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AKAPs: The Architectural Underpinnings of Local cAMP signaling

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Abstract

The cAMP-dependent protein kinase A (PKA) is targeted to specific compartments in the cardiac myocyte by A-kinase anchoring proteins (AKAPs), a diverse set of scaffold proteins that have been implicated in the regulation of excitation-contraction coupling and cardiac remodeling. AKAPs bind not only PKA, but also a large variety of structural and signaling molecules. In this review, we discuss the basic concepts underlying compartmentation of cAMP and PKA signaling, as well as a few of the individual AKAPs that have been shown to be functionally relevant in the heart.

Keywords

PKA; AKAP; cAMP; compartmentation; scaffold; signaling

In the heart, stimulation of G_s-protein coupled receptors by catecholamines, prostanoids and peptide hormones all increase levels of the second messenger cyclic-adenosine monophosphate (cAMP). Through cAMP signaling pathways, these receptors differentially control inotropy (strength of muscle contraction), lusitropy (rate of relaxation), and chronotropy (heart rate), as well as myocyte hypertrophy (growth), metabolism, and cell survival. cAMP is synthesized by adenylyl cyclases (AC) and degraded by phosphodiesterases (PDE), both present in a large number of isoforms subject to diverse modes of regulation [1, 2]. Direct cAMP targets include protein kinase A (PKA), the small G protein guanine nucleotide exchange factor Epac, and hyperpolarization-activated and cyclic-nucleotide gated ion channels. PKA holoenzyme contains two regulatory “R” and two catalytic “C” subunits. cAMP binding to R-subunits causes release of the C-subunits and activation of the protein kinase. Over thirty years ago, it was recognized that stimulation of β-adrenergic receptors, but not prostanoid receptors, was inotropic, even though both increased intracellular cAMP levels and activated PKA [3–5]. Together, these fundamental

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None.

observations raised a profound question which has been the subject of intense research to this day: how can a diffusible second messenger that activates a diffusible protein kinase differentially activate downstream signaling pathways that control distinct cellular processes?

One mechanism by which cAMP signals are spatially and temporally restricted is through the formation of multimolecular complexes or “signalosomes” by A-kinase anchoring proteins (AKAP) [6, 7]. There are over fifty known AKAPs (including alternative-spliced forms) that target PKA to different sites within the cell [8]. While AKAPs share in common their ability to bind PKA, they are remarkably diverse scaffold proteins. Within each signalosome, AKAPs couple PKA to different substrates, enhancing the rate and fidelity of their phosphorylation by the kinase. By incorporating select ACs, AKAPs direct the specific phosphorylation of these substrates in response to relevant stimuli [9]. In contrast, which phosphodiesterase is present in individual AKAP complexes affects the duration, amplitude, and spatial extent of cAMP signaling, as well as defining crosstalk with other signaling pathways [10, 11]. By bringing together different combinations of upstream and downstream signaling molecules, AKAPs provide the architectural infrastructure for specialization of the cAMP signaling network.

Compartmentation of the Second Messenger cAMP

Cyclic AMP is a small, freely diffusible hydrophilic molecule. While it is straightforward that through direct protein-protein interactions, scaffold proteins may physically organize the cAMP machinery within individual compartments, it is less intuitive how inappropriate signaling between adjacent compartments is prevented. This “compartmentation” can be imposed by either physical or functional barriers [12]. Functional compartmentation involves the production of areas of cAMP rarefaction by distinct pools of PDE anchored by AKAPs and other scaffold and adaptor proteins. Investigations using ion channels and fluorescence resonance energy transfer (FRET)-based cAMP and PKA activity sensors have demonstrated the importance of this mechanism in cardiac myocytes [12]. The importance of PDEs to cAMP compartmentation was first demonstrated in live cells in 1996 when Jurevicius and Fischmeister showed that local application of the β-adrenergic receptor agonist isoproterenol resulted in local L-type Ca^{2+} currents that were more generalized after addition of the general PDE inhibitor IBMX [13]. Of the PDEs expressed in myocytes, PDE3 and PDE4 family members account for the majority of activity [14–16]. In particular, PDE4 is responsible for limiting the distance that cAMP signals may diffuse when generated by β–adrenergic receptors [17, 18]. Type I PKA (PKAI) contains RIα or RIβ subunits, while type II PKA (PKAII) contains RIIα or RIIβ subunits; of these, RIα and RIIα are expressed in the heart [19]. Recently, Benedetto, et al., compared PKAI and PKAII compartments by fusing RI and RII-docking domains to the Epac1-camps FRET sensor [20]. “RII_epac” sensor was concentrated at the M-line and less so at the Z-line of the sarcomere, while “RI_epac” displayed a striated pattern that overlapped both the M and Z-lines. Although PKAI, but not PKAII, is typically soluble upon tissue fractionation [21], they showed using fluorescence recovery after photobleaching (FRAP) that PKAI is also anchored by AKAPs in myocytes, albeit more loosely than PKAII. Reminiscent of early data showing that particulate PKA is activated preferentially by β-adrenergic receptors [22], RII_epac was selectively activated by isoproterenol and inhibited by PDE4, while RI_epac was activated by isoproterenol, PGE_1, glucagon and GLP-1 and preferentially inhibited by PDE2. Together, these results argue that multiple cAMP compartments regulated by distinct sets of receptors and PDEs may be present within the same organelle in a cell.

Physical compartmentation utilizes membrane barriers or the direct transfer of cAMP between AC and cAMP targets that are physically associated [23]. Although less well

studied than PDEs, the differential association of ACs with select downstream pathways can contribute to specificity in cAMP signaling, promoting the generation of threshold cAMP fluxes near relevant targets. With the exception of soluble AC, all ACs are transmembrane proteins localized primarily at the cell membrane, providing close coupling of cAMP production to G-protein coupled receptors as well as calcium ion influx [24]. In myocytes, the plasma membrane is extended by the transverse tubule system deep into the interior of cell, thereby permitting the synthesis of cAMP within compartments close to intracellular targets. In particular, dyads composed of transverse tubules and either the sarcoplasmic reticulum or the outer nuclear membrane may facilitate the coupling of cAMP signaling to calcium ion release important for the contractile cycle and other functions such as hypertrophy [25, 26]. Of the nine AC isoforms known to be expressed in the heart, type 5 and type 6 cyclases account for the majority of AC activity [27]. These cyclases are similarly regulated, including activation by $G_{\alpha s}$ and $G_{\beta\gamma}$, and inhibition by $G_{\alpha i}$, PKA feedback phosphorylation and Ca^{2+} [28]. However, AC5 and AC6 do not exhibit functional redundancy *in vivo*. For example, in response to transverse aortic constriction, a model for chronic pressure-overload, $AC5^{-/-}$ mice exhibited decreased myocyte apoptosis and increased Bcl2 expression, while $AC6^{-/-}$ mice exhibited decreased hypertrophy and unchanged Bcl2 expression [29, 30]. Together, these findings suggest that different cAMP compartments may be defined by different combinations of ACs and PDEs targeted by individual scaffold proteins. Like PDEs, clustering of ACs by AKAPs and other scaffolds within different plasmalemmal microdomains may account for their differential function [26, 31].

PKA binding to AKAPs

PKA R-subunits contain N-terminal docking (residues 1–23) and dimerization domains (24–44) followed by an inhibitor site and two cAMP binding domains (residues 158–426) that bind and inhibit a C-subunit [32]. AKAPs vary greatly in structure, localization, and the proteins for which they serve as a scaffold. What they share in common is an amphipathic, 14–18 amino acid residue motif capable of binding the PKA R-subunit docking domain. Most AKAPs show a preference for PKAII, while a few bind both PKAI and PKAII. The structure of PKA bound to AKAPs has been studied in great detail [33]. The N-terminal docking and dimerization domain of the R-subunit dimer forms a X-type, antiparallel four-helix bundle with a hydrophobic groove that binds the hydrophobic face of the AKAP amphipathic helix [34]. Differences in the depth of the groove and the conformation of the extreme N-terminal residues in the RI and RII dimer account for the differences in affinity for AKAPs between PKAI and PKAII [35].

Synthetic peptides based on AKAP sequences have proven instrumental in the elucidation of PKA anchoring-dependent events [8]. These peptides can either bind both PKAI and PKAII or show high specificity for a single type of PKA [36–38]. For example, expression by adenoviral infection of the Ht31 PKA-binding peptide that is selective for PKAII globally competed PKAII-AKAP complex formation in adult cardiac myocytes [39]. When introduced by *in vivo* adenoviral gene transfer into rat hearts, Ht31 inhibited isoproterenol-induced phosphorylation of troponin I (cTnI), phospholamban and the ryanodine receptor (RyR2) [40]. While baseline $+dP/dt_{max}$, $-dP/dt_{max}$ and left ventricular end-diastolic pressure, but not ejection fraction, were decreased, at high doses of isoproterenol, ejection fraction and stroke volume were elevated. The authors suggest that the paradoxically increased inotropy in the face of inhibited PKA activity may be due to increased cTnI N-terminal proteolysis, which can in turn promote cardiac function. More recently, the cell-permeable peptide “TAT-AKAD” that binds both RI and RII with nanomolar affinity has been introduced into isolated myocytes and Langendorff-perfused hearts [41]. As with Ht31, cTnI and phospholamban phosphorylation were inhibited. Remarkably, TAT-AKAD

inhibited both the contraction and relaxation rates and shortening of paced myocytes. Importantly, TAT-AKAD inhibited β-adrenergic induced ex vivo heart rate, peak pressure, $+dP/dt_{max}$ and $-dP/dt_{max}$. Together, these results imply that AKAP anchoring of PKA is critical for cardiac function. There are at least 15 AKAPs expressed in the heart (see Table). In the discussion that follows, we now review what is known about some of these individual AKAPs. While global peptide disruptors are important research tools, future clinical therapy will likely target individual AKAP complexes dedicated to distinct cellular functions, thereby avoiding the complexities and side-effects of broad-based signaling inhibition.

AKAP79/150/75

The *AKAP5* gene product (AKAP79 in humans, AKAP150 in rodents, AKAP75 in bovine) is the best described AKAP and has recently been shown to be critical for sympathetic control of cardiac myocytes (Figure 1) [31]. It is a prime example of a scaffold protein that facilitates and integrates upstream signaling with far-reaching downstream effects on cellular function. Associated with a variety of ion channels and membrane receptors in excitable cells (neurons and myocytes), AKAP79/150/75 binds and integrates cAMP, calcium, and phospholipid signaling through PKA, protein kinase C and the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin [42]. AKAP79/150/75 is targeted to the plasma membrane through the direct binding of phospholipids and is a major determinant of PKA anchoring at the neuronal post-synaptic density where it plays an important role in learning and memory [43–45]. Early on, it was shown that AKAP79/150/75 can bind both β_1 and β_2-adrenergic receptors in heterologous systems, facilitating both downstream signaling and PKA-regulated downregulation and desensitization [46–48]. Later, in the first demonstration of an AC-AKAP complex, it was discovered that AC5/6 associated directly with AKAP79/150/75, coupling cAMP production to PKA activation and negative feedback regulation of the cyclase [49]. Interestingly, AKAP79/150/75 is present in cardiac myocytes with β_1-adrenergic receptors at caveolin-3-deficient synapses formed with sympathetic neurons in co-culture [50].

Although AKAP79/150/75 has been the subject of intense investigation of over 20 years, the specific function of this AKAP in the cardiovascular system has only recently been addressed. In the past, AKAP79/150/75 appeared in the cardiac literature mainly due to the use of its consensus PxIxIT calcineurin-binding domain as a potent inhibitor of calcineurin activity. Expression of the AKAP79/150/75 site, like other calcineurin-binding peptides, inhibits NFAT-dependent myocyte hypertrophy [51]. Experiments using a AKAP79/150/75 knock-out mouse have more directly tested whether this scaffold is important in the heart and vasculature. AKAP79/150/75 facilitates the association of the L-type Ca^{2+} channel with PKA and PKC in excitable cells, potentiating channel opening by direct phosphorylation of the channel [52–54]. In arterial smooth myocytes, AKAP79/150/75 expression was required for the generation of persistant Ca^{2+} "sparklets" that promote vascular tone, as well as angiotensin II-induced hypertension [55]. Interestingly, in this cell type, anchoring by AKAP79/150/75 of PKC, but not PKA, was critical for promoting Ca^{2+} influx through the channel. In contrast, Nichols et al, recently demonstrated that in cardiac myocytes, AKAP79/150/75 mediates the association of $\beta_{1/2}$-adrenergic receptors, AC5/6, PKAII, calcineurin and a caveolin-3-associated sub-population of $Ca_v1.2$ L-type Ca^{2+} channels, facilitating the selective PKA phosphorylation of that pool of ion channels in response to β-adrenergic stimulation [31]. Importantly, β-adrenergic stimulation of Ca^{2+} transients was absent in myocytes from AKAP79/150/75 knock-out mice, even though the overall stimulated influx of Ca^{2+} through L-type Ca^{2+} channels remained intact. Further, β-adrenergic stimulation of Ca^{2+} transients was normal in myocytes isolated from an AKAP79/150/75 knock-in mouse in which the AKAP79/150/75 PKA binding domain is deleted, suggesting that overall Ca^{2+} transients depend on the scaffold, but not PKA-

phosphorylation of $Ca_v1.2$. Remarkably, even though AKAP79/150/75 does not form a complex with RyR2 and phospholamban, AKAP79/150/75 knock-out resulted in decreased sarcoplasmic reticulum loading and spontaneous Ca^{2+} release through ryanodine receptors in response to β-adrenergic stimulation, as well as reduced PKA phosphorylation of both RyR2 and phospholamban. Although this study raises more questions than it answers, including the unclear relationship of $Ca_v1.2$ pools associated with or separate from AKAP79/150/75 to Ca^{2+} transients, it does establish the central role of a single AKAP in orchestrating the sympathetic stimulation of Ca^{2+} transients in adult myocytes [31].

AKAP18/15

AKAP79/150/75 is not the only AKAP associated with Ca_v1 channels. AKAP18/15 (*AKAP7*) is expressed in myocytes as multiple alternatively-spliced forms that are differentially localized to the plasmalemma and the sarcoplasmic reticulum. AKAP18/15α, the smallest AKAP with only 81 amino acids, is localized to the plasma membrane by dual N-terminal myristoylation and palmitoylation [56]. A leucine zipper motif mediates binding of the scaffold to the Ca_v1 family of voltage-gated channels, facilitating PKA phosphorylation and potentiation of the channels [56–58]. Interestingly, in myocytes, the distal C-terminal domain of $Ca_v1.2$ containing the AKAP18α binding site is often cleaved from the remainder of the pore-forming subunit, associating non-covalently and auto-inhibiting the channel in the absence of cAMP elevation [59]. Importantly, introduction of a competing leucine zipper peptide (AKAP18/15α residues 38–54) into adult cardiac myocytes that blocks association of the scaffold with the C-terminal $Ca_v1.2$ domain inhibits β-adrenergic stimulation of I_{Ca} [60].

AKAP18/15δ is a longer isoform of AKAP18/15 that is targeted to the sarcoplasmic reticulum [61]. When not phosphorylated, phospholamban binds and inhibits the sarco-endoplasmic reticulum Ca^{2+}-ATPase 2A (SERCA2A). Upon sympathetic stimulation, PKA phosphorylation causes the dissociation of phospholamban from the pump, increasing Ca^{2+} re-uptake into internal stores (lusitropy) and cardiac contractility. AKAP18/15δ binds phospholamban and activates SERCA2 by promoting PKA phosphorylation of phospholamban [61]. The requirement for AKAP18/15δ for adrenergic-stimulated reuptake in cultured myocytes was confirmed by AKAP18/15δ RNA interference. Recently, we reported that AKAP18/15δ also binds protein phosphatase 1 and its inhibitor Inhibitor-1, facilitating PKA phosphorylation of the inhibitor and inactivation of the phosphatase [62]. This phosphatase is responsible for the inhibition of SERCA2A through phospholamban dephosphorylation [63]. Thus, by promoting Inhibitor-1 phosphorylation, AKAP18/15δ may further increase lusitropy. Taken together, *in vitro* evidence suggests that AKAP18/15 isoforms are important for both the regulation of Ca^{2+} influx through L-type Ca^{2+} channels and Ca^{2+} re-uptake through SERCA2A. *In vivo* evidence is, however, pending to support these hypotheses.

Yotiao

Repolarization of the cardiac myocyte after contraction is facilitated by the slow outward potassium ion current (I_{Ks}) contributed by the KCNQ1-KCNE1 channel. β-adrenergic stimulation increases I_{Ks} through PKA phosphorylation of KCNQ1, an event important for reducing action potential duration during states of increased chronotropy. Mutations in the subunits of this channel induce Long QT syndrome due to prolonged myocyte repolarization and are a cause of fatal cardiac arrhythmia. Through the binding of a leucine zipper motif on KCNQ1, Yotiao (*AKAP9*; AKAP350, AKAP450) is the AKAP responsible for targeting PKA to the I_{Ks} channel [64]. Importantly, mutations in either the human KCNQ1 leucine zipper (G589D) or the corresponding yotiao binding site (S1570L) block association of the

scaffold with the channel, inhibiting KCNQ1 phosphorylation and inducing Long QT syndrome, and making yotiao the first AKAP known to be associated with the etiology of a human disease [64, 65].

Like AKAP79/150/75, yotiao binds other signaling molecules besides PKA, including AC (types 1–3 and 9), the phosphodiesterase PDE4D3 and protein phosphatase 1 that can affect localized PKA phosphorylation [64, 66, 67]. Interestingly, yotiao is also a PKA substrate and phosphorylation of yotiao affects I_{Ks} independently of PKA phosphorylation of the channel [68].

Sarcomeric AKAPs

Several sarcomeric proteins are phosphorylated in response to β-adrenergic stimulation, including cardiac troponin I (cTnI), myosin binding protein C and titin [69]. Recently, cardiac troponin T (cTnT) was shown to be dual AKAP capable to binding either PKAI or PKAII [69]. Remarkably, deletion of cTnT Lys-210, a mutation found in human familial dilated cardiomyopathy that reduces the phosphorylation of cTnI and myosin binding protein C, inhibited binding of PKA to cTnT.

The intermediate filament protein synemin is a PKAII AKAP that is localized to the Z-line, intercalated discs, and the sarcolemma [70]. The targets of PKA-anchored synemin remain unknown. In contrast, the large 449 kDa AKAP and PKA substrate myospryn is a calpain 3 and titin-binding protein [71]. A gene polymorphism for myospryn has been associated with left ventricular hypertrophy in hypertension [72].

AKAP-Lbc

AKAP-Lbc (*AKAP13*; Brx-1, and proto-Lbc) is essential for cardiac development and pathologic myocyte hypertrophy [73, 74]. AKAP-Lbc is perhaps better known for Ht31 (human thyroid clone 31), an AKAP-Lbc fragment containing its PKA-binding site [75]. The Ht31 peptide was used in early studies to define the determinants of PKA anchoring and to demonstrate the functional importance of anchored PKA in various cell types [6]. Later, full-length clones for this scaffold were isolated and the AKAP was recognized to be a longer splice variant of the Lbc proto-oncogene expressed most highly in the heart [76]. AKAP-Lbc is a homo-oligomeric Rho-selective guanine nuclear exchange factor (Rho-GEF) that binds $G\alpha_{12/13}$, inducing Rho activity and stress fiber formation in lysophosphatidic acid-stimulated fibroblasts [76, 77]. One target for AKAP-Lbc activated Rho is PKNα, which in turn regulates a MLTK, MKK3 and p38α cascade associated with the scaffold [78]. Interestingly, when PKA phosphorylated, AKAP-Lbc activation of RhoA is inhibited by the binding of the small protein 14-3-3 [79, 80]. As a result, cAMP signaling inhibits RhoA activation through AKAP-Lbc.

In cardiac myocytes, AKAP-Lbc expression is required for α-adrenergic-induced hypertrophy and is itself increased in expression by hypertrophic stimuli [74]. Although AKAP-Lbc mediates α-adrenergic-stimulated RhoA activation, [74]. AKAP-Lbc Rho-GEF activity does not appear to be required for hypertrophic signaling, but instead depends on anchoring of PKD1 [81]. Besides being a Rho-GEF, AKAP-Lbc facilitates the activation of PKD1 by PKCη bound to the scaffold [82]. Together with PKA, activated AKAP-Lbc anchored PKD promotes the nuclear export of the class II histone deacetylase HDAC5 through 14-3-3 binding, resulting in increased MEF-2-dependent gene transcription [81]. The requirement for AKAP-Lbc *in vivo* in adult pathologic cardiac hypertrophy has not yet been tested, since the AKAP-Lbc knock-out mouse was embryonic lethal at embryonic day 10.5–11.0 [73]. Nevertheless, the hearts of AKAP-Lbc null embryos displayed thin walls,

were dilated, and developed a pericardial effusion. Accordingly, null myocytes displayed decreased sarcomere formation and reduced MEF2C expression.

Recently, signaling through AKAP-Lbc has been shown to be even more extensive. AKAP-Lbc can bind both the Ras effector Raf and the scaffold kinase suppressor of Ras (KSR-1) that anchors MEK1/2 and ERK1/2, thereby, bringing together the entire classical ERK1/2 cascade [83]. In HEK293 cells, AKAP-Lbc was required for efficient mitogen stimulation of ERK1/2 signaling. Furthermore, phosphorylation of KSR by PKA associated with the complexes sustained ERK1/2 activation. While this pathway has not been studied in myocytes, it will be important to study AKAP-Lbc activation of ERK1/2 signaling in the heart, especially in light of recent studies that ERK1/2 regulates the balance between concentric and eccentric cardiac myocyte growth [84].

mAKAPβ

mAKAPβ (*AKAP6*; AKAP100 is a C-terminal fragment of mAKAP) is a scaffold protein for a large signalosome located at the striated myocyte nuclear envelope that we have proposed serves as a gatekeeper for transcription factors involved in cardiac remodeling [85, 86]. The mAKAPβ signalosome contains all of the required machinery for cAMP synthesis, degradation and function, including AC5, PDE4D3, PKAII, and Epac1 [10, 26, 87, 88]. In addition to these enzymes, mAKAPβ also can bind the following molecules important for cardiac remodeling and contractility: calcineurin Aβ, protein phosphatase 2A (PP2A), NFATc transcription factor family members, MEK5, ERK5, and the type 2 ryanodine receptor (RyR2) [10, 89–93]. Recently, we have discovered mAKAPβ also contributes to the regulation of the transcription factor hypoxia-inducible factor 1α (HIF-1α) by binding HIF-1α and ubiquitin E3 ligases [94], while others have reported that mAKAPβ can also be co-immunoprecipitated with the sodium/calcium exchanger NCX1 and the CaN substrate myopodin [95, 96].

Work on the mAKAPβ signalosome has contributed importantly to our understanding of how AKAPs may modulate local cAMP fluxes (Figure 2). The direct binding of PDE4D3 to mAKAPβ was the first example of a PDE-AKAP complex [87]. As part of a negative feedback loop, PDE4D3 is activated by PKA phosphorylation at serine residue 54 (S^{54}), resulting in increased cAMP degradation [16, 97]. PDE4D3 mediates the recruitment of an ERK5 signaling module to the complex [10]. Upon activation, ERK5 phosphorylates PDE4D3 S^{597}, inhibiting PDE4D3 activity and increasing cAMP activation of PKA. Together these results suggest that upstream cAMP and ERK5 signals will synergistically activate mAKAPβ-bound PKA, a mechanism potentially important in the chronically stressed heart exposed to increased circulating catecholamine levels and IL-6 type cytokines. Besides ERK5, mAKAPβ-associated PDE4D3 binds Epac1 directly, thus establishing the mAKAPβ signalosome as the first identified to include two different cAMP effectors (PKA and Epac1) [10]. Through Rap1, Epac1 inhibits ERK5, as part of another negative feedback loop intrinsic to the mAKAPβ complex. Because Epac1 is a relatively low affinity cAMP target, Epac1 feedback may be important for homeostasis when sympathetic stimulation is maximal, such as during cardiac decompensation. Recently, we showed that PDE4D3 S^{54} is dephosphorylated by PP2A [98]. PP2A bound to mAKAPβ contains a B56δ-subunit that confers PKA activation, comprising an incoherent feedforward loop that opposes PDE4D3 activation. Moreover, we have also found that mAKAPβ-bound AC5 is inhibited by PKA phosphorylation, constituting yet another negative feedback loop [26]. Mathematical modeling suggests that negative feedback and incoherent feedforward loops are characteristic of systems that exhibit high sensitivity (the amplitude of the output in response to a stimulus) with precise adaptation (the ability of a signaling system to reset to baseline after termination of a stimulus) [99]. Through the organization of these interlinked feedback

and feedforward loops, the mAKAPβ signalosome should confer tight control of local cAMP levels in response to stress-induced neuroendocrine and paracrine stimuli. cAMP fluxes local to mAKAPβ are likely to be autonomous of cAMP fluxes elsewhere in the myocyte that are regulated by signalosomes containing different sets of signaling enzymes.

An important substrate for mAKAPβ-bound PKA is RyR2 (Figure 2B), a Ca^{2+}-induced Ca^{2+} release channel potentiated by PKA phosphorylation [90, 91]. RyR2 is important for excitation-contraction coupling at the sarcoplasmic reticulum (SR), but is also present within dyads formed between perinuclear transverse tubules and the outer nuclear membrane [25]. Since mAKAPβ is primarily located at the nuclear envelope through the binding of the KASH domain protein nesprin-1α [100], we propose that mAKAPβ complexes include a small subset of RyR2 molecules present within these perinuclear dyads [91]. There mAKAPβ-RyR2 complexes may modulate the activity of hypertrophic transcription factors that translocate to the nucleus. Upon norepinephrine stimulation, active calcineurin Aβ is recruited to mAKAPβ complexes where the phosphatase can promote the dephosphorylation of NFATc3 [93]. Accordingly, mAKAPβ RNAi inhibits NFATc nuclear translocation and transcriptional activity, as well as the adrenergic and cytokine-induced hypertrophy of cultured neonatal rat ventricular myocytes [10, 89, 93].

Although *in vivo* mAKAPβ function remains to be determined, it is interesting that AC5, calcineurin Aβ, NFATc2 and NFATc3 knock-out mice are resistant to pathologic cardiac hypertrophy [101–104]. In addition, a human nesprin-1 missense mutation has been described in a patient with dilated cardiomyopathy [105]. Conversely, unstressed PDE4D knock-out mice exhibit a progressive, exaggerated age-dependent cardiomyopathy [106]. mAKAPβ is one of the least abundant AKAPs in the heart (MSK, unpublished observations). In rescue experiments, we have found that myocytes expressing full-length mAKAPβ mutants lacking either the PKA or calcineurin Aβ binding domains have impaired hypertrophic signaling [89, 93]. It is remarkable that specific disruption of such a small pool of anchored PKA can have such an obvious effect on cellular phenotype. It is possible that in the future, drug therapy directed at specific AKAPs such as mAKAP may provide a strategy for the treatment or prevention of pathologic cardiac remodeling and heart failure in the absence of effects on cardiac contractility.

Summary

Through research on AKAPs, our view of the cell has changed dramatically. Traditionally, intracellular signal transduction was perceived to be driven primarily by the abundance of relevant molecules and the catalysis of specific post-translational modifications, as these molecules freely diffused within the cytosol. It has become clear that through scaffold and adaptor proteins that much of intracellular signaling actually occurs in defined compartments in which relevant molecules, that are either recruited or constitutively associated, interact due to specific protein-protein interactions. Thus, enzyme specificity is conferred as much by co-localization as by the intrinsic selectivity of the active site. Given that the inhibition of the catalytic activities of many enzymes is clinically problematic due to their pleiotropism, we propose that the disruption of unique protein-protein interactions is an alternative therapeutic strategy permitting the selective inhibition of cellular processes. While intracellular protein-protein interactions have not been traditionally considered feasible drug targets, recent work, including the inhibition of NOTCH transcriptional complexes by synthetic peptides, has shown that protein binding may be specifically inhibited *in vivo* with directed physiologic effects [107]. A more complete understanding of the structure-function relationships underlying AKAP complexes will result in the development of a new generation of therapeutics that moves clinical medicine beyond receptor ligands and enzyme inhibitors.

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Abbreviations

AC	adenylyl cyclase
AKAP	A-kinase anchoring protein
CaN	calcineurin
NFAT	nuclear factor of activated T-cell
PDE	phosphodiesterase
PKA	protein kinase A
RyR2	ryanodine receptor

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Research Highlights

- In this review, we discuss A-kinase anchoring proteins expressed in the heart.
- AKAPs are important for cAMP compartmentation.
- AKAP scaffold proteins confer specificity and fidelity to cAMP signaling.

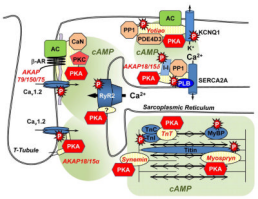

Figure 1. AKAPs Involved in Sympathetic Regulation of Excitation-Contraction Coupling

AKAP79/150/75 and AKAP18/15α can regulate the L-type Ca^{2+} channel, yotiao the KNCQ1 slow outward potassium ion current, and AKAP18/15δ phospholamban and Ca^{2+} reuptake. Troponin T, myospryn, and synemin are sarcomeric AKAPs. The AKAP responsible for PKA phosphorylation of RyR2 at the sarcoplasmic reticulum is unclear, albeit AKAP5 knock-out mice have diminished RyR2 phosphorylation [31].

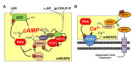

Figure 2. Signal integration by the mAKAPβ signalosome

The yellow boxes represent the mAKAPβ signalosome. **A.** There is evidence for three conjoined negative feedback loops intrinsic to the mAKAPβ complex that control local cAMP levels: (1) AC5, cAMP, PKA; (2) cAMP, PKA, PDE4D3; and (3) cAMP, Epac1, Rap1, MEK5, ERK5, PDE4D3. βAR stimulation will activate AC5, resulting in cAMP production and PKA activation. PKA phosphorylation inhibits AC5 and activates PDE4D3 activity, resulting in decreased cAMP accumulation [31, 97, 108]. The MEK5/ERK5 MAPK pathway is activated in myocytes by α_1AR and gp130/LIF-R agonists [109]. Activation of ERK5 will lead to PDE4D3 inhibition and increased PKA activity [10]. When high cAMP levels activate the guanine nucleotide exchange factor Epac1, Rap1 will inhibit the ERK5 pathway, reversing the ERK5-mediated inhibition of PDE4D3 and limiting downstream signaling. In addition, there is an incoherent feedforward loop that will oppose PKA phosphorylation of PDE4D3 resulting from PKA phosphorylation and activation of PP2A in the complex. An incoherent feedforward loop is present when two pathways lead to the same effector with opposite results [99]. Compare PKA-PDE4D3 and PKA-PP2A-PDE4D3. **B.** Calcineurin Aβ (CaNAβ) may serve as an mAKAPβ signalosome effector. RyR2 bound to mAKAPβ is PKA phosphorylated when myocytes are stimulated by β-agonists, potentially increasing local Ca^{2+} levels [89]. Norepinephrine-treatment of myocytes results in CaNAβ recruitment into the complex, where it can catalyze the dephosphorylation and nuclear translocation of NFATc transcription factors [93]. While not illustrated in this Figure, ERK5 and HIF-1α are also potential effectors for mAKAPβ complexes.

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Table

AKAPs Expressed in the Heart.

Gene Name	Protein Names and Isoforms	Binding Partners	Localization
AKAP1	D-AKAP1, s-AKAP84, AKAP121, AKAP149	PKA I and II, Protein tyrosine phosphatase PTPD1, Src, PKCα, Lfc, PDE7A, RSK1, PP1, PP2A, CaN, RNA, AMY-1	Mitochondrion, Endoplasmic Reticulum, Nuclear Envelope
AKAP5	AKAP79, AKAP75, AKAP150	PKAII, PKC, CaN, KCNQ2, Ca_V1, β-AR, AC, SAP-97	Plasmalemma and T-tubules
AKAP6	mAKAPβ	PKAII, AC5, PDE4D3, PP2A B56δ, RyR2, CaNAβ, NFATc, HIF-1a, VHL, Siah2, Epac1, Rap1, ERK5, MEK5, RSK3, PDK1, NCX1, Nesprin-1α, myopodin	Nuclear Envelope
AKAP7	AKAP15, AKAP18 (α,β,γ,δ)	PKAII, Ca_V1, Na Channel, phospholamban, Inhibitor-1, PP1	Plasmalemma and sarcoplasmic reticulum
AKAP8	AKAP95	PKAII, PDE7A, MCM2, p68 RNA helicase, HDAC3, AMY-1, Cyclin D/E	Nucleus
AKAP9	Yotiao, AKAP450, AKAP350, CG-NAP, Hyperion	PKAII, AC, PP1, PP2A, PDE4D3, KCNQ1, IP_3R, PKCε, PKN, Casein kinase 1, chloride intracellular channel (CLIC)	Plasmalemma, Centrosomes, Golgi
AKAP10	D-AKAP2	PKA I and II, Rab11, Rab4, PDZK1	Outer Mitochondrial Membrane
AKAP11	AKAP220	PKAII, PP1, GSK3β	Peroxisomes
AKAP12	Gravin, SSeCKS	PKAII, PDE4D, PKC, Src, CaN, β_2AR, Calmodulin, Cyclin D	Actin cytoskeleton, Plasma membrane
AKAP13	AKAP-Lbc, Ht31, BRX-1	PKAII, $G_{\alpha 12}$, Rho, PKNα, MLTK, MKK3, p38α, KSR1, Raf, MEK1/2, ERK1/2, 14-3-3, PKCη, PKD	Cytoskeleton
ARFGEF2	BIG2	PKAI and II, formin binding protein 3, PDE3A, TNFR1	Cytoplasm, Internal Membranes including Golgi
EZR	Ezrin	PKAI and II, CFTR, EBP50/NHERF, NHE3, calmodulin, Rho kinase, Actin, α_1AR, E-cadherin, β-catenin, EGFR, Fas-R, PKCα, S100, FAK, SAP-97, Moesin, Radixin	Cytoskeleton
MAP2	MAP2B	PKAII, tubulin, Ca_v1	microtubules
CMYA5	Myospryn	PKAII, Dysbindin, Titin, Calpain-3	Sarcomere
SPHKAP	SKIP	PKAI, sphingosine kinase type 1,	Cytosolic
SYNM	Synemin	PKAII, desmin, vimentin, dystrobrevin, desmuslin, zyxin, talin, vinculin,	Intercalated discs, sacrolemma, Z-lines, intermediate filaments
TNNT2	Troponin T	Troponin I, Troponin C, Actin	Sarcomere Thin Filaments

AC, AC; AMY-1, associate of myc-1; AR, adrenergic receptor; CaN, calcineurin (PP2B); Ca_V1, L-type Ca^{2+} channel; NCX1, sodium-calcium exchanger; PKC, protein kinase C; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; Siah2, seven in absentia homolog; RyR2, ryanodine receptor; VHL, von Hippel-Lindau tumor suppressor