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MINIREVIEW

Adenylyl Cyclase–A-kinase Anchoring Protein Complexes: The Next Dimension in cAMP Signaling

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ABSTRACT

The formation of multiprotein complexes is a repeated theme in biology ranging from the regulation of the extracellular signal-regulated kinase 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. 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 spatiotemporal aspects of cAMP signaling adds yet another dimension to the classic 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 β1 adrenergic receptors (ARs), β2AR, 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 seem to use the same intracellular intermediates but activate different sets of genes in granulose cells (Conti, 2002). Measurements of cAMP using cyclic nucleotide-gated channels (CNGs or HCN) or fluorescence resonance energy transfer reporters based on PKA or EPAC have provided direct evidence for limited cAMP diffusion throughout the cell (Fischmeister et al., 2006; Berrera et al., 2008). Where cAMP is produced is also critical, because 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 focuses 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.

cAMP 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 Gα_s (Kamenetsky et al., 2006). The topology of transmembrane ACs consists of a variable intracellular N terminus and two large cytoplasmic domains separated by two membrane-spanning domains (six trans-

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ABBREVIATIONS: PKA, protein kinase A; AC, adenylyl cyclase; AKAP, A-kinase anchoring protein; AR, adrenergic receptor; CaN, calcineurin; EPAC, exchange protein activated by cAMP; GPCR, G protein-coupled receptor; PDE, phosphodiesterase; PKC, protein kinase C; ERK, extracellular signal-regulated kinase.

membranes 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 Gα_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 Fig. 1 (Sadana and Dessauer, 2009). Therefore, one emerging question is how all of the regulation of AC is coordinated? In addition, 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 the localization of ACs, AC regulators, and downstream effectors of cAMP to specific regions of the plasma membrane (i.e., lipid rafts), and the 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 (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 that these structures are required for at least some forms of regulation. In addition, AC6 shows differential coupling to GPCRs that cosegregate with AC6 in lipid rafts, enhancing βAR 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 reorganization 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 Gα_s·$\beta\gamma$ (Levitzki, 1988). Since that time, bioluminescence resonance energy transfer studies suggest that stable complexes occur between G$\beta\gamma$ subunits and AC2 (Rebois et al., 2006), and these complexes are probably assembled before insertion into the plasma membrane (Dupré et al., 2007). There is also evidence for AC complexes containing GPCRs (Lavine et al., 2002) or downstream signaling components. Coimmunoprecipitation of AC1 and ERK1/2 explains the selective activation of ERK signaling in human embryonic kidney 293 cells by AC1 but not AC2, 5, or 6 (Gros et al., 2006). ACs can form even larger complexes containing βAR, G proteins, PKA, phosphatases, and L-type Ca^{2+} channels to possibly facilitate highly spatially restricted signaling in neurons (Davare et al., 2001). The question becomes how these megacomplexes are assembled. The remainder of this review focuses 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 AKAPs. Since their initial discovery in 1982 (Theurkauf and Vallee, 1982), more than 50 AKAPs have been identified that are highly divergent, with the exception of a signature PKA regulatory subunit docking motif (Wong and Scott, 2004; McConnachie et al., 2006). AKAPs are localized to numerous cellular sites, including the plasma membrane (AKAP79, Yotiao, AKAP18, and Gravin) (Klauck et al.,

