AKAP12 Signaling Complex: Impacts of Compartmentalizing cAMP-Dependent Signaling Pathways in the Heart and Various Signaling Systems

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ABSTRACT: Heart failure is a complex clinical syndrome, represented as an impairment in ventricular filling and myocardial blood ejection. As such, heart failure is one of the leading causes of death in the United States. With a mortality rate of 1 per 8 individuals and a prevalence of 6.2 million Americans, it has been projected that heart failure prevalence will increase by 46% by 2030. Cardiac remodeling (a general determinant of heart failure) is regulated by an extensive network of intertwined intracellular signaling pathways. The ability of signalosomes (molecular signaling complexes) to compartmentalize several cellular pathways has been recently established. These signalosome signaling complexes provide an additional level of specificity to general signaling pathways by regulating the association of upstream signals with downstream effector molecules. In cardiac myocytes, the AKAP12 (A-kinase anchoring protein 12) scaffolds a large signalosome that orchestrates spatiotemporal signaling through stabilizing pools of phosphatases and kinases. Predominantly upon β-AR (β2-adrenergic-receptor) stimulation, the AKAP12 signalosome is recruited near the plasma membrane and binds tightly to β-AR. Thus, one major function of AKAP12 is compartmentalizing PKA (protein kinase A) signaling near the plasma membrane. In addition, it is involved in regulating desensitization, downregulation, and recycling of β-AR. In this review, the critical roles of AKAP12 as a scaffold protein in mediating signaling downstream GPCRs (G protein–coupled receptor) are discussed with an emphasis on its reported and potential roles in cardiovascular disease initiation and progression.

Key Words: adrenergic receptor ■ AKAP12 ■ compartmentalization ■ gravin ■ PKA ■ signaling pathways ■ signalosome

AKAPs (A-kinase anchoring proteins) (AKAPs) belong to a family of scaffolding proteins that organize complex signal transduction events descending from cell surface–stimulated receptors. AKAPs mediate sequestering of protein kinases, phosphatases, and signal termination molecules, with their target substrates. Thus, AKAPs coordinate phosphorylation and dephosphorylation states within the cell to confer the specificity of intracellular events. When first identified, regardless of the general structural diversity, they were functionally characterized on the basis of their ability to bind to PKA (protein kinase A). This binding capacity is mainly attributed to their highly conserved amphipathic helix that anchors to PKA regulatory subunits. In addition, AKAPs localize to diverse subcellular locations, including, but not limited to, the plasma membrane, mitochondria, nuclear envelope, and cytoskeleton.

Briefly, on their binding to the regulatory subunit of PKA, AKAPs localize the activation of PKA catalytic subunits, utilizing the specific targeting sequences located near their N terminus. This explains how such a common signaling pathway (PKA signaling) can serve many different functions with high selectivity.
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Nonstandard Abbreviations and Acronyms

| AKAP      | A-kinase-anchoring protein |
|-----------|---------------------------|
| β-AR      | β-adrenergic receptor     |
| ERK       | extracellular signal–regulated kinase |
| GPCR      | G protein–coupled receptor |
| PKA       | protein kinase A          |
| PKC       | protein kinase C          |
| PLN       | phospholamban             |
| SERCA     | sarco/endoplasmic reticulum |
| Ca^{2+}–ATPase |                         |
| SR        | sarcoplasmic reticulum    |
| VIP       | vasoactive intestinal peptide |

However, later studies have shown that these scaffold proteins further can bind to other substrates and even homo- or heterodimerize/oligomerize, indicating a more complex spatial and temporal signaling profile.9,10–12

Kinases and phosphatases are key regulators of enzymatic activities and protein interactions. AKAPs colocalize these enzymes by playing a major role in influencing cellular activities, where any deviation from their normal function may induce states of imbalance and disease. Several AKAPs have been identified within the past decade. However, their full function/mechanism of action in different systems under physiologic conditions and possible involvement in pathophysiologic states is not fully understood. Thus, in this review we covers some of the major AKAPs, with a stronger emphasis on AKAP12 and its possible associated cardiovascular pathways/effects.

