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AKAP Phosphatase Complexes in The Heart

John M. Redden and Kimberly L. Dodge-Kafka*

Pat and Jim Calhoun Center for Cardiology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, Connecticut 06030

Abstract

Directed protein phosphorylation is indisputably critical for a multitude of cellular processes. A growing body of research demonstrates A Kinase Anchoring Proteins (**AKAPs**) to mediate a significant number of phosphorylation events in the heart. By acting as molecular tethers for the regulatory subunit of PKA, AKAPs focus the kinase's actions onto specific substrates. In the time since their discovery, the AKAP model has evolved in appreciation of the broader role these scaffolds play in coordinating multiple signaling enzymes in order to efficiently regulate dynamic cellular processes. The focus of this review is on the emerging role of AKAPs in regulating the three main cardiac phosphatases: Protein Phosphatase 1 by AKAP18 and Yotiao, and Protein Phosphatases 2A and 2B by mAKAP.

Keywords

AKAP; Phosphatase; PP1; PP2A; PP2B; AKAP18; mAKAP; Yotiao

Introduction

Directed protein phosphorylation is indisputably critical for a multitude of cellular processes. This is perhaps best exemplified in the myocardium, in which cAMP-dependent Protein Kinase (**PKA**) acutely modulates contractile dynamics through phosphorylation of accessory proteins and ion channels that comprise the myocyte's contractile machinery.¹ Interestingly, this same cAMP effector also contributes to the onset of cardiac disease. A growing body of research demonstrates A kinase anchoring proteins (**AKAPs**) to mediate a significant number of phosphorylation events in the heart.^{2,3} By acting as molecular tethers for the regulatory subunit of PKA, AKAPs focus the kinase's actions onto specific substrates. In the time since their discovery, the AKAP model has evolved in appreciation of the broader role these scaffolds play in coordinating multiple signaling enzymes in addition to PKA, including other kinases, phosphodiesterases (**PDE**), and adenylyl cyclases (**AC**).^{4,5} AKAPs are poised, thus, to integrate cAMP signaling at all levels in order to tightly regulate protein phosphorylation and cell function. However, cellular homeostasis is maintained by crosstalk between kinases and phosphatases. Regulation of the latter, while of paramount importance, remains understudied. The focus of this review is on the emerging role of AKAP phosphatase complexes in the regulation of cardiac physiology and pathology.

*Corresponding Author dodge@uchc.edu telephone: (860) 679 – 2452 fax: (860) 679 – 1426.

Conflicts of Interest:

The authors declare no conflicts of interest.

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Muscle contraction is a Ca^{2+} dependent process, and cardiac muscle is no exception. Each beating of the heart is made possible by the transduction of electrical current into mechanical force. This process, known as excitation-contraction coupling, results in a rhythmic cycling of Ca^{2+} between the cytosol and the sarcoplasmic reticulum (SR).⁶ This complex chain of events is initiated by entry of extracellular Ca^{2+} through L-type Ca^{2+} channels (LTCC) located on the sarcolemma. The unique morphology of the cardiac myocyte, characterized by deep invaginations in the sarcolemma known as transverse tubules, places the sarcolemma in close proximity to the SR membrane. As a result, the LTCC is spatially coupled to an integral Ca^{2+} release channel on the SR known as the ryanodine receptor (RyR). This allows a relatively small Ca^{2+} influx to increase the local concentration of Ca^{2+} around the RyR to a level sufficient to activate calcium induced calcium release, and trigger depletion of SR Ca^{2+} stores. This robust wave of Ca^{2+} transverse the cytosol to interact with the myofilaments, specifically Troponin C, and initiate contraction of the myocyte. Muscle relaxation is characterized by the removal of Ca^{2+} from the cytosol and restoration of resting potential, which occurs primarily through the sarcoplasmic reticulum Ca^{2+} /ATPase (SERCA), and to a lesser extent through the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and other channels. The function of SERCA is regulated by a small phosphoprotein known as phospholamban (PLB), which is inhibitory to SERCA activity in its dephosphorylated state and permissive upon phosphorylation.⁶

The heart is a dynamic organ that responds to physiological demands made by the body's fight or flight response. Sympathetic innervation coordinates the release of catecholamines, which elicit rapid increases in heart rate, stroke volume and cardiac output by up to three times during exercise.⁷ These hormones act primarily upon β -adrenergic, G-protein coupled receptors to generate cAMP and activate PKA, which deftly manipulates contractility via phosphorylation of the contractile machinery and its accessory proteins at nearly every step. For example, phosphorylation of the LTCC, RyR, troponin-I (TnI), PLB and other proteins by PKA enhances the kinetics of excitation-contraction coupling, thus increasing the strength and speed of contraction.⁶

Deregulation of this pathway leads to the uncoupling of the contractile process and contributes to the onset of heart failure. Cardiac stressors such as hypertension and myocardial infarction necessitate an increase in signaling through the β -adrenergic pathway. This compensatory response allows the heart to transiently meet physiological demands. Chronic activation of this signaling cascade, however, sets in motion the deadly processes of cardiac remodeling and pathological hypertrophy.⁸ Prolonged PKA activity nucleates a negative feedback loop that leads to the downregulation of several proteins including the β_1 adrenergic receptor, which impedes Ca^{2+} cycling and contractility⁹. This vicious cycle leads to more signaling, further dysfunction, and ultimately to heart failure.

AKAPs are intricately involved in the regulation of PKA, and so make significant contributions to the efficacy of the fight or flight response by focusing PKA action on important substrates. The role of PKA in progression of heart failure gives them therapeutic appeal as well. AKAPs known to be expressed in the heart include mAKAP¹⁰, AKAP18¹¹, AKAP79¹², D-AKAP1/2¹³, AKAP95¹⁴, AKAP220¹⁵, AKAP-LBC¹⁶, gravin¹⁷, ezrin¹⁸, yotiao, synemin¹⁹, SPHKAP²⁰, and BIG2²¹. Though a comprehensive discussion of AKAP mediated phosphorylations in the myocardium falls outside the scope of this review, it has been discussed elsewhere in detail.^{2,3,22} The focus of this discussion is on the phosphatases that oppose kinase action and maintain the basal phosphorylation state. In the heart, there are three main serine/threonine phosphatases that account for the bulk of phosphatase activity: Protein Phosphatase type 1 (PP1), and the type 2 phosphatases PP2A and PP2B (calcineurin). As the gatekeepers of protein phosphorylation, AKAPs are involved in the regulation of these important phosphatases as well.

Cardiac Phosphatases

i. Protein Phosphatase 1 (PP1)—PP1 is a prominent, ubiquitously expressed Ser/Thr phosphatase that is highly conserved among all eukaryotic organisms. The PP1 holoenzyme is comprised of a catalytic and regulatory subunit (**Figure 1**). The four isoforms of the catalytic subunit (PP1 α , PP1 β , PP1 γ 1, PP1 γ 2) arise from one of three highly conserved genes. Although PP1 has already been implicated in a multitude of cellular processes including ion channel function, cytoskeletal dynamics, metabolism, cell cycle regulation, and apoptosis, several lines of evidence demonstrate PP1 to be the predominant phosphatase governing cardiac contractility.²³ Early pharmacological studies utilizing okadaic acid, a non-specific PP1 inhibitor, revealed that this phosphatase's inhibition enhanced contractile parameters of *ex-vivo* heart muscle and isolated ventricular myocytes.²⁴ Subsequent biochemistry characterized the altered phosphorylation profile associated with PP1 inhibition, demonstrating increased phosphorylation of critical substrates such as PLB, LTCC, TnI, and others. More than a decade after these seminal observations were made, the first transgenic mice were bred to specifically determine the role of PP1 in the heart. These PP1 α overexpressing mice exhibit a three fold increase in phosphatase expression and activity, and a phenotype which underscores the importance of this enzyme.²³ In agreement with the earlier studies, the transgenic mice have depressed cardiac function, as measured at the organ level, and reduced PLB phosphorylation. A causal link between heart failure and PP1 activity is established by observations that human patients with end-stage heart failure show a 37% increase in PP1 expression, and blunted contractile responses.²⁵ Most importantly, these data suggest that PP1 could be an attractive therapeutic target.²⁶ Lending support to this, in a murine aortic constriction model of heart failure, it was observed that specific inhibition of PP1 partially rescued the severe PLB dephosphorylation that is a hallmark characteristic of heart failure. Accordingly, these mice had enhanced cardiac function, and augmented β -adrenergic responses.²⁷ In light of PP1's tight evolutionary conservation, inhibition of PP1 may ameliorate the severity and progression of human heart failure as well.

ii. Protein Phosphatase 2A (PP2A)—Like PP1, PP2A is a highly abundant Ser/Thr phosphatase that is ubiquitously expressed. The holoenzyme is assembled as either a heterodimer comprised of a scaffold and catalytic subunit, or *in vivo* as a heterotrimer, which additionally associates with a regulatory subunit (**Figure 1**).²⁸ The catalytic subunit exists in two isoforms (PP2A α , PP2A β) arising from the essential genes PP2CA or PP2CB. Although both PP2A α and PP2A β are present in cardiac tissue, PP2A α is an order of magnitude more abundant.²⁹ PP2A maintains broad range of cellular processes in many tissues. In fact, its inhibition can disrupt metabolism, genomic stability, cytoskeletal organization and a number of other cellular processes.²⁸ PP2A is being actively studied by cancer biologists for its role in tumorigenesis, tumor suppression, and apoptosis.³⁰ Of particular interest, however, is the role of PP2A in the myocardium. The study that first implicated PP1 in the regulation of contractility utilized Okadaic acid, which can also inhibit PP2A at low concentrations.²⁴ Thus it was speculated based on that evidence that PP2A, like PP1, could potentially play an important role in the heart. In support of this, a later study conducted using the more selective PP2A inhibitor Fostriecin determined that PP2A is able to reduce infarct size following global ischemia when administered prophylactically or immediately following ischemic insult.³¹ Ultimately, these observations led researchers to generate transgenic PP2A overexpressing mice to investigate its role in the heart. The results of this study confirmed that PP2A activity produces a cardiac phenotype. The mice had significant reductions in phosphorylation of PLB and TnI, and prematurely developed cardiac hypertrophy.³² Upon comparison of these findings to the available data on PP1, it seems that in an overexpression context PP2A and PP1 have some degree of redundancy. However, the observance of increased necrosis and fibrosis appears to be unique to PP2A

transgenic mice. A significant volume of work now demonstrates receptor mediated PP2A action on a number of cardiac substrates including the LTCC, RyR, and TnI, and delineate its role in a number of cardiac pathologies including sepsis³³, infarction & ischemia, hypertrophy, and cardiomyopathy.

iii. Protein Phosphatase 2B (PP2B, Calcineurin)—PP2B, also known as calcineurin (CnA), is another ubiquitous Ser/Thr phosphatase that is most abundantly expressed in the brain and heart. As shown in Figure 1, it is composed of a catalytic and regulatory subunit (CNA and CNB, respectively). The catalytic subunits may arise from one of three possible genes (α , β , γ), or in the case of the regulatory subunit, one of two (α , β).²⁹ Although the catalytic subunits share structural similarities with PP1, PP2B is unique from both PP1 and PP2A in that it contains an auto-inhibitory element which renders isolated PP2B catalytically inactive. This inhibition is removed upon the binding of Ca^{2+} -Calmodulin (CaM) to the holoenzyme, making the phosphatase catalytically competent.²⁹ Activated PP2B is well characterized in the brain, hence its alias calcineurin. Its Ca^{2+} sensitivity implicates it in a number of Ca^{2+} dependent processes including memory formation and neurogenesis, which have been well defined in the 30 years since its discovery.^{34,35} In contrast to the brain, the diversity of PP2B isoform expression is narrowed in the heart. In fact, only the α and β catalytic subunits, and the β regulatory subunit are translated in the mammalian heart.³⁶ In contrast to PP1 and PP2A, PP2B has highly selective pharmacological inhibitors such as cyclosporin A and FK506, which facilitated the early observation that the primary effector of PP2B is calcineurin-nuclear factor of activated T - cells (NFAT), a master regulator of gene transcription.³⁷ Consequently, inhibition of PP2B β prevents nuclear accumulation of NFAT. Left unabated, the cardiac consequences of nuclear NFAT accumulation are great.³⁶ This was elucidated using PP2B β overexpressing mice, which rapidly develop decompensated cardiac hypertrophy and die prematurely. The massive hypertrophic response observed was determined to be the result of NFAT activating the GATA-4 and MEF-2 families of transcription factors. Most strikingly, PP2B is necessary and sufficient to nucleate this process. This adverse signaling cascade now comprises the dogma of pathological hypertrophy. To that end, circumventing this process is a promising strategy to combat the cardiac dysfunction that occurs in a hypertrophic failing heart.

iv. Regulation—Considerable progress has been made in defining the physiological function of these three important phosphatases in the heart. However, the current difficulty facing the field is to understand the dynamics of phosphatase regulation, so that this wealth of information may be put to therapeutic use.³⁸ This presents an intriguing challenge, since the number of genes identified to encode phosphatases is dwarfed by the kinome by an order of magnitude.²⁹ Though the catalytic subunits of phosphatases arise from just a handful of genes, they regulate a multitude of cellular processes. In light of this, the off-target consequences of *global* phosphatase inhibition may detract from its therapeutic appeal. However, despite their multifunctionality, phosphatases do not act promiscuously. While it is true that the catalytic subunit of a phosphatase can often act on many substrates *in vitro*, cellular phosphatases exist as holoenzymes. The association of regulatory and targeting subunits promotes the formation of unique complexes in distinct cellular microenvironments. It is believed that there are over 100 PP1 regulatory subunits and over 50 for PP2A; new regulators continue to be discovered at an incredible rate.^{39,40} Taking into account the large number of molecular permutations made possible by these regulatory subunits, it is extremely likely that phosphatase complexes act with exquisite specificity in a cellular context.^{29,38} A cautious interpretation of findings from overexpression and knockout studies is therefore necessary, since it is unclear how well this specificity is preserved under conditions where these regulatory subunits may be saturated or otherwise

perturbed. Understanding the spatiotemporal underpinnings of this specificity is a budding area of research, since it could provide a means to contextually regulate phosphatases, and more accurately determine their contributions to these local signaling environments. Recent studies conducted on various members of the prototypical family of protein scaffolds, AKAPs, clearly demonstrate that these proteins have a significant role to play in phosphatase regulation. The AKAP-phosphatase interactions currently characterized in the heart, discussed herein, effectively demonstrate how signal propagation is regulated at multiple levels within the cell.

AKAP18: A Sarcoplasmic Reticulum Signaling Nexus

i. AKAP18 (AKAP15, AKAP7)—The name AKAP18 refers to a family of alternatively spliced AKAPs arising from a single AKAP7 gene. Like most AKAPs, AKAP18 preferentially regulates the RII subunit of PKA. After being cloned independently by two laboratories, AKAP18 was characterized as a low molecular weight AKAP highly enriched in the brain and heart.^{11,41} A combination of biochemistry and genetic manipulation revealed that it was targeted to the plasma membrane via a dual lipid modification (palmitoylation and myristoylation) on the N-terminal amino acid residues. This localization was discovered to facilitate binding and cAMP-dependent regulation of the LTCC.^{42,43,44} Several studies demonstrate that AKAP18 α 's regulation of CaV1.2 channels is translatable to the heart. In fact, the LTCC's of a transgenic mouse with ablated AKAP18/LTCC binding are not sensitive to cAMP stimulation, and the mice also suffer from cardiac hypertrophy⁴⁵.

Three other splice variants of AKAP18, β , γ , and δ , are also characterized. At 20kD, AKAP18 β is structurally and functionally similar to AKAP18 α , though it contains an additional 28 amino acids sufficient to target this isoform to the apical membrane in polarized epithelia.⁴² The physiological consequences of this unique targeting are not yet known. Of the larger isoforms, the 44kD AKAP18 γ was initially thought to have a predominantly cytoplasmic localization when it was discovered. However, recent studies link AKAP18 γ to the regulation of insulin secretion in INS-1E cells.⁴⁶ AKAP18 δ , which is completely conserved except for an additional 27 amino acids, is believed to localize to the inner medullary collecting duct of the kidney where it regulates Aquaporin-2 shuttling in response to vasopressin.⁴⁷ This suggests that large isoforms of AKAP18 may serve differential functions depending on the tissue type they are expressed in.

Further support for this idea comes from the important role these larger isoforms play in the heart. As previously mentioned, Phospholamban is the primary downstream target of β -adrenergic signaling on the SR membrane, and the efficacy of this PKA mediated effect is directly correlated to cardiac function.^{48,49} In 2007, a large isoform of AKAP18 was visualized by immunofluorescence to be in close proximity to PLB and SERCA2a. Immunoprecipitation and cAMP agarose purifications confirmed that an AKAP18-PLB-SERCA2a complex existed on the SR membrane of ventricular myocytes.⁵⁰ Recruitment of AKAP18 to the SR membrane is facilitated by its direct interaction with PLB. Importantly, ablation of this binding by peptide competitors resulted in a 50% reduction in β -adrenergic action on PLB, and significantly depressed SERCA2a activity.⁵⁰ Extending these findings, a new study suggests that AKAP18 complexes may have an expanded role in PLB regulation as coordinators of not only its upstream kinase (PKA), but also its opposing phosphatase PP1.⁵¹

ii. AKAP18 Regulates I-1, PP1 activity—Protein phosphatase inhibitor -1 (I-1) is the first characterized PP1 regulatory subunit that was identified over 30 years ago as a regulator of glycogen metabolism⁵². This small 19kD protein is remarkable for both its thermal stability and its specific, potent inhibition of PP1. The multiple phosphorylation

sites on I-1 dictate its inhibitory function, and its role as an amplifier of the β -adrenergic response. Upon phosphorylation on residue Thr35 by PKA, I-1 robustly inhibits PP1 activity by acting on the catalytic site of the phosphatase.^{40,52} Phosphorylation of other residues by PKC or Cdk5 preclude this effect.^{53,54} Although all reports indicate that I-1 expression in the myocardium is quite low, a number of studies have established that it has a significant role to play in cardiac function.^{55,56,57,58} For example, I-1 knockout animals have significantly depressed cardiac function that is attributed to enhanced PP1 activity on SR associated proteins.²³ These animals also exhibit a blunted β -adrenergic response. Conversely, a constitutively active form of I-1 improved cardiac function by potentiating PKA action on SR substrates.⁵⁹ This mouse model also boasts an improved prognosis following ischemia/re-perfusion injuries versus wild type. In light of these findings and others, several authors have suggested that I-1 could have therapeutic utility for the treatment of heart disease.^{26,60}

However, despite this demonstrated role in cardiac function, the spatiotemporal details of I-1's activation and activity remain unclear. In 2010, these details were demystified to some extent when I-1 was found to co-purify on cAMP-agarose with the RII subunit of PKA, with AKAP18 as the intermediary protein.⁵¹ Both AKAP18/I-1 and AKAP18/PP1 complexes were able to be isolated from rat heart homogenates. I-1 was found to bind directly to AKAP18. Since the large isoforms of AKAP18 are enriched at the SR via association with PLB, this was strong evidence that protein scaffolding could account, at least in part, for the distinctly SR associated effects of I-1 manipulation observed by other authors. In agreement with the AKAP paradigm, AKAP18 expression enhanced the phosphorylation of I-1 at Thr35 by PKA by 48% in a cell system. Interestingly, no binding was observed between recombinant AKAP18 γ and PP1c. It therefore appears that PP1's association with AKAP18 is not direct, but rather through an unknown accessory protein. Despite this, the I-1 mediated inhibition of PP1 activity associated with the complex was contingent upon PKA binding to AKAP18. Thus, although AKAP18 is not a PP1 regulatory subunit in itself, it does regulate phosphatase activity by coordinating the phosphorylation of I-1 at Thr35 (**Figure 2**). The direct interaction of AKAP18 with the unknown accessory protein(s), which recruit PP1 to the complex, could also play some role in phosphatase regulation as well, though this is highly speculative without knowing the identity of that protein. Nevertheless, it will be of great interest to see this model of PP1 regulation tested in cardiac myocytes. Its validation would poise AKAP18 as a bidirectional modifier of PLB phosphorylation, and a nexus of inotropic signaling on the SR membrane.

mAKAP: Signaling Node at the Nuclear Envelope

i. mAKAP—In 1999 the full length, 230kD mAKAP β protein was discovered and found to localize to the nuclear envelope of cardiac myocytes.¹⁰ An alternatively spliced transcript gives rise to mAKAP α , an isoform which contains an additional 244 amino acids that is expressed in the brain.⁶¹ Underlying its distinctly nuclear localization is a triple spectrin repeat domain, which binds and tethers mAKAP to the integral nuclear membrane protein Nesprin1 α .⁶² Due to its large size, mAKAP is known to coordinate the actions of many enzymes including the kinases PKA, MEK5, ERK5, PDK-1, and RSK3, the phosphatases PP2A and PP2B, and the phosphodiesterase PDE4D. Other associated signaling enzymes include the guanine exchange factor Epac, GTPase Rap1, the adenylyl cyclase AC5, transcription factor HIF1 α , and the ryanodine receptor (RyR2). Taking all of this into consideration, the primary function of mAKAP β is thought to be the integration of cAMP and Ca²⁺ signals in the perinuclear region of the cell.⁶³

ii. mAKAP Regulation of PP2A—mAKAP has been recognized to maintain a perinuclear cAMP gradient since it was discovered to bind the phosphodiesterase PDE4D3

nearly a decade ago.⁶⁴ This compartmentalization provides a functional barrier for an otherwise freely diffusible second messenger, which is important since cAMP can mediate a broad range of cellular processes.⁶⁵ As adrenergic receptor signaling attenuates, cAMP is hydrolyzed by PDEs in order to terminate the cellular response. Interestingly, mAKAP orchestrates a negative feedback loop that bolsters this process (**Figure 3**).⁶⁶ Scaffolding of PDE4D3 enhances its phosphorylation by PKA, which increases both its affinity for mAKAP and its catalytic activity towards cAMP. This prevents loitering of activated cAMP and PKA, and contributes to the temporal fidelity of receptor mediated signaling. Interestingly, these findings were recently extended in a manuscript reporting that PP2A regulates PDE4D activity associated the mAKAP complex.⁶⁷ Specifically, isolated mAKAP complexes had a 30% increase in PDE activity upon treatment with okadaic acid. The phosphatase mediating this was determined to be PP2A because treatment with a specific PP2A inhibitor (PP2A inhibitor-1) completely inhibited all phosphatase activity. Similarly, PP2A was discovered to co-precipitate with mAKAP from rat heart, and this association was required for modulation of mAKAP-PDE activity by okadaic acid. Treatment of mAKAP immunoprecipitates with PKI suggested that the activity of mAKAP bound PP2A was itself modulated by PKA. This was confirmed when the PKA sensitive, PP2A regulatory subunit, B56 δ , was found to co-immunoprecipitate with mAKAP.⁶⁷ This subunit has been characterized in the heart, where it localizes to the perinuclear regions of the cell.⁶⁸ These observations are in agreement with immunofluorescence data showing overlap of mAKAP and PP2A on the nuclear envelope.⁶⁷ In agreement with the PKA/AKAP precedent, B56 δ phosphorylation and inhibition of PP2A was enhanced by its binding to mAKAP. Collectively these findings demonstrate how regulation of an mAKAP anchored phosphatase, PP2A, can regulate phosphodiesterase activity and the spatiotemporal compartmentalization of cAMP signals in cardiac myocytes (**Figure 3**).

iii. mAKAP Regulation of PP2B—Following a discussion on the regulation of cAMP at the nuclear envelope, an obvious question that arises concerns the ultimate targets of PKA action in this region of the cell. In essence, what process is being regulated that would warrant such exquisite control? Aside from PKA, the first known binding partner of mAKAP was nuclear associated RyR2.⁶⁹ The function of the RyR is to release Ca²⁺ from intracellular stores, a process that is reported to occur with greater frequency upon phosphorylation at Ser2809. It was revealed using siRNA that mAKAP was involved in the regulation of RyR phosphorylation by PKA, and also in the regulation of this perinuclear Ca²⁺ pool.⁶² This is noteworthy since the Ca²⁺ sensitive phosphatase PP2B has also been found in immunoprecipitated mAKAP complexes isolated from rat heart, along with RyR2. Recent studies have expanded on these findings by demonstrating a direct interaction between mAKAP β and PP2B β . A model has been proposed in which adrenergic signaling acts through the mAKAP complex to phosphorylate RyR2, release Ca²⁺, and activate complex associated PP2B (**Figure 4**).⁶² Supporting this are in vitro studies showing that mAKAP β preferentially binds activated PP2B β . Chronic β -adrenergic stimulation is known to underlie human heart failure, and this effect can be recapitulated in cultured cells. In support of the proposed model, inhibition of PP2B (using cyclosporin A) or RyR2 (using ryanodine) blunted isoproterenol and phenylephrine induced hypertrophy in cultured myocytes, suggesting that in this context PP2B is not activated in the absence of mAKAP. Importantly, a comparable effect was observed when mAKAP/PKA anchoring was disrupted, suggesting that mAKAP coordinates pro-hypertrophic phosphorylation of RyR2.⁶² Most strikingly, however, it was found that mAKAP knockdown prevents the nuclear accumulation of NFAT in cultured cardiac myocytes that is characteristic of PP2B activation. Expression of a mutant mAKAP that cannot bind PP2B β produces the same effect⁷⁰. These observations that mAKAP is a necessary requirement for NFAT translocation and transcriptional activity highlight the importance of mAKAP/PP2B

interactions for the onset of cardiac hypertrophy, and potentially identify a new, druggable target that could specifically inhibit this process.

Yotiao: Dynamic Regulator of KCNQ1

Yotiao is a high molecular weight (230kD) AKAP that was originally discovered in the brain, where it was found to interact with the NR1 subunit of the NMDA receptor.⁷¹ It was subsequently revealed that Yotiao could interact with the type 1 inositol 1,4,5-trisphosphate receptor (IP₃) via the channel's leucine zipper motif and mediate its phosphorylation by PKA.⁷² However, regulation of the IP₃ receptor by this AKAP has not been studied in the heart. Nevertheless, a role for Yotiao in cardiac tissue was identified in 2002, when co-immunoprecipitation studies revealed that it organized a macromolecular signaling complex around the KCNQ1 potassium channel.⁷³ This channel is responsible for producing slow outward potassium currents responsive to β -adrenergic stimulation. Electrophysiological recordings determined that these currents are cAMP and okadaic acid sensitive only when KCNQ can interact with Yotiao.⁷³ Subsequent biochemistry revealed that Yotiao tethers both PKA, PDE4D, PP1 to the KCNQ channel in order to dynamically regulate its function through phosphorylation of Serine 27 (**Figure 5**).^{73,74} Proper function of this channel is known to play a critical role in cardiac physiology, since known human gene mutations disrupting its function result in inherited arrhythmia known as long QT syndrome. Interestingly, one characterized mutation in the Yotiao ablates binding to KCNQ1, which leads to uncoupling of β -adrenergic signaling and arrhythmia.^{75,76} This is the first example of an AKAP loss of function mutation discovered in humans, and highlights the importance protein scaffolding to cellular homeostasis.

Interactions of Unknown Cardiac Consequence

Other cardiac expressed AKAPs are known to bind phosphatases, though these interactions have not yet been studied in the context of the heart. For example, AKAP79 (AKAP5, AKAP150) is well studied in neuronal tissues, where it is believed to regulate the process of synaptic plasticity.^{77,78,79} Enzymes known to associate with AKAP79 include PKA, PKC, CaM, AC5, AC6, and PP2B. Yet despite the tremendous amount of work that has been done in neurons, few studies have specifically explored its role cardiac myocytes. It is believed that AKAP79 organizes a signaling complex around the LTCC in cardiac myocytes that is critical to the β -adrenergic response.⁸⁰ However, the role of AKAP79 associated PP2B in this tissue has yet to be established, as is a model reconciling the overlapping functions of AKAP79 and AKAP18 in LTCC regulation.

Conclusion

Though significant progress has been made in defining the role of AKAP-phosphatase complexes in cardiac function and dysfunction, many more are likely to be discovered and characterized in the coming years. However, even with respect to those that have been presently studied, many questions remain unanswered. For example, little is known about how AKAP signalosomes are dynamically regulated. Despite the potential for a single AKAP to bind multiple signaling enzymes simultaneously, it is still unclear if this is the case *in vivo*. Current technologies make this question difficult to answer definitively. Understanding the intricacies of phosphatase regulation by AKAPs is critically important, since it could ultimately reveal consequences of scaffolding on phosphatases that extend beyond mere targeting. For example, is an anchored phosphatase inhibited or active? If multiple enzymes are present in the complex, does AKAP binding direct phosphatase action towards one substrate vs. another and if so, how? It is likely that the answers to these questions will come from perturbing specific AKAP/phosphatase interactions. Unfortunately, traditional methods used to study protein/protein interactions, such as gene

silencing, are difficult to apply to these studies. Knockdown of an AKAP, for example, would affect the regulation of all proteins in the complex and could confound data analysis. Reagents are needed to achieve targeted disruption of some AKAP binding partners while leaving others intact. Peptide competition is one approach to achieve this goal. For example, the Ht31 peptide, derived from the amino acid sequence of an AKAP, is commonly used to outcompete all PKA/AKAP interactions. This strategy has been applied to specific AKAP complexes as well, as in the case of AKAP18/PLB. Recently, small molecule inhibitors have been explored and early findings appear quite promising.⁸¹ Identification of novel reagents is important given the enthusiasm surrounding AKAPs as therapeutic targets. One major problem facing drug design is specificity. In fact, the most commonly prescribed therapeutics to treat heart failure act broadly, with many off target effects. The next generation of therapeutics must be designed with greater appreciation for the distinct cellular microenvironments that utilize common signaling enzymes to different ends. This is especially true of the cardiovascular system, given the dual role of many signaling enzymes in both contractility and heart failure. The role of AKAPs in the formation and maintenance of these microenvironments is therefore of paramount importance, and a necessary consideration during therapeutic design.

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Figure 1. Schematic Diagram of Three Major Cardiac Phosphatases

A. Protein Phosphatase 1 (PP1) is a heterodimer consisting of a catalytic (PP1c) and regulatory (R) subunit. **B.** Protein Phosphatase 2A (PP2A) is a heterotrimer *in vivo* consisting of a scaffolding subunit (A), regulatory subunit (B), and catalytic subunit (PP2Ac). **C.** PP2B is a heterodimer consisting of a catalytic subunit (CnA), and a regulatory subunit (CnB). Activation of PP2B also requires binding of Ca^{2+} /Calmodulin (CaM).

Figure 2. AKAP18: A Sarcoplasmic Reticulum Signaling Nexus

Calcium reuptake into the SR occurs through the Sarcoplasmic Reticulum ATPase (SERCA2a). Phospholamban (PLB) is inhibitory to SERCA2a activity in its dephosphorylated state, which is maintained by Protein Phosphatase 1 (PP1). In response to β -adrenergic stimulation, PLB is phosphorylated by cAMP-dependent protein kinase (PKA) and inhibition of SERCA2a is alleviated. Inset: AKAP18 is localized to the SR membrane through direct association with PLB. This interaction enhances phosphorylation of PLB and enhances SERCA2a activity. AKAP18 was also found to bind to Protein Phosphatase Inhibitor 1 (I-1), and increase its phosphorylation at Thr35. This phosphorylation makes I-1 a potent inhibitor of complex associated PP1, which may promote phosphorylation of PLB.

Figure 3. mAKAP Regulation of PP2A and Nuclear cAMP Gradients

mAKAPβ is localized to the nuclear envelope through association with Nesprin1α. It recruits the phosphodiesterase 4D (PDE4D) and B56δ regulatory subunit of PP2A. In an unstimulated condition, PDE hydrolyzes cAMP to prevent activation of PKA. This process is maintained by PP2A, which keeps PDE4D dephosphorylated. Upon β-adrenergic stimulation, cAMP levels rise to release the catalytic subunits of PKA to phosphorylate complex associated proteins. Phosphorylation of PDE4D increases cAMP hydrolysis, and phosphorylation of B56δ inhibits PP2A activity. Collectively, these mAKAP orchestrated events facilitate transient increases in cAMP signaling.

Figure 4. mAKAP Regulation of PP2B and Induction of Hypertrophy

β-adrenergic stimulation activates mAKAP bound PKA, which phosphorylates the Ryanodine Receptor (RyR2) at Ser2809. This phosphorylation triggers the release of perinuclear Ca²⁺, which activates PP2B. PP2B dephosphorylates NFAT, leading to its nuclear translocation. Once inside the nucleus, NFAT activates hypertrophic gene expression. Displacement of PP2B from mAKAP curtails this adverse signaling event, and prevents the onset of hypertrophy.

Figure 5. Yotiao Regulates KCNQ1

The I_{ks} channel is responsible for gating inward current. The N-terminus of the α-subunit, KCNQ1, contains a phosphorylation site at Ser27. Phosphorylation of this residue by PKA increases channel activity. PP1 opposes phosphorylation at this residue. The AKAP Yotiao binds to KCNQ1 and recruits PKA and PP1 to the complex to regulate this phosphorylation/dephosphorylation. Ablation of Yotiao binding results in a loss of cAMP sensitive current.