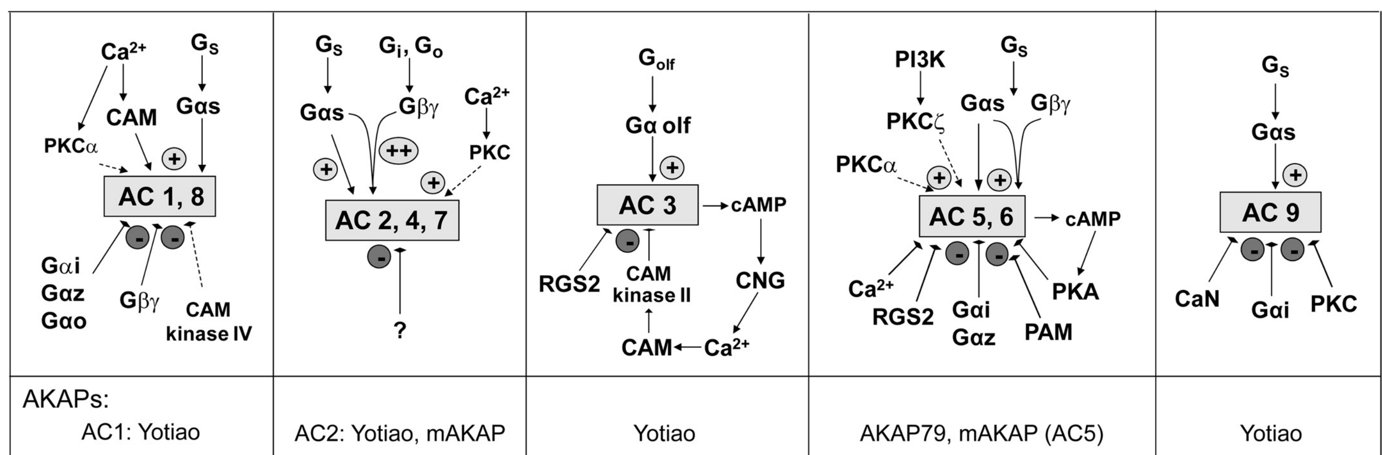

Fig. 1. Regulation of AC isoforms. General patterns of regulation are shown for individual isoforms and, where appropriate, closely related ACs. Broken lines indicate modes of regulation that differ between grouped isoforms. AKAPs known to associate with AC isoforms are indicated. For simplicity, not all forms of regulation are shown. CAM, calmodulin; CNG, cyclic nucleotide-gated channel; G_s, heterotrimeric Gα_s · $\beta\gamma$; PAM, protein activator of myc; PI3K, phosphatidylinositol-3-kinase; RGS, regulator of G protein signaling.

1996; Fraser et al., 1998; Lin et al., 1998; Grove and Bruchey, 2001) as well as Golgi, centrosome, nucleus, mitochondria, and cytosol (Feliciello et al., 2001). Those AKAPs that are located at the plasma membrane use a number of different strategies for docking, including myristoylation (AKAP18 and Gravin) (Lin et al., 1996; Fraser et al., 1998), 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 (Smith et al., 2006a).

Evidence for AC-AKAP Interactions

Our recent evidence suggests that membrane-bound ACs are precoupled to AKAP complexes to potentially generate a local pool of cAMP. Because 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 (Sarkar et al., 1984; Bregman et al., 1989; Carr et al., 1992). AKAP79 anchors calcineurin (CaN, also known as protein phosphatase 2B), PKC, β2AR, and β1AR, in addition to AMPA-type glutamate receptors (via PSD-95 and SAP97), L-type Ca^{2+} channels, M-type K^{+} channels, and the transient receptor potential vanilloid receptor 1 channel (capsaicin receptor) (Fig. 2A) (Dodge and Scott, 2000; Smith et al., 2006a; Jeske et al., 2008; Schnizler et al., 2008; Zhang et al., 2008). Because of 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 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 (Iwami et al., 1995; Chen et al., 1997). PKA anchoring facilitates the preferential phosphorylation of AC5/6 in rat brain extracts, and AKAP79

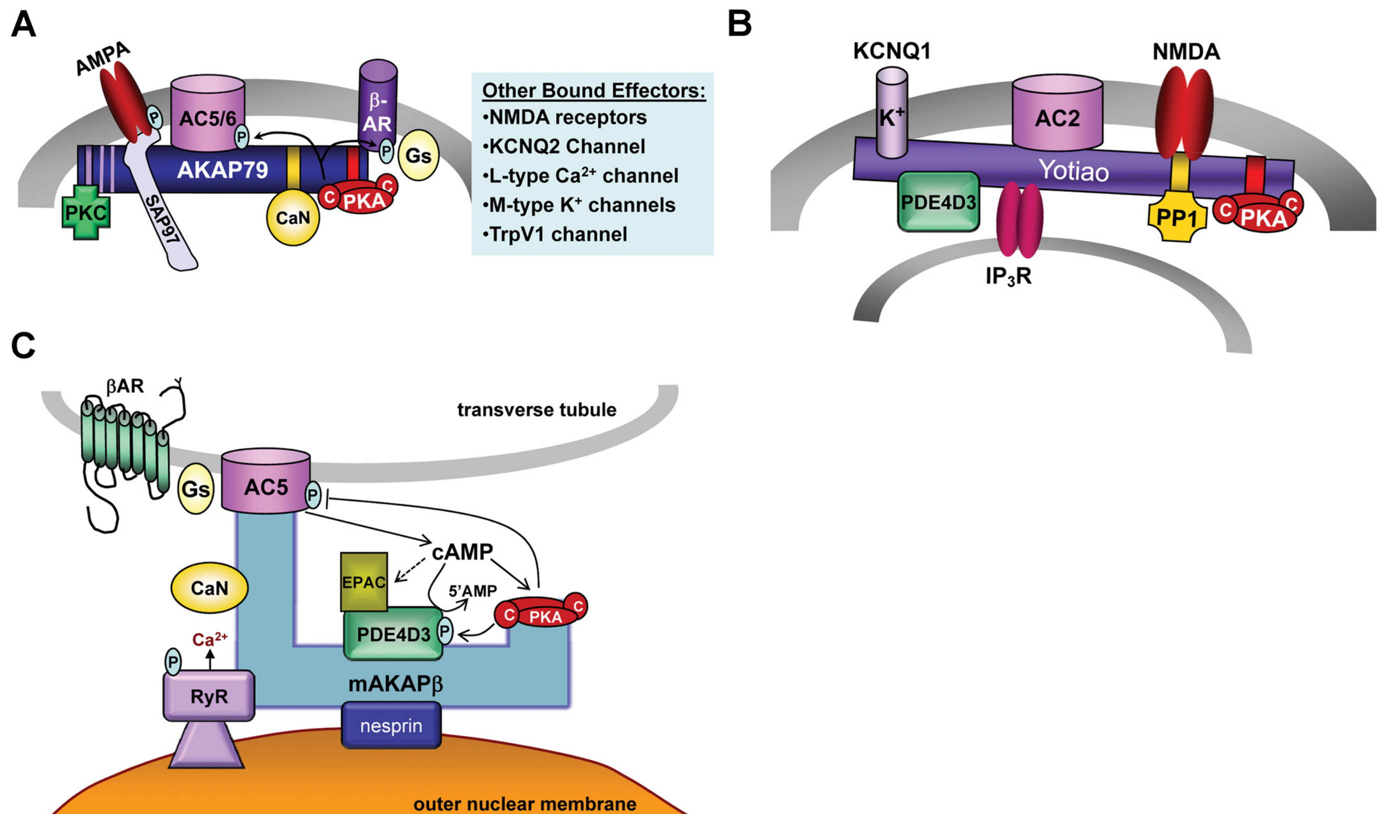

Fig. 2. AC-AKAP assembled complexes. A, AKAP79 coordinates different protein combinations to tailor effector regulation in different tissues. Anchored PKA can phosphorylate and inhibit bound AC5/6 and desensitize anchored βAR, in addition to regulation of associated downstream effectors. B, Yotiao binds 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 Ca^{2+} activation of 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 Kapiloff MS, Piggott LA, Sadana R, Li J, Heredia LA, Henson E, Efendiev R, and Dessauer CW (2009) An adenylyl cyclase-mAKAPβ signaling complex regulates cAMP levels in cardiac myocytes. *J Biol Chem* **284:**23540–23546. Copyright © 2009 American Society for Biochemistry and Molecular Biology. Used with permission.]

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 feedback 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 fluorescence resonance energy transfer reporter) (Bauman et al., 2006). Knockdown of endogenous AKAP79 increased cAMP levels and sustained PKA activity upon stimulation with β2-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 β2-AR provides negative feedback on AC5 and generates a burst of cAMP synthesis. This is a fairly rapid process, because 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. Although 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 kDa). 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, 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; Westphal et al., 1999; Marx et al., 2002; Tu et al., 2004; Terrenoire et al., 2009). 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), whereas anchored protein phosphatase 1 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, because these isoforms have no reported sensitivity to PKA. However, it is unlikely to be a consequence of direct interaction with Yotiao but rather is 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).

mAKAPβ. As discussed above, not all AKAPs are localized to the plasma membrane. The cardiac splice variant of muscle AKAP (mAKAPβ) 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 (McCartney et al., 1995; Kapiloff et al., 1999; Ruehr et al., 2003; Schulze et al., 2003; Pare et al., 2005b). mAKAPβ 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) (Dodge-Kafka and Kapiloff, 2006; Bauman et al., 2007). By additionally anchoring calcineurin and components of the ERK pathway, mAKAPβ complexes respond to several classes of intracellular receptors. mAKAPβ 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, mAKAPβ is required for full induction of cardiac hypertrophy and the activation of calcineurin/nuclear factor of activated T cells transcription by β-adrenergic agonists (Pare et al., 2005a). Thus, mAKAPβ integrates cAMP signaling with that of calcium and MAP kinases.

Despite the intracellular location of mAKAPβ, AC activity strongly associates with mAKAPβ in heart and isolated cardiac myocytes (Kapiloff et al., 2009). Four different antibodies against mAKAPβ, or its nuclear envelope tether nesprin, immunoprecipitate significant AC activity in heart. mAKAPβ associates with AC5 and AC2 but surprisingly not AC6 or AC1. The predominant complex in heart is mAKAPβ-AC5, because mAKAPβ-associated AC activity is completely absent in AC5 knockout hearts. AC5 directly interacts with amino acid 275 to 340 of mAKAPβ, a region that does not overlap with binding sites for other known mAKAP-associated proteins. Similar to the regulation of ACs by other AKAPs, mAKAPβ inhibits AC5 but not AC2 activity. This inhibition is lost upon deletion of the PKA anchoring site on mAKAPβ, consistent with a PKA-dependent mechanism of inhibition observed previously 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 mAKAPβ 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 II type 1 receptors, endothelin receptors, metabotropic glutamate receptors, and prostaglandin receptors (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 endoplasmic reticulum, then the C-terminal tail containing G-protein interaction sites would face the cytoplasm (Boivin et al., 2008). The same would seem to 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 catecholamines.

What Dictates Specificity for AC-AKAPs?

Each AKAP seems 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, and each displays very different regulatory patterns (Fig. 1) (Piggott et al., 2008). Because 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 mAKAPβ 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 mAKAPβ to bind AC5 versus AC6 (Piggott et al., 2008; Kapiloff et al., 2009). However, additional domains of AC (C1 and C2) participate in interactions between AC5 and mAKAPβ (Kapiloff et al., 2009). Thus, interactions with the N termini probably dictate the observed specificity among AC isoforms, but clear differences in the mechanism of binding exist.

A general "AC binding motif" on AKAPs has also not been identified. This is in part due 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 or with AKAP79. Different AKAPs seem to use different mechanisms to interact with the same AC isoform, because 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 as to 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 heterodimerization of AC isoforms that might be facilitated by AKAP interactions (Willoughby and Cooper, 2007).

Physiological Relevance for AC-AKAP Complexes

AKAP79-AC5/6. PKA-anchoring to AKAP79 plays an important role in hippocampal long-term potentiation, phosphorylation of L-type Ca^{2+} channels, and TrpV1 regulation, because deletion of the PKA binding site on AKAP79/150 (AKAP150Δ36) resulted in a significant disruption of these functions (Lu et al., 2007, 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 AKAP150Δ36 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 transient receptor potential vanilloid receptor 1 regulation by PKA in AKAP150Δ36 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, AKAP150Δ36 mice lack persistent Ca^{2+} sparklets and have lower intracellular calcium because of 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 (Tyr808–957) 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 Tyr808–957 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.

mAKAPβ-AC5. The deletion of AC5 results in protection from cardiac stress and hypertrophy as a result of age-induced cardiomyopathy or in response to pressure overload through aortic banding (Okumura et al., 2003; Yan et al., 2007). Knockdown of mAKAPβ 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 mAKAPβ may be required for the transduction of sympathetic hypertrophic cAMP signaling in cardiac myocytes. This concept was tested using the AC-mAKAP binding domain (245–340 of mAKAPβ) to disrupt association between AC5 and mAKAPβ (Kapiloff et al., 2009). Overexpression of AC-mAKAP binding domain in cardiac myocytes using adenoviral expression resulted in increased basal and isoproterenol-stimulated cAMP, presumably because of a relief of mAKAPβ 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-mAKAPβ 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 seems 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 (Fischmeister et al., 2006; Smith and Scott, 2006; Berrera et al., 2008). 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 mAKAPβ-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 more than 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., 2003)? One future challenge is to overcome the limitations of AC antibodies and to define other possible membranes and organelles in which transmembrane ACs may reside and the modes of AC regulation that occur at these sites.

In addition, how dynamic are AKAP-generated complexes? Microdomains created as a result of 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, and cadherins (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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