AKAP–PKA COMMUNICATION PLATFORM

Recent studies have shown that distinct AKAPs bind to different protein kinases/phosphatases, uniquely colocalizing them to their targets. An exception is PKA, which is the common protein kinase that all AKAPs share the capacity to bind.3 Considering the diverse network through which PKA signals, it is peculiar how it can still be highly specific to its targets. Further understanding of binding/signaling mechanisms through PKA gives insights into the general AKAP signalosome.

Briefly, PKA holoenzyme (a heterotetramer) contains 2 possible regulatory subunits, either RI or RII.13 The regulatory subunits keep the enzyme in a dormant state at low levels of cAMP through inhibiting the 2 catalytic subunits (PKAc).13 When PKAc subunits are activated, they phosphorylate serine/threonine residues on their multiple target substrates, triggering downstream signaling pathways.14

To manage a more distinct signal specificity of such diverse pathways, adjustments take place within the cell. Such adjustments include sequestration of AKAP complexes near their targets, a local increase in cAMP levels, and the appearance of termination mechanisms.13,15 Although the local increase of cAMP levels postactivation of the upstream regulators of PKA may assure some level of specificity, yet another signal termination mechanism must exist to maintain homeostasis. AKAPs assure this through not only linking PKA to its upstream modulators but also to signal termination molecules such as phosphodiesterase through scaffolding to them as well.16

So far, all studied AKAPs contain the highly conserved sequence of 14 to 18 amino acids, usually Gln4–Lys21, which forms a 5-turn amphipathic (alpha) helix.17 This helix is important because of its ability to slide into the binding pocket near the N terminus of PKA.17,18 The majority of AKAPs preferentially anchor the RII dimer of the PKA holoenzyme.19 However, D-AKAP1 and D-AKAP2 are capable of binding to either subtype of PKA-regulatory subunits.19 Furthermore, recent studies have indicated that AKAP11 and sphingosine kinase–interacting protein selectively bind to the RIα subunit of PKA, forming a dynamic cytoplasmic signaling complex.14,20 However, it remains unknown whether AKAP anchoring to either RI or RII subunits of PKA has differential effects on its signaling outcomes. Nonetheless, it is established that the binding of AKAPs to the RI or RII subunits neither affected the tertiary nor the quaternary conformations of the dimer.18 On the contrary, exposure to elevated levels of cAMP induces such change.21 Thus, AKAPs are not essential for the phosphorylation/activation of PKA. Rather they are crucial to spatially restrict the activation of PKA to areas where cAMP levels are high with the stimulation of upstream proteins/receptors.21

As mentioned previously, AKAPs can directly bind and sequester various signaling proteins. AKAPs can bind protein kinases (PKC [protein kinase C] and mitogen-activated protein kinase), phosphatases, cAMP phosphodiesterase (PDE), guanosine triphosphate–binding proteins, and adaptor proteins. PDEs reduce the local levels of cAMP available for triggering downstream signaling pathways.22 Considering that PKA is mainly affected by cAMP levels, crosstalk between PKA and PDE is crucial to forming a feedback loop, which is believed to be mainly controlled through their colocalization by AKAPs at the same subcellular compartments.23
Another key signaling transduction mechanism that ultimately activates nuclear transcription factors is regulated by the calcium–calmodulin-dependent serine–threonine protein phosphatase 2B/calcineurin. Certain AKAPs, such as AKAP79/150, anchor protein phosphatase 2B/calcineurin to facilitate the downstream signaling. Ultimately, this signaling complex activates effector molecules within the nucleus, including the nuclear factor of activated T cells. This diversity of AKAPs and their complexes indicates their ability to control a vast array of opposing signaling pathways within the cells to maintain homeostasis. Regardless, the exact signaling mechanism driving their ability to compartmentalize the complexes near their targets is still speculative because of the high structural diversity of AKAPs and their anchoring domains. It is worthy of mention that the presence of adenyl cyclase subtypes reported in some AKAP (AKAP79, Yotiao, and mAKAP) complexes demonstrates a possible constitutional adenyl cyclase–AKAP binding that maintains these unique clusters near their targets at basal levels where cAMP will be increased once the upstream receptors are stimulated. However, this scheme cannot be generalized for all AKAPs. For instance, myristylation of N-terminal sequences, along with the presence of basic amino acid–rich regions (polybasic domains) within the N terminus on AKAP12 is sufficient to mediate this localization near the β2-AR.

Notably, the AKAP12 association with PDE4D3 and β2-AR (β2-adrenergic receptor) recruitment was enhanced with activation of PKA. PDE4 is exceptionally important in cardiovascular signaling, which is delineated by its signaling under cardiac diseases. For instance, under cardiac hypertrophy, PDE4 activity is decreased and associated with propagation of heart failure. Furthermore, acute PDE inhibition enhances cardiomyocyte function, whereas chronic inhibition results in increased mortality. This complexity may be explained by the compartmentalized controls of cAMP levels by PDE4, in an agonist-dependent manner downstream of β2-AR. Such properties make PDE4 a favorable partner for many AKAPs.

**AKAP FUNCTIONAL ROLES**

Generally, signaling interactions are governed by the global abundance of proteins in addition to their specific interactions. The specificity of such interactions is fundamental to maintain homeostasis and is controlled by the organized subcellular microdomains, coordinated by several scaffolding proteins, some of which are AKAPs. AKAPs dictate how, when, and where each member of their complexes will initiate a

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**Table 1. AKAP-Reported Functions in Various Signaling Systems**

| System         | AKAP             | Effect                                                                 | Mechanism                                                                 |
|----------------|------------------|------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Nervous32–34   | AKAP5 (AKAP79/150) | Promotes synaptic plasticity; essential for NMDA receptor–mediated LTD and normal motor coordination | Scaffolding of PKA, PKC, and PP2B to phosphorylate glutamate receptors       |
| Immune35,36    | Ezrin            | Suppression of T-cell replication                                       | Scaffolding of PKA to phosphorylate C-terminal Src kinase                  |
| Reproductive37–39 | D-AKAP1 | HIV progression             | Cofactor of HIV reverse transcriptase                                        |
| Reproductive37–39 | AKAP1 | Defective maturation of ovaries                                         | Disrupted association of PKA to AKAP1                                     |
| Reproductive37–39 | AKAP3 | Impaired sperm motility                                                 | PK3 interference with AKAP3–PKA binding                                    |
| Reproductive37–39 | AKAP4 | Impaired sperm motility                                                 | Failure of association of glycolytic enzymes to the fibrous sheath        |
| Endocrine38,39 | AKAP79/150       | Regulation of insulin secretion                                         | Reduction of PP2B activity                                                 |
| Endocrine38,39 | AKAP18α/γ        | Enhance/reduction of insulin secretion respectively                      | Controlling glucagon-like peptide–1–mediated insulin secretion             |
| Renal40,41     | AKAP185          | Trafficking of aquaporins AQP2 toward plasma membrane                  | PKA Phosphorylation at serine (S)256, S261, S264, and S269 at AQP2        |
| Respiratory42   | AKAP-Lbc         | Redistribution of AQP2 from intracellular vesicles to the periphery of medullary collecting duct principal (IMCD) cells | PKA phosphorylation of RhoA at S188 inducing its inhibition               |
| Gastrointestinal43–45 | AKAP150 | Regulation of pepsinogen secretion                                      | Unclear                                                                    |
| Liver47        | Ezrin            | Gap junction modulation                                                  | PKA phosphorylation of connexin 43                                        |

AKAP indicates A-kinase anchoring protein; AQP2 aquaporins; LTD, long-term depression; NMDA, N-methyl-D-aspartate receptor; PKