et al., 2006). The existence of signaling complexes

involving AC was first proposed in 1988 for AC and G α · β γ (Levitzki, 1988). Since that time BRET studies suggest that stable complexes occur between G β γ subunits and AC2 (Rebois et al., 2006) and these complexes are likely assembled before insertion into the plasma membrane (Dupre et al., 2007). There is also evidence for AC complexes containing GPCRs (Lavine et al., 2002) or downstream signaling components. Co-immunoprecipitation of AC1 and ERK1/2 explains the selective activation of ERK signaling in HEK293 cells by AC1 but not AC2, 5, or 6 (Gros et al., 2006). ACs can form even larger complexes containing β AR, G proteins, PKA, phosphatases, and L-type Ca²⁺ channels to possibly facilitate highly spatially restricted signaling in neurons (Davare et al., 2001). The question becomes how these mega-complexes are assembled. The remainder of this review will focus on AKAPs that scaffold components of the cAMP signaling pathways, including AC, to achieve temporal and spatial specificity.

AKAPs Anchor PKA and Other Components of cAMP Signaling

Compartmentalization of PKA signaling is accomplished by means of A-kinase anchoring proteins (AKAPs). Since their initial discovery in 1982 (Theurkauf and Vallee, 1982), over 50 AKAPs have been identified that are highly divergent with the exception of a signature PKA regulatory subunit docking motif (reviewed in (McConnachie et al., 2006; Wong and Scott, 2004)). AKAPs are localized to numerous cellular sites including the plasma membrane (AKAP79, Yotiao, AKAP18, and Gravin; (Fraser et al.,

1998; Grove and Bruchey, 2001; Klauck et al., 1996; Lin et al., 1998)) as well as Golgi, centrosome, nucleus, mitochondria, and cytosol (reviewed in (Feliciello et al., 2001)). Those AKAPs that are located at the PM use a number of different strategies for docking, including myristoylation (AKAP18 and Gravin; (Fraser et al., 1998; Lin et al., 1996)), polybasic regions (AKAP79 and Gravin; (Streb and Miano, 2005; Tao et al., 2006)), or as yet unknown mechanisms (Yotiao). One of the important features of AKAP complexes is the intricate feedback loops that are assembled to control temporal aspects of cAMP signaling. For example, the assembly of protein kinases and phosphatases or PKA and PDEs ensures only local fluctuations in signal output and the possibility for oscillating pulses of activity (reviewed in (Smith et al., 2006a)).

Evidence for AC-AKAP Interactions

Our recent evidence suggests that membrane-bound ACs are pre-coupled to AKAP complexes to potentially generate a local pool of cAMP. Since AKAPs target PKA to specific substrates and targeting is an important aspect of PKA's ability to sense cAMP gradients, it makes sense that AC might also be in close proximity to these same molecules.

AKAP79/150: The initial concept of AC-AKAP interactions was tested with AKAP79/150 (AKAP150 is the rat ortholog of human AKAP79; also known as AKAP5). It is highly expressed in neurons, particularly postsynaptic densities of excitatory synapses, and at lower levels in heart and other non-neuronal tissues (Bregman et al., 1989; Carr et al., 1992; Sarkar et al., 1984). AKAP79 anchors calcineurin (CaN, also

known as protein phosphatase 2B), PKC, β 2AR, and β 1AR, in addition to AMPA-type glutamate receptors (via PSD-95 and SAP97), L-type Ca^{2+} channels, M-type K^{+} channels, and the TRPV1 channel (capsaicin receptor) (see Fig 2A; (Dodge and Scott, 2000; Jeske et al., 2008; Schnizler et al., 2008; Smith et al., 2006a; Zhang et al., 2008)). Due to its multivalent nature, AKAP79 coordinates different enzyme combinations to modulate the activity of anchored channels, tailoring regulation to individual effectors (Hoshi et al., 2005). This provides the ideal scaffold for regulating AC activity as well.

Substantial biochemical evidence using forskolin-agarose or immunoprecipitations to purify AC and associated AKAP79/150 and/or PKA activity from rat brain extracts cells supports a complex between AC5/6 and AKAP79 (Bauman et al., 2006). Reciprocal immunoprecipitations of AKAP79 contain significant AC5 activity. Direct phosphorylation of AC5 or AC6 by PKA inhibits cAMP synthesis (Chen et al., 1997; Iwami et al., 1995). PKA anchoring facilitates the preferential phosphorylation of AC5/6 in rat brain extracts and AKAP79 expression inhibits AC5 activity in a PKA-dependent manner. Both the inhibition and phosphorylation of AC5 by AKAP79-anchored PKA are abolished upon mutation of the PKA phosphorylation site on AC5.

The PKA-dependent feed-back regulation of cAMP synthesis that is assembled on AKAP79 was demonstrated in cells using two different reporters (cyclic nucleotide gated channels and the PKA AKAR2 FRET reporter; (Bauman et al., 2006)). Knockdown of endogenous AKAP79 increased cAMP levels and sustained PKA activity upon stimulation with β 2-AR agonists. Rescue of AKAP79 depletion by the rat ortholog AKAP150 was dependent on the presence of the PKA anchoring site. Thus PKA phosphorylation of AKAP79-bound AC and/or β 2-AR provides negative feedback on

AC5 and generates a burst of cAMP synthesis. This is a fairly rapid process, as PKA activity returned to baseline within 4 min of stimulation when AKAP79 was present. Additional AC5 regulation may also occur through anchored PKC and/or calcineurin. Thus, AKAP79/150 clearly shapes the dynamics of cAMP accumulation. While AKAP79 does not alter the initial rate of PKA activation, it facilitates the subsequent inhibition of AC5/6 by PKA and ultimately the decay of PKA activity.

Yotiao: Another plasma membrane associated AKAP is Yotiao (smallest splice variant of AKAP9 family; 250 kD). It is found in both brain and heart, accumulating near neuromuscular junctions (Lin et al., 1998; Schmidt et al., 1999). Yotiao anchors PKA, protein phosphatase 1 (PP1), PDE4D3, the NR1 subunit of the NMDA receptor, IP3 receptor, and the K⁺ channel subunit KCNQ1 which is responsible for I_{Ks} currents in the heart (Fig 2B; (Lin et al., 1998; Marx et al., 2002; Terrenoire et al., 2009; Tu et al., 2004; Westphal et al., 1999)). Yotiao is required for sympathetic regulation of I_{Ks} currents which shape the duration of action potentials (Chen and Kass, 2006). Inherited mutations in KCNQ1 and/or Yotiao that disrupt binding to one another are associated with long QT syndrome, a disease characterized by cardiac arrhythmias and sudden death (Chen and Kass, 2006; Chen et al., 2007); emphasizing again the requirement for assembled complexes in the temporal regulation of PKA activity. Yotiao also brings together opposing regulators to control downstream effectors. For example, PKA phosphorylation of NR1 potentiates NMDA receptor activation (Tingley et al., 1997), while anchored PP1 reduces channel activity (Westphal et al., 1999).

The tight control of PKA activity described above strongly suggests that AC must also be part of this complex. Immunoprecipitation of Yotiao from brain and heart

identified significant associated AC activity (Piggott et al., 2008). Yotiao can associate with AC isoforms 1, 2, 3, and 9, but not 4, 5, and 6. Yotiao binds directly to AC2, as assessed by binding assays using purified fragments of the two proteins. Expression of Yotiao inhibited the activity of AC 2 and 3, but not AC 1 or 9; serving purely as a scaffold for these latter isoforms under the stimulatory conditions tested. The mechanism for inhibition of AC 2 and 3 is unknown, as these isoforms have no reported sensitivity to PKA. However, it is unlikely to be a consequence of direct interaction with Yotiao, but rather due to regulatory proteins recruited to the scaffolding protein. The assembly of both AC and PDE forms another feedback loop to tightly control cAMP-dependent PKA activity (Piggott et al., 2008; Terrenoire et al., 2009).

mAKAP β : As discussed above, not all AKAPs are localized to the plasma membrane. The cardiac splice variant of muscle AKAP (mAKAP β) is anchored to the nuclear envelope by the membrane spanning protein, nesprin, and is found at lower levels in the sarcoplasmic reticulum of cardiac myocytes (Kapiloff et al., 1999; McCartney et al., 1995; Pare et al., 2005b; Ruehr et al., 2003; Schulze et al., 2003). mAKAP β anchors a finely tuned series of feedback loops to regulate three cAMP-binding proteins, PKA, EPAC (a cAMP-dependent Rap exchange factor), and PDE4D3 (Fig. 2C; (Bauman et al., 2007; Dodge-Kafka and Kapiloff, 2006)). By additionally anchoring calcineurin and components of the ERK pathway, mAKAP β complexes respond to several classes of intracellular receptors. mAKAP β expression at the nuclear envelope is required for cytokine-induced hypertrophy, which is sensitive to EPAC activation by cAMP (Dodge-Kafka et al., 2005). In addition, mAKAP β is required

for full induction of cardiac hypertrophy and the activation of calcineurin/NFAT transcription by beta-adrenergic agonists (Pare et al., 2005a). Thus mAKAP β integrates cAMP signaling with that of calcium and MAP kinases.

Despite the intracellular location of mAKAP β , AC activity strongly associates with mAKAP β in heart and isolated cardiac myocytes (Kapiloff et al., 2009). Four different Abs against mAKAP β , or its nuclear envelope tether nesprin, immunoprecipitate significant AC activity in heart. mAKAP β associates with AC5 and AC2, but surprisingly not AC6 or AC1. The predominant complex in heart is mAKAP β -AC5, as mAKAP β -associated AC activity is completely absent in AC5 knock-out hearts. AC5 directly interacts with aa 275-340 of mAKAP β , a region that does not overlap with binding sites for other known mAKAP-associated proteins. Similar to the regulation of ACs by other AKAPs, mAKAP β inhibits AC5, but not AC2 activity. This inhibition is lost upon deletion of the PKA anchoring site on mAKAP β , consistent with a PKA-dependent mechanism of inhibition previously observed for AKAP79 (Bauman et al., 2006).

In the cardiac myocyte, the transverse tubular system consists of invaginations within the plasma membrane that bring it adjacent to the sarcoplasmic reticulum, which is contiguous with the outer nuclear membrane. The model presented in Fig 2C suggests that AC5, located on transverse-tubules or the plasma membrane (Gao et al., 1997), interacts with mAKAP β on the nuclear envelope when these structures are close in space. This is perhaps the simplest explanation for the organization of this complex. However, there are also reports of AC activity in nuclear membrane preparations, which is supported by immunocytochemistry of AC5 on the nuclear envelope of cardiac

myocytes (Belcheva et al., 1995; Boivin et al., 2006). In addition, numerous GPCRs are also targeted to the perinuclear region including α - and β -adrenergic receptors, angiotensin 2 type I receptors, endothelin receptors, metabotropic glutamate receptors, and prostaglandin receptors (reviewed in (Boivin et al., 2008)). What remains unclear is the orientation of these receptors and/or AC components. If receptors maintain the topology found within the ER, then the C-terminal tail containing G-protein interaction sites would face the cytoplasm (reviewed in (Boivin et al., 2008)). Presumably, the same would be true for AC5. However the physiological relevance of this localization is unknown, particularly in terms of the activation of GPCRs and ACs on the nuclear membrane by membrane-impermeable agonists such as catacholamines.

What Dictates Specificity for AC-AKAPs?

Each AKAP appears to bind a unique subset of AC isoforms. There are few common threads among the ACs recognized by Yotiao and/or mAKAP. Yotiao bound AC 1, 2, 3, and 9, each display very different regulatory patterns (Fig. 1; (Piggott et al., 2008)). Since the C1 and C2 domains of ACs are highly conserved and form the catalytic site, the N terminus is the most logical binding site for obtaining specificity. This region is highly variable and could serve to differentiate AC isoforms. Both Yotiao and mAKAP β interact with the N-terminus of AC2 and AC5, respectively, providing a means for Yotiao to selectively interact with AC2 over the closely related isoform AC4, or mAKAP β to bind AC5 versus AC6 (Kapiloff et al., 2009; Piggott et al., 2008). However,

additional domains of AC (C1 and C2) participate in interactions between AC5 and mAKAP β (Kapiloff et al., 2009). Thus interactions with the N-termini likely dictate the observed specificity among AC isoforms but clear differences in the mechanism of binding exists.

A general “AC binding motif” on AKAPs has also not been identified. This is due in part to the limited sequence homology between AKAPs. Although the AC binding domain has been identified on both Yotiao (for AC2; 808-957) and mAKAP (for AC5; 245-275), no sequence similarity exists between these regions, nor with AKAP79. Different AKAPs appear to use different mechanisms to interact with the same AC isoform, since the mAKAP binding site for AC5 cannot compete for AKAP79 interactions with AC5 (Kapiloff et al., 2009). In addition, it is clear that different ACs interact with different regions on the same AKAP. For example, the N-terminus of AC2 effectively competes for Yotiao-AC2 binding and inhibition but not for Yotiao-AC3 inhibition, indicating unique binding sites for the two ACs (Piggott et al., 2008). With clearly distinct AC binding domains on Yotiao, the question arises whether multiple ACs can bind at once. Although steric hindrance may be an issue, this is certainly a possibility that cannot be ruled out. In fact, there are reports of homo- and hetero-dimerization of AC isoforms that might be facilitated by AKAP interactions (reviewed in (Willoughby and Cooper, 2007)).

Physiological Relevance for AC-AKAP Complexes

AKAP79-AC5/6 – PKA-anchoring to AKAP79 plays an important role in hippocampal LTP, phosphorylation of L-type Ca^{2+} channels, and TrpV1 regulation, as deletion of the PKA binding site on AKAP79/150 (AKAP150 Δ 36) resulted in a significant disruption of these functions (Lu et al., 2007; Lu et al., 2008; Schnizler et al., 2008). Deletion of the entire AKAP79 scaffold results in mislocalization of PKA in neurons, altered AMPA receptor modulation, reductions in memory retention, defects in motor coordination and strength, resistance to muscarinic induced seizures, and protection against angiotensin II-induced hypertension (Navedo et al., 2008; Tunquist et al., 2008). Thus, although AKAP79 has no catalytic activity of its own, it is required to facilitate the coordinated regulation of many physiological events.

Several of the AKAP79 phenotypes have similarities with knockouts of AC5 or AC6. For example, both AKAP150 Δ 36 mice and AC5 knockouts show reduced inflammatory thermal hypersensitivity in response to prostaglandin E2 or formalin, respectively (Kim et al., 2007; Schnizler et al., 2008). These effects correlate with the loss of TRPV1 regulation by PKA in AKAP150 Δ 36 dorsal root ganglia. AC5 and AKAP150 are highly expressed in striatum and both exhibit defects in motor coordination when deleted (Iwamoto et al., 2003; Tunquist et al., 2008). In heart, AKAP150 Δ 36 mice lack persistent Ca^{2+} sparklets and have lower intracellular calcium, due to a loss of PKA regulation of L-type Ca^{2+} channels. Deletion of AC6 results in reduced Ca^{2+} transients and other defects associated with calcium handling in cardiac myocytes (Tang et al., 2008). The significant overlap between a subset of AC5/6 and AKAP79 phenotypes in brain and to a lesser extent heart suggests that many, but not

all, of the cAMP-dependent processes associated with these proteins may require scaffolding of AC5/6 to AKAP79/150 to properly regulate cAMP dynamics.

Yotiao-AC2 – Yotiao plays a clear role in the sympathetic regulation of the I_{KS} current that is responsible for the normal repolarization of the heart (Chen and Kass, 2006; Chen et al., 2007). Of the Yotiao-interacting ACs, only AC2 and AC9 are expressed in the adult cardiac myocytes, albeit at lower levels than AC5/6. Thus these AC isoforms may participate in the temporal PKA-regulation of I_{KS} function that is balanced by the anchored PDE and phosphatase present in the Yotiao complex.

Yotiao is also highly expressed in brain. The AC2 binding site on Yotiao (Y808-957) effectively competes for AC2-Yotiao interactions and reverses inhibition of AC2 by Yotiao when added to membranes (Piggott et al., 2008). Disruption of Yotiao-AC interactions in brain using purified Y808-957 gives rise to a 40% increase in AC activity upon stimulation. Thus AC activity is clearly regulated by association with Yotiao in brain tissue. Interactions with other AKAPs, such as AKAP79, may give rise to similar modes of regulation. The interaction of ACs and Yotiao may play a role in NMDA regulation. Depending on the associated AC, either feed-forward (AC1), feed-back (AC9), or calcium insensitive pathways (AC2) could be assembled on Yotiao to regulate downstream effector activity.

mAKAP β -AC5 – The deletion of AC5 results in protection from cardiac stress and hypertrophy due to age-induced cardiomyopathy or in response to pressure overload through aortic banding (Okumura et al., 2003; Yan et al., 2007). Knockdown of mAKAP β in cardiac myocytes also protects against cytokine- or adrenergic-induced hypertrophy (Dodge-Kafka et al., 2005; Pare et al., 2005a). Therefore, binding of AC5

to mAKAP β may be required for transduction of sympathetic hypertrophic cAMP signaling in cardiac myocytes. This concept was tested using the AC-mAKAP binding domain (ACBD; 245-340 of mAKAP β) to disrupt association between AC5 and mAKAP β (Kapiloff et al., 2009). Overexpression of ACBD in cardiac myocytes using adenoviral expression resulted in increased basal and isoproterenol-stimulated cAMP, presumably due to a relief of mAKAP β inhibition of AC5 and/or loss of PDE4D3 control of cAMP levels at the complex. This is analogous to the increase in AC activity exhibited by disruption of AC2-Yotiao interactions in brain (Piggott et al., 2008). Disruption of AC5-mAKAP β interactions also led to an increase in basal protein synthesis and cardiac myocyte cell size, consistent with the increased levels of cAMP. Thus the regulation of AC5 via mAKAP-anchored proteins appears to be critical for maintaining a delicate balance between cAMP production (via AC5) and potentially degradation (via anchored PDE4D3) to control anchored PKA signaling and ultimately the hypertrophic response.

Concluding Remarks and Future Directions

It is increasingly appreciated that cAMP is restricted in its diffusion throughout the cell and that AKAP scaffolding proteins contribute to the temporal and spatial regulation of cAMP signaling (Berrera et al., 2008; Fischmeister et al., 2006; Smith and Scott, 2006). When an AKAP tethers both PKA and its substrate, the rate of substrate phosphorylation by PKA is enhanced (Zhang et al., 2001). The addition of AC to this complex not only provides added feedback regulation of cAMP production, but may also

alter the kinetics of PKA signaling, as demonstrated for AKAP79-AC complexes (Bauman et al., 2006). In addition, scaffolding of AC may provide spatial resolution for cAMP effector proteins such as PKA, EPACs, and cyclic nucleotide-gated ion channels. For mAKAP β -AC5, this results in cAMP generation near the nuclear envelope, perhaps when appropriate membrane surfaces are in close proximity (Kapiloff et al., 2009). However, what about other cellular sites? There are over 30 mammalian AKAPs located on structures as diverse as Golgi, microtubules, centrosomes, peroxisomes, nucleus, mitochondria, or even within bulk cytosol. Does scaffolding of ACs represent a general paradigm for AC functions or is it a unique property of a subset of ACs and AKAPs. If cAMP diffusion is truly limited, how does AKAP-anchored PKA present at cellular sites other than the plasma membrane sense cAMP generated (presumably) at the cell surface? Does the bicarbonate-sensitive soluble AC which can be found in mitochondria, nuclei, and other subcellular organelles scaffold to AKAPs (Zippin et al., 2002)? Certainly one future challenge is to overcome the limitations of AC antibodies and define other possible membranes and organelles where transmembrane ACs may reside and the modes of AC regulation that occur at these sites.

Additionally, how dynamic are AKAP-generated complexes? Microdomains created due to lipid rafts are highly transient in nature, but it is unclear whether AC association with scaffolds exhibit dynamic or stable interactions. AKAPs such as AKAP79 and Gravin are known to shuttle on and off the plasma membrane. For example, AKAP79 is targeted to postsynaptic membranes via associations with the actin cytoskeleton, phosphatidylinositol-4,5-bisphosphate (PIP₂), and cadherins

(reviewed in (Dell'Acqua et al., 2006)). Brief NMDA activation leads to persistent redistribution of AKAP79/PKA and dissociation from cadherin and F-actin complexes and release of calcineurin (Smith et al., 2006b). The ability of AKAPs to suppress endogenous AC activity suggests that these complexes may be quite stable under at least some conditions, but what about after stimulation? One of the real challenges for the future is to determine what combination of ACs, PKA substrates, and AKAPs are required to control the numerous cAMP-dependent physiological events.

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Footnotes

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Figure Legends

Figure 1. Regulation of AC isoforms. General patterns of regulation are shown for individual isoforms and, where appropriate, closely related ACs. Dashed lines indicate modes of regulation that differ between grouped isoforms. AKAPs known to associate with AC isoforms are indicated. Note, for simplicity not all forms of regulation are shown. Abbreviations include, AC, adenylyl cyclase; CaN, calcineurin; CAM, calmodulin; CNG; cyclic nucleotide gated channel; G_s, heterotrimeric G_sβγ; G_α, alpha subunit of G_s; PAM, protein activator of myc; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase C; RGS, regulator of G protein signaling.

Figure 2. AC-AKAP Assembled Complexes. **A**, AKAP79 coordinates different protein combinations to tailor effector regulation in different tissues. Anchored PKA can phosphorylate and inhibit bound AC5/6 and desensitize anchored βAR, in addition to regulation of associated downstream effectors. **B**, Yotiao binds to AC2, in addition to AC 1, 3, and 9. The anchoring of a PKA-regulated PDE sets up potential feedback regulation of cAMP levels independent of Yotiao-mediated inhibition of AC2. **C**, mAKAPβ complexes assembled on the nuclear envelope. In this model, βAR-stimulated AC5 increases cAMP to activate anchored PKA and potentially EPAC. PKA phosphorylation of the ryanodine receptor (RyR) increases channel activity to allow for Ca²⁺ activation of calcineurin (CaN). Several feedback loops are also initiated including PKA-dependent inhibition of AC5 to decrease cAMP synthesis and activation of PDE4D3 by PKA to increase cAMP breakdown. The binding of ERK1/2 to PDE4D3 is not shown. Adapted from (Dodge-Kafka and Kapiloff, 2006) and (Kapiloff et al., 2009).

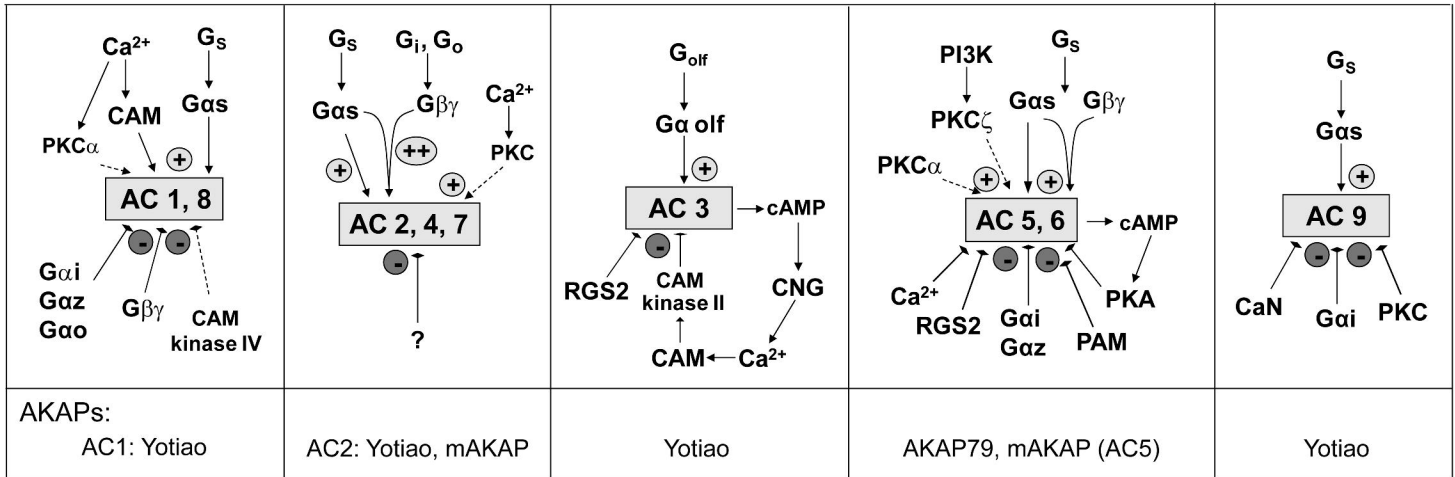


Figure 1

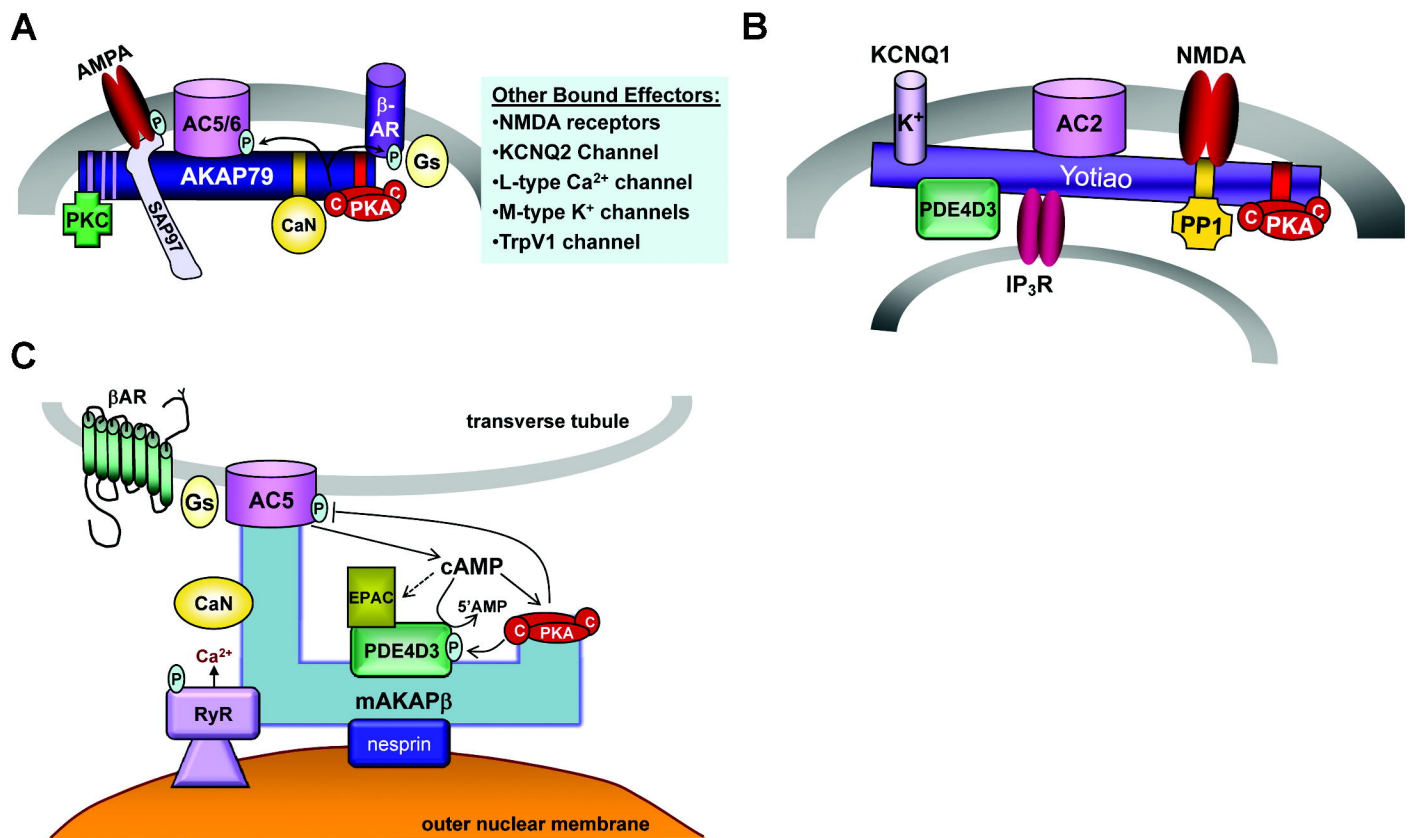


Figure 2