This Review is part of a thematic series on **Microdomains in Cardiovascular Signaling**, which includes the following articles:
Caveolae and Caveolins in the Cardiovascular System
Focal Adhesion: Paradigm for a Signaling Nexus
Vesicular Trafficking of Tyrosine Kinase Receptors and Associated Proteins in the Regulation of Signaling and Vascular Function

Compartmentation of Cyclic Nucleotide Signaling in the Heart: The Role of A-Kinase Anchoring Proteins

Targeting Cyclic Nucleotide Signaling
G Protein–Coupled Receptor Trafficking

Kathy K. Griendling and David A. Kass, Editors

Compartmentation of Cyclic Nucleotide Signaling in the Heart

The Role of A-Kinase Anchoring Proteins

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Abstract—The activation of the cyclic nucleotide protein kinase A (PKA) and PKG by their respective second messengers is responsible for the modulation of many cellular functions in the heart including cardiac hypertrophy, strength of contraction, and ion flux. However, several studies have revealed that a general increase in cyclic nucleotide concentration in the cell is not sufficient for the specific regulation of target proteins. These studies found that PKA and PKG must be colocalized with their targets to ensure spatial–temporal control of substrate phosphorylation. This compartmentation of cyclic nucleotide signaling is accomplished by tethering the protein kinases with their respective substrates through the association with scaffolding proteins. For cAMP signaling, A-kinase anchoring proteins (AKAPs) provide a molecular mechanism for cAMP compartmentation, allowing for the precise control of PKA-mediated phosphorylation events. (cAMP, PKA, AKAP, PKG). **(*Circ Res*. 2006;98:993-1001.)**

Key Words: cGMP ■ ion channels ■ protein kinase A phosphorylation ■ signal transduction ■ signaling pathways

The cAMP-dependent protein kinase is a broad-specificity Serine/threonine protein kinase that is responsible for the physiological actions of many hormones, neurotransmitters, and growth factors. Protein kinase A (PKA) is particularly important in the heart because cAMP signaling regulates both calcium dynamics, and the rate and force of heart contraction. However, pioneering work from several laboratories demonstrates that not all agonists that increase cAMP concentration elicit similar PKA-mediated responses. This finding leads us to question how signaling specificity can be accomplished by a broad-action kinase like PKA.[1–3] It is now appreciated that scaffolding, anchoring, and adaptor proteins that function to sequester signaling enzymes into discrete subcellular compartments maintain localized pools of kinase activity.[4] The molecular architecture of these localized kinase signaling complexes accounts for the spatial–temporal control over substrate phosphorylation, which is seen in response to different hormonal stimulation. Specifically in the cAMP pathway, the clustering of PKA into discrete intracellular compartments is accomplished through its association with A-kinase anchoring proteins (AKAPs).[5] This molecular configuration takes advantage of the formation of microdomains of cAMP and focuses the actions of PKA toward

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particular substrates.[6] This review concentrates on the role of AKAPs in cardiac physiology.

A-Kinase Anchoring Proteins

Initially controversial was the idea that the actions of the freely diffusible second messenger cAMP are compartmentalized in the heart. However, several key studies have demonstrated the localized actions of cAMP in the heart. Whole-cell patch recordings of frog hearts demonstrate the localized activation of calcium channels by localized stimulation of β-adrenergic receptors.[7] Furthermore, elegant live cell imaging studies that monitor the activation of PKA in rat neonatal cardiac myocytes clearly demonstrate that β-adrenergic stimulation activates a specific subpopulation of PKA moieties.[8] This localization of PKA is a direct result of the association between the kinase and the AKAPs.

AKAPs are a diverse group of proteins characterized solely by their ability to bind PKA. The PKA heterodimer is composed of a regulatory (R)-subunit dimer, with each R-subunit binding a catalytic (C)-subunit.[9] Three C-subunit genes (Cα, Cβ, and Cγ) have been characterized.[9] Cα and Cβ are 93% identical and ubiquitously expressed. However, Cγ is roughly only 80% identical to the other subunits and is found exclusively in the testis.[10]

Four R-subunit genes (RIα, RIIα, RIβ, and RIIβ) have been identified.[9] RI associates with other RI-subunits, whereas RII binds only other RII-subunits.[9] The predominant R isoforms, RIα and RIIα, are expressed in most tissues, whereas RIβ and RIIβ are found mostly in the central nervous system and reproductive tissues.[9,11,12] A fundamental difference between the subunits is that RI-subunits are found predominantly in the cytosol, whereas RII is particulate bound.[9] Although the relative distribution of the R-subunits is cell dependent, early studies using various animal hearts found that up to 50% of the cAMP-dependent protein kinase activity was associated with the particulate fraction.[13] Furthermore, the particulate bound kinase was almost entirely of the type II isozyme.[13] Several studies have found individual hormones preferentially activate a particular R-subunit. This is represented in the cardiac myocytes, where treatment with isoproterenol activates the particulate PKA-RII while PKA-RI is activated by prostaglandin E_2.[1–3] These differences in the R-subunits helps to explain the differences between the global affects of PKA acting through cytosolic RI and the localized effects of PKA-RII on specific sites in the cell.

The localization of the RII-subunit is directed through the association with AKAPs. RII has a higher binding affinity for AKAPs than RI, allowing for the cellular placement of these holoenzymes.[14,15] An amphipathic helix found on most AKAPs is responsible for binding the N-terminal domain of the RII homodimer.[16,17] Reagents have been developed to interrupt this interaction. Specifically, a peptide that mimics the RII-binding domain of the human thyroid AKAP Ht31 (termed Ht31 peptide) competes with a full-length AKAPs for the binding of RII both in vitro and in vivo.[17,18] Overexpression of Ht31 disrupts all known AKAP-PKA interactions, signifying the importance of AKAPS on localized signaling.[14] In particular, adenovirus mediated gene expression of the Ht31 peptide in cardiac myocytes was shown to disrupt PKA binding to AKAPs in the cell.[19] Global disruption of PKA anchoring affected the kinetics of the myocyte contraction cycle and blocked the phosphorylation of the PKA substrates troponin I and myosin binding protein C.[19] However, no affect of the peptide was seen on other PKA substrates, such as phospholamban.[19] These results clearly demonstrate that anchored pools of PKA regulate specific, localized phosphorylation events without interfering with global cAMP signaling.

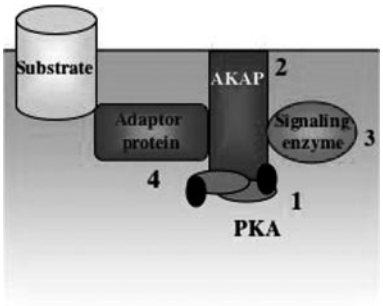

Figure 1. The four properties of AKAPs: (1) the first property of AKAPs is that they bind to the R-subunit of PKA; (2) unique targeting domains determine the cellular location of individual AKAPs; (3) the ability to associate with other signaling enzymes demonstrates the third property of AKAPs; (4) AKAPs can bind to additional adaptor or scaffolding proteins to form multiprotein networks that tether target substrates to their regulatory kinases.

There is limited sequence conservation of the RII binding domain on individual AKAPs and each AKAP has a distinct binding affinity for RII.[15] Additionally, the PKA-induced phosphorylation of the RII-subunit increases the RII/AKAP binding affinity.[20] These kinetics are particularly important in the failing heart, where desensitization of β-adrenergic signaling is seen. In fact, the phosphorylation of RII is decreased in tissue isolated from human failing heart, suggesting a decrease or redistribution of anchored PKA signaling under these conditions.[21]

The PKA anchoring model has been refined over the past decade and currently incorporates four important concepts (Figure 1). First, a protein is classified as an AKAP if it associates with the R-subunit of PKA inside cells. Second, unique targeting domains contained within each AKAP participate in either protein/protein or protein/lipid interactions, dictating the subcellular localization of PKA.[5,22] The third component of AKAPs is the ability to coordinate multiple signaling pathways through the anchoring of additional signaling enzymes such as phosphatases, phosphodiesterases (PDEs), and other kinases.[5,22] The ability of AKAPs to form much larger, multiprotein units through the interaction with other adapter and scaffolding proteins demonstrates the fourth property of AKAPs.[23,24] Collectively, these four properties allow AKAPs to function as signal processing units, linking upstream activators with their downstream targets and allowing for specificity of signaling and integration of multiple signaling events.

Several AKAPs have been identified in adult cardiac myocytes, including ezrin, AKAP-lymphoblastoma crisis (Lbc), AKAP15/18, muscle-selective AKAP (mAKAP), Yotiao, and AKAP220.[25–31] The physiological significance of each AKAP is currently under investigation. Evidence exists for the presence of currently unidentified AKAPs. For example, AKAP responsible for the phosphorylation of the sarcomeric proteins is currently unknown. This review will catalog

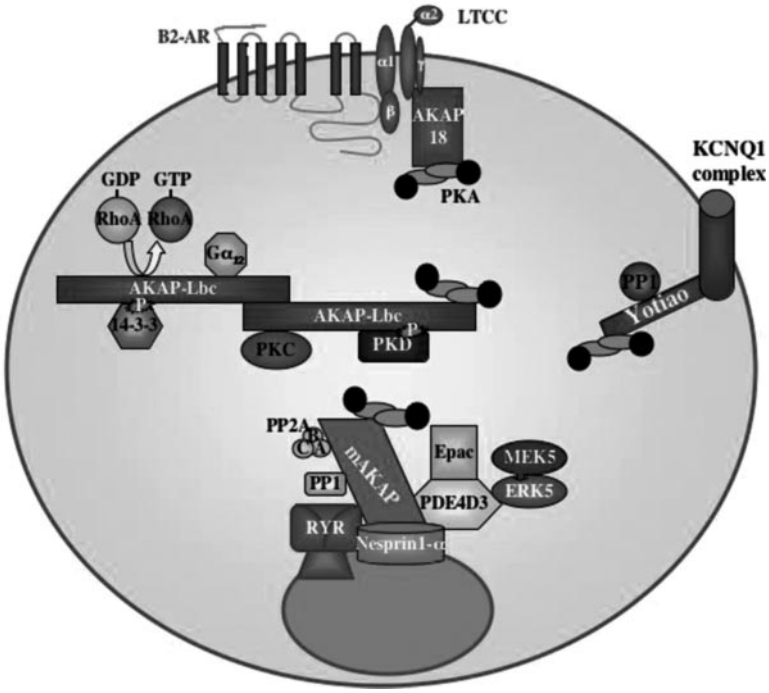

Figure 2. Schematic diagram of the AKAP signaling complexes in the heart. The primary function of an AKAP is to direct PKA actions toward a defined substrate usually found within the complex. The AKAPs discussed in this review are shown at their respective locations. The AKAP15/18 complex consists of the β2-AR and the L-type calcium channel. AKAP-Lbc is diffusely seen in the myocyte and contains a Rho-GTPase activity. Other known partners of the AKAP-Lbc complex include Rho-A, PKC, and 14-3-3. The phosphorylation of the potassium channel, KCNQ1, is regulated by the association of the channel with the AKAP Yotiao and PP1. Finally, the multiprotein mAKAP complex contains two scaffolding proteins: mAKAP and PDE4D3. mAKAP dictates the cellular location of the complex and binds PKA, PP2A, PP1, and the RyR. PDE4D3 tethers both Epac and ERK5 to the complex. Binding of mAKAP to Nesprin1-α is responsible for the nuclear localization of mAKAP. LTCC indicates L-type calcium channel.

our current understanding of some of the more characterized AKAPs in the heart: AKAP15/18, AKAP-Lbc, Yotiao, and mAKAP (Figure 2).

A-Kinase Anchoring Protein 15/18

AKAP18 has a low molecular weight, an AKAP of 81 amino acids. It has been independently cloned in two separate laboratories and given the names of AKAP15 or AKAP18 (referred to here as AKAP15/18).[26,27] However, several alternative splice variants of this scaffolding protein have been identified and are expressed in the cardiac myocyte.[32] AKAP15/18 is located exclusively in the plasma membrane of the heart, where covalently attached lipid moieties are inserted directly into the plasma membrane and anchor the scaffolding protein.[26,27] This molecular configuration allows for the specific regulation of plasma membrane substrates.

The first identified target of AKAP15/18 was the L-type calcium channel.[26,27] This ion channel is the major voltage-gated calcium channel in the heart and is responsible for the plateau phase of the action potential.[33] Previous work has established that AKAP-anchored PKA phosphorylation of the channel increases the probability of an open channel, allowing for potentiation of the inward calcium current.[34] Through the use of both biochemical and electrophysiological experiments, AKAP15/18 was identified to be the AKAP responsible for mediating this effect on the channel.[26,27] The PKA that is associated with AKAP15/18 is responsible for phosphorylation of the calcium channel at Serine 1928 on the α_{1C}-subunit and multiple sites on the β_2-subunit.[27] The direct binding between AKAP15/18 to the L-type calcium channel is mediated through a leucine zipper motif found in the C terminus of the α_1-subunit of the L-type calcium channel (amino acids 1774–1841), whereas the binding domain on the anchoring protein is contained in amino acids 25–54.[35] Interestingly, when a synthetic peptide mimicking the channel binding domain on AKAP15/18 was introduced into MM14 skeletal myotubes, the voltage-dependent potentiation of the channel was inhibited.[35] Furthermore, disruption of the L-type calcium channel and AKAP15/18 association by the peptide blocked β-adrenergic regulation of the channel in ventricular myocytes.[35,36] Therefore, the anchoring of PKA to the calcium channel by AKAP15/18 allows for a specific and rapid response to small, localized changes in cAMP concentration mediated by activation of the adrenergic receptor.

Additional complex partners have been identified in the heart through the use of subcellular fractionation techniques. These additional proteins include the protein phosphatase 2B (PP2B), also known as calcineurin, and the β_2-adrenergic receptor.[37] Currently, it is unknown whether these interactions are direct or mediated through adaptor proteins. However, direct association of the β-adrenergic receptor with the L-type calcium channel has been shown in the brain, suggesting a multiprotein complex consisting of the adrenergic receptor, the L-type calcium channel, and AKAP15/18 may exist in the heart.[38] Together, these data propose that AKAP15/18 may play a crucial role in the regulation of calcium dynamics in the heart and may mediate the inotropic actions of the β_2 receptor.

AKAP-Lbc

For the past 10 years, the identification of a partial protein fragment that bound PKA, termed Ht31, has been used as an important tool to elucidate not only the molecular mechanisms of PKA anchoring but to determine the biological significance of anchored PKA on many important physiological events.[16–19] It was not until recently that a database search revealed the similarity between Ht31 and the guanine nucleotide exchange factor Lbc,[39] revealing the function of full-length Ht31 as a Rho-selective guanine nucleotide exchange factor. AKAP-Lbc, as this AKAP is now known, is a large, 320-kDa protein. It contains several recognizable interaction domains, including consecutive Dbl and Pleck-

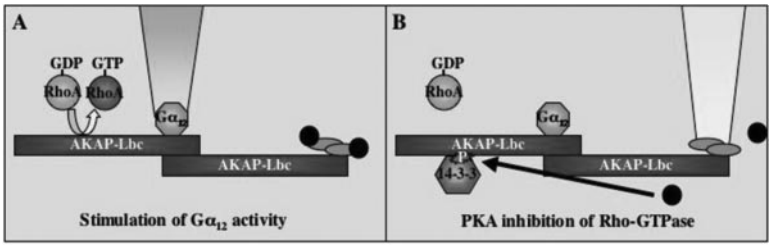

Figure 3. Model of AKAP-Lbc regulation of Rho Signaling. A, The AKAP-Lbc dimer is activated in response to stimulation by $G\alpha_{12}$ (shown by orange gradient). Once active, AKAP-Lbc interacts with Rho and induces the exchange of GDP for GTP on the small G-protein. The active, GTP-bound Rho is then released from the complex. B, Under conditions of active PKA (shown by yellow gradient), AKAP-Lbc is phosphorylated at Serine 1565 by PKA, recruiting 14-3-3 to the complex. 14-3-3 then exerts an inhibitory affect on AKAP-Lbc GTPase activity.

strin homology domains, that are involved in the activation of the small G-protein RhoA.[39] Furthermore, the upstream activator of Rho, $G\alpha_{12}$, also associates with AKAP-Lbc, suggesting the anchoring protein functions to couple G-protein activation with Rho signaling.[39]

Several previous findings have established that PKA has an antagonistic effect on Rho signaling.[40,41] It is now appreciated that AKAP-Lbc–bound PKA decreases the activation of Rho by blocking the Rho-guanine nucleotide exchange factor (GEF) activity of the scaffolding protein (Figure 3).[42,43] PKA phosphorylation of Serine 1565 on AKAP-Lbc stimulates the recruitment of 14-3-3 to the complex, which, in turn, exhibits an inhibitory function on Rho activation. The inhibitory effect of 14-3-3 is also dependent on oligomerization of AKAP-Lbc as well as the anchoring of PKA to the complex.[42,44] These data demonstrate that AKAP-Lbc integrates two effector pathways to couple cAMP signaling with that of the small G-protein Rho, allowing for the coordinated regulation of G-protein function. However, the physiological consequence of AKAP-Lbc Rho-GEF activity on heart function has yet to be determined. Because Rho signaling is intricately involved in the induction of cardiac hypertrophy, we can hypothesize that AKAP-Lbc may act as an adaptor module that facilitates G-protein activation of Rho, thereby enhancing the cardiotrophic affects of the small G-protein.[45]

The AKAP-Lbc complex has now been expanded to include the binding of both PKC and PKD and has been shown to play an important part in the coordinated activation of PKD.[46] First, the anchoring protein aids in the activation of PKD through the recruitment of both PKD and its upstream activator PKCη to the AKAP-Lbc complex. This molecular arrangement is common among scaffolding proteins and allows for the linear transduction of signals from one kinase to another.[4] Next, AKAP-Lbc facilitates the release of active PKD from the complex into the cytoplasm. This action is accomplished when PKA phosphorylates Serine 2737 on AKAP-Lbc.[46] These two actions, shown in Figure 4, allows AKAP-Lbc to not only orchestrate the specific activation of PKD but to contribute to the amplification of PKD signaling by processing multiple PKD enzymes in a short amount of time.

Yotiao

The two phases of the myocyte action potential determine the size and duration of cardiac contraction. Depolarization of the membrane potential is initiated by inward Na^+ channel currents and is maintained by calcium movement through the L-type calcium channel.[47] The delayed rectifier K^+ current (I_K) is primarily responsible for repolarization of the plasma membrane.[47] The KCNQ1 I_K is responsible for the slow component of the I_K, and regulation of this channel controls the duration of the action potential in the heart. The importance of this channel in normal physiological processes is illustrated by the identification of mutations of KCNQ1 in long-QT syndrome, an inherited cardiac arrhythmia that usually results in sudden death during increased sympathetic stimulation.[48–50] KCNQ1 current is potentiated by PKA, and the channel is both associated with and regulated by AKAP-bound PKA.[29] Recently, it was determined that the channel binds to the AKAP Yotiao in the heart.[51] Yotiao was first identified as a 210-kDa protein that associated with the *N*-methyl-D-aspartate receptor in neurons and functioned to tether a kinase/phosphatase signaling complex to the ion channel.[52] Anchored protein phosphatase 1 (PP1) is the predominant enzyme that regulates the ion channel under basal conditions, limiting the effects of active PKA until increases in cAMP overpower the action of the phosphatase (see Figure 2 for a diagrammatic representation of the complex).[52] The affect of anchored PP1 on KCNQ1 is currently unknown.

PKA was shown to significantly increase channel currents through the phosphorylation of KCNQ1 at Serine 27.[51] Yotiao is responsible for linking the kinase with the channel. Disruption of the channel association with Yotiao blocked the cAMP enhancement of channel activity.[51] The binding of the AKAP with KCNQ1 is mediated by a conserved "leucine zipper" coiled–coiled motif, and mutation of a single amino acid (G589D) disrupts channel association with Yotiao. Importantly, this mutation is found in 508 of 939 established Finnish Lung QT syndrome (LQTS) patients,[53] suggesting that PKA targeting to the channel is necessary for proper channel function in vivo.[51] This finding is the first genetic evidence demonstrating the physiological importance of PKA targeting and gives significant weight to the anchoring hypothesis.

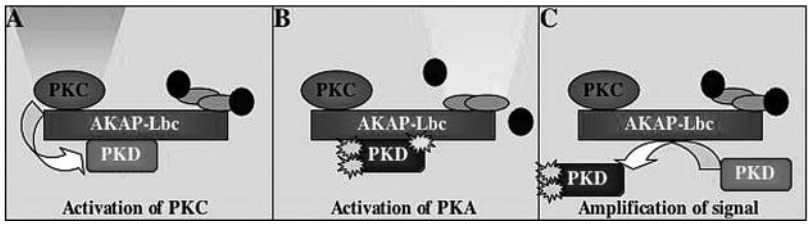

Figure 4. Amplification of PKD signaling by AKAP-Lbc. A, Stimulation of PKC (shown by orange gradient) results in the phosphorylation of PKD at two sites. B, It takes the coordinated activation of PKA (shown by yellow gradient) and phosphorylation of AKAP-Lbc at Serine 2737 to release the active PKD from the complex. C, The release of active PKD opens up the PKD binding site on AKAP-Lbc, allowing for the stimulation of another PKD and amplification of PKD signaling

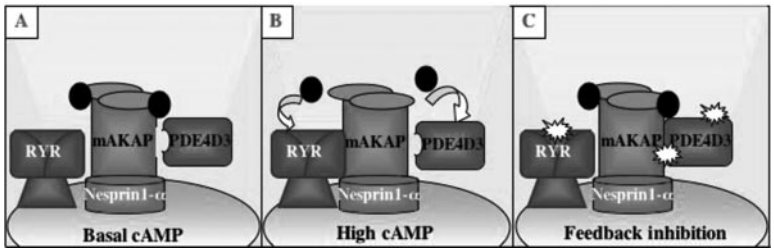

Figure 5. Negative feedback inhibition of mAKAP-bound PKA by PDE4D3. A, Under resting concentrations, the PDE keeps cAMP concentrations low and PKA inactive. B, However, stimulation of the myocyte and the increase in cAMP concentrations overpower the activity of the PDE, allowing for activation of PKA and phosphorylation of target substrates. C, PKA phosphorylation of PDE4D3 at Serine 54 increases the catalytic activity of the PDE, whereas phosphorylation of Serine 13 increases the association of the PDE with mAKAP. These two phosphorylation events induce a negative feedback loop to turn off PKA activity.

Although these data provide genetic evidence of the importance of AKAP/channel interaction on the electrophysiology of the myocyte, direct physiological models are not yet available to test how the G589D mutation alters the sympathetic response of the myocyte action potential. Beginning to address these questions, McCulloch et al used an integrative computational model of β-adrenergic signaling, excitation–contraction coupling, and action potential propagation to investigate the physiological consequence of the G589D mutation.[54] Although complex disruption did not prolong the QT interval at rest, QT prolongation and transient after-depolarizations were seen after β-adrenergic stimulation. This suggests that complex formation links β-adrenergic signaling to myocyte electrophysiology.[54]

New evidence suggests that Yotiao is not only responsible for the phosphorylation of the KCNQ1 channel itself but also acts as an effector of the channel, helping to translate the phosphorylation event into altered channel activity. In cells that express a Serine to aspartic acid mutation at the PKA phosphorylation site on KCNQ1, the coexpression of Yotiao with the mutated channel increased current and slowed channel deactivation compared with cells expressing the channel alone.[55] Although it is still unclear whether this is the result of protein/protein interactions or the recruitment of additional signaling enzymes, it does demonstrate another physiological role of AKAPs for the translation of channel function.

Muscle-Selective AKAP

mAKAP is a 255-kDa protein localized to the nuclear envelope and sarcoplasmic reticulum (SR) in the heart, skeletal muscle, and brain.[56,57] As shown in Figure 2, the mAKAP complex consists of PKA, PDE4D3, the calcium-activated calcium channel ryanodine receptor (RyR), the phosphatases PP2A, PP1, and PP2B, nesprin-1α, the GTP exchange protein Epac (exchange protein activated by cAMP), extracellular signal-regulated kinase 5 (ERK5), and the upstream activator of ERK5, mitogen-activated protein kinase/ERK kinase 5.[58–61]

The binding of PDE4D3 to mAKAP highlights the complexity of PKA signaling by setting up a unique negative feedback mechanism that regulates the activity of the associated PKA (Figure 5).[60,61] Under basal conditions, the tethered PDE maintains low resting cAMP levels sufficient for the prevention of PKA activation. However, stimulation of the cell and the resulting increase in cAMP concentration overpowers the basal catalytic activity of the PDE, allowing for the release of active C-subunit and the phosphorylation of substrate proteins. PDE4D3 itself contains two very important PKA phosphorylation sites.[62] The first site at Serine 54 increases the maximum velocity of the PDE 2- to 3-fold.[62] Therefore, phosphorylation at this site favors the reformation of the PKA holoenzyme, shutting off PKA signaling. The second PKA phosphorylation site at Serine 13 increases the binding affinity of the PDE for the mAKAP complex, enhancing the negative feedback loop.[63]

To determine the dynamics of this negative feedback mechanism, Dodge-Kafka et al used live cell imaging to monitor the temporal activation of PKA.[61] These studies used a vector named A-kinase activity receptor (AKAR) that consisted of a cyan fluorescent protein (CFP) linked to a citrine fluorescent protein (YFP), with a forkhead homology domain and PKA phosphorylation site in the linker region (Figure 6A). Increases in cAMP- and PKA-dependent phosphorylation of the PKA site on the vector results in engagement of the forkhead homology domain, allowing for fluorescent energy transfer between the CFP and YFP moieties. To determine the importance of anchoring PKA in close proximity to the substrate, the AKAR vector was modified to include the mAKAP–PKA binding site before the CFP (AKAR-PKA; Figure 6B). A schematic representation of their data is shown in Figure 6D. Stimulation of cAMP signaling in human cell line cells expressing AKAR-PKA resulted in a large, prolonged activation of anchored PKA. Next, the importance of anchored phosphatase to control PKA activity was tested. The PD4D3 binding domain on mAKAP was added to the YFP moiety, leading to the creation of the vector AKAR-PKA-PDE (Figure 6C). Importantly, the association of PDE4D3 to the same complex as PKA resulted in a tempered pulse of PKA activity in cells expressing AKAR-PKA-PDE, suggesting that the colocalization of kinase and PDE is a critical component for turning off PKA signaling (Figure 6D, red line). These studies demonstrate that cAMP compartmentation by the mAKAP complex regulates not only the spatial location of PKA activity but controls the timing of activation as well.

Interestingly, PDE4D3 itself acts as a scaffolding protein, recruiting ERK5 to the mAKAP complex.[61] Two ERK binding domains on the PDE mediate the direct interaction. A mutation of either domain blocks the association of ERK5 with the complex.[61] Similarly to PKA, ERK5 also regulates PDE4D3 catalytic activity.[64] Phosphorylation of Serine 579 by the mitogen-activated protein (Map) kinase decreases cAMP hydrolysis by 40%.[61] The resulting increase in cAMP concentration sufficiently stimulates the release of PKA C-subunit, demonstrating that by tethering PDE4D3, ERK5, and PKA to the same anchoring protein, mAKAP facilitates the bidirectional regulation of PDE activity and dictates local cAMP concentrations.

Changes in cAMP concentrations have also been shown to regulate the activity of Map kinases in a context-specific manner. For the mAKAP complex, increases in cAMP concentration block the serum-induced increase in associated ERK5 activity. Surpris-

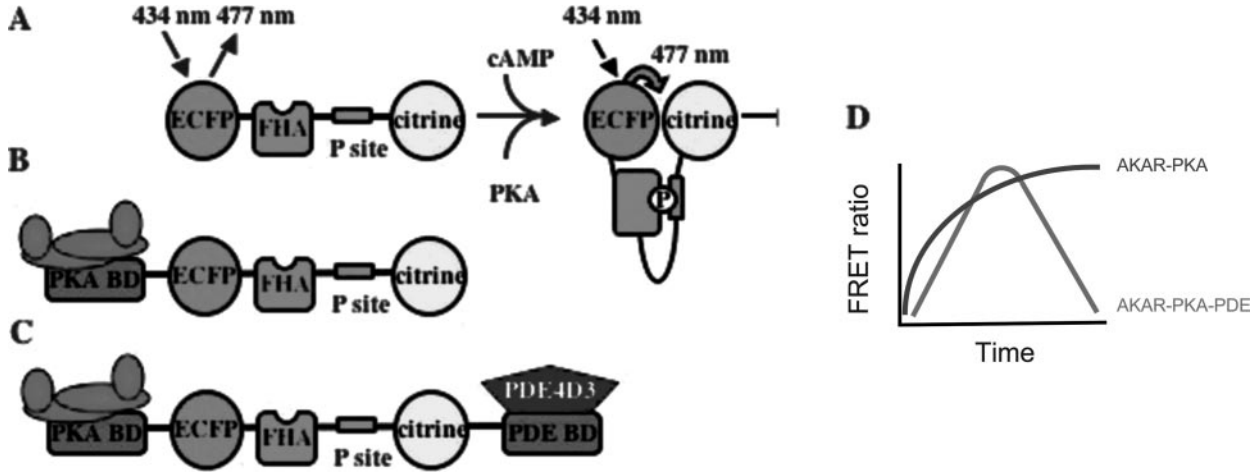

Figure 6. Temporal control of mAKAP-bound PKA activity. A, Diagram depicting AKAR. B, The association of the mAKAP PKA binding domain to AKAR results in the localization of PKA to AKAR in cells. C, AKAR-PKA-PDE contains both the mAKAP PKA binding domain and the PDE4D3 binding domain. D, Schematic representation of data using the AKAR-PKA-PDE vector in human cells. Cells were stimulated with 10 μmol/L forskolin. Fluorescent energy transfer was measured as performed by Dodge-Kafka et al.[61] ECFP indicates cyan fluorescent protein.

ingly, this mechanism is independent of the actions of PKA.[61] However, another cAMP effector was shown to mediate the cAMP inhibitory affect. Epac is a cAMP-stimulated small guanine nucleotide exchange factor for the small G-protein Rap1.[65,66] Activation of Epac with specific cAMP analogs mimicked the attenuation of ERK5 stimulation. Furthermore, association of Epac with the mAKAP complex is also mediated by a direct association of the exchange protein with PDE4D3, again demonstrating the anchoring functions of the PDE.[61]

The theory of compartmentation suggests that cAMP-mediated events in the cell that are responsive to different durations and concentrations of cAMP can emanate from the same microdomain. This theory is illustrated by the mAKAP complex, in which three distinct cAMP binding proteins coexist, each with different ranges of activation.[61] At the beginning of a stimulus in which cAMP concentration is in the nanomolar range, PKA is the first enzyme to become active. However, as the stimulus continues, the concentration of cAMP increases into the micromolar range, allowing for the sequential activation of PDE4D3 and Epac.[67] At the height of the cAMP signal, PKA-induced phosphorylation of PDE4D3 and the resulting increase in cAMP hydrolysis would start to shut off the stimulus, terminating the actions of Epac and PDE4D3 before deactivating PKA.[62,68]

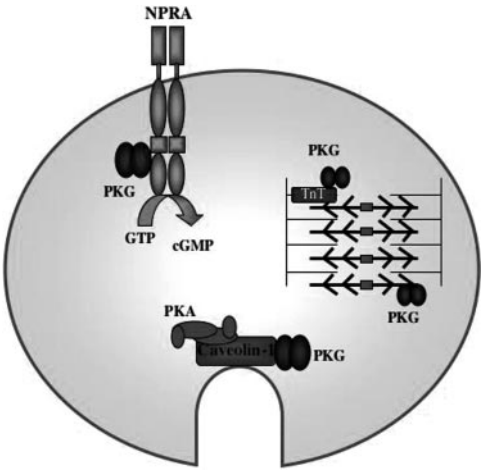

Figure 7. Proposed PKG binding complexes. PKG has been shown to directly associate with sarcomeric proteins troponin T (TnT) and myosin as well as the natriuretic peptide receptor. A complex of PKA, PKG, and caveolin-1 has been identified in rat aorta endothelial cells.

Immunohistochemistry of cardiac tissue has determined that mAKAP is located at both the nuclear envelope and SR.[56,59,69] Targeting of mAKAP to the nuclear envelope is accomplished through the direct association of the spectrin repeat domain on mAKAP to the nuclear-associated Nesprin-1α.[70] Nesprin-1α is inserted into the nuclear envelope by a conserved C-terminal Klarsicht-related transmembrane domain and has been identified in complexes with both laminin A/C and emerin.[71] Disruption of mAKAP/Nesprin binding, by the overexpression of the mAKAP spectrin repeat domain, is sufficient to displace mAKAP from the nuclear envelope, suggesting that the localization of mAKAP to the nuclear envelope is saturable. The molecular mechanism responsible for targeting mAKAP to the SR is presently unclear but may involve direct association of the anchoring protein with the RyR through a conserved leucine zipper motif.[72]

The identification of such a large complex at both the nuclear envelope and the SR suggests diverse roles for mAKAP at both locations. At the SR, the mAKAP complex plays a role in the calcium dynamics of the heart through the regulation of the RyR. PKA phosphorylation of the RyR at Serine 2808 increases the probability of an open channel as well as the sensitivity of the RyR to calcium-dependent activation.[59] mAKAP links PKA to the RyR, and mAKAP-bound PKA has been shown to phosphorylate both skeletal and cardiac isoforms of the RyR.[56,69] PKA hyperphosphorylation of the RyR has been demonstrated in failing hearts, leading to depletion of the SR calcium stores and an increased diastolic SR calcium leak.[59] This hyperphosphorylation in failing hearts is attributable to a decrease in the level of PDE4D3 associated with the RyR, demonstrating the physiological significance of the PKA negative feedback loop detailed above.[73] In fact, deletion of the PDE4D gene in mice results in a progressive cardiomyopathy, accelerated heart failure after myocardial infarction, and cardiac

arrhythmias.[73] These data strongly suggest that regulation of RyR phosphorylation by the mAKAP complex has a significant impact on SR calcium dynamics, and disruption of cAMP dynamics results in progression of cardiac disease.

An understanding of the cellular function of the nuclear localized mAKAP is lacking, but new evidence suggests the complex may regulate cardiac hypertrophy.[61,74] RNA interference of mAKAP expression in rat neonatal ventriculocytes blocked the ability of several prohypertrophic agonists to stimulate both the increase in myocyte size and fetal gene expression seen in hypertrophy.[61,74] Although the precise mechanism used by the complex to induce hypertrophy is presently unknown, one may hypothesize that by localizing the kinases ERK5 and PKA within close proximity to nuclear targets such as transcription factors, mAKAP may facilitate the increase in fetal gene expression necessary for the induction of hypertrophy.

cGMP-Dependent Protein Kinase Anchoring Protein in the Heart

Although the precise functions and regulation of the cAMP-dependent kinase have been well studied, an understanding of the molecular mechanisms that regulate the cGMP dependent kinase is lacking. PKG has been shown to regulate a wide variety of processes including smooth muscle relaxation, platelet aggregation, and calcium homeostasis.[75–77] PKA and PKG share similar structural properties, suggesting the two kinases may be phylogenetically related.[78] Although both enzymes consist of two R- and two C-subunits, the R- and C-subunits of PKG are contained within the same polypeptide chain, indicating that PKG exists as a homodimer. Furthermore, the two kinases have similar consensus phosphorylation sites, where both kinases can efficiently phosphorylate the same substrate sequence in vitro.[79] These similarities between PKA and PKG suggest that proteins analogous to AKAPs must exist with the ability to localize the actions of PKG to discrete domains (Figure 7).

In the first screen used to identify cGMP-dependent protein kinase anchoring proteins (GKAPs), a protein overlay assay determined myosin directly associated with the kinase.[80] Furthermore, a genetic yeast-2-hybrid screen identified troponin T as a PKG binding protein and determined that the interaction is dependent on a leucine zipper motif.[81] This study found that although troponin T is not a substrate for PKG, troponin I is effectively phosphorylated by the kinase when it is colocalized with troponin T.[81] These data suggest that PKG anchoring to the contraction machinery through the association with GKAPs may be important for the regulation of muscle contraction.

Another important cardiac target for PKG binding is the natriuretic peptide receptor A (NPRA).[82] Through the use of the yeast-2-hybrid, a direct association of PKG with the NPRA was identified. This finding was extended to mammalian cells when PKG activity was copurified with NPRA immunoprecipitated from stably transfected human embryonic kidney 293 cells. Furthermore, activation of the receptor by atrial natriuretic peptide (ANP) but not NO induces the translocation of PKG from the cytosol to the plasma membrane. These data demonstrate how compartmentation of cGMP signaling distinguishes natriuretic peptide affects from those of NO. This helps to explain early observations that cGMP signaling displays potent biological affects despite extremely low concentrations of the cyclic nucleotide.

The association of both PKG and PKA to the same scaffolding complex would allow for the localized control of cyclic nucleotide signaling. Support for this hypothesis was shown by the identification of a complex isolated from rat aorta consisting of PKA, PKG, and caveolin-1. The disruption of the complex decreased the NO-induced relaxation of the tissue.[83] Additionally, PKG was shown to associate with the post synaptic density protein, disc large, zo-1 domain containing protein Na^+/H^+ exchanger regulatory factor 2, a protein already known to associate with the AKAP ezrin.[84,85] These data suggest that AKAPs may not only function to localize PKA signaling but PKG as well.

Conclusions

Early work on second messenger signaling in the heart found the freely diffusible second messenger cAMP could elicit localized actions of PKA, suggesting that microdomains of cAMP signaling mediate the localized action of PKA. Several recent studies have helped to unravel how the cellular placement of PKA through the association with AKAPs helps define second messenger compartmentation in the heart. However, our understanding of the physiological importance of AKAPs is currently in its infancy. Therefore, of high importance is the generation of mice lacking individual AKAP genes. These mice will not only provide insight into the mechanisms of AKAP functions but may yield genetic models of specific cardiac diseases. Of equal importance is the understanding of the alterations of AKAP signaling during heart failure. PKA-induced phosphorylation of RII increases the association of the subunit with the PKA-anchoring domain peptide Ht31.[21] Decreases in RII phosphorylation were also demonstrated in human hearts with dilated cardiomyopathy.[21] These data suggest the association of PKA with AKAP decreases in heart failure and may contribute to the decrease in PKA-dependent phosphorylation observed previously. An additional question to address is whether other changes in AKAP signaling complexes occur during heart failure. Does the expression or location of AKAPs change, or are there alterations in the association of other signaling components with AKAPs? This information will be helpful as we move forward in our treatment of heart failure.

Additional future directions in the field of AKAPs should include the understanding of the molecular mechanisms that mediate cross-talk between each of the signaling pathways associated with the different AKAPs. For example, how does the association of PP1 with the KCNQ1 complex affect channel potentiation and PKA phosphorylation? Does mAKAP-bound ERK5 regulate RyR phosphorylation in the complex? The full extent of the role of AKAPs in orchestrating cross-talk will not be fully appreciated until many of these questions have been addressed.

It is also imperative to quantitatively address how the different AKAPs not only spatially restrict cAMP signaling but how they regulate the temporal actions of PKA as well. The use of the AKAR vector to study the cAMP signals associated with the mAKAP complex is the first quantitative measurement of the cAMP transients associated with a particular AKAP. This

use of this technology will provide novel information of the real-time dynamics of cell signaling.

Although our understanding of cAMP compartmentation has come a long way since it was first suggested >30 years ago, our understanding of cGMP signaling has not. Only a few GKAPs have been identified, and we have no information on the molecular mechanisms of PKG binding to GKAPs. Furthermore, the basic question of cGMP compartmentation needs to be addressed. Do localized pools of cGMP regulate specific targets of PKG action? The complete understanding of cyclic nucleotide compartmentation will not only help us understand the complexity of second messenger signaling but will also define the molecular basis of many cardiac diseases and progress toward the identification of novel targets for drug design to treat cardiac disease.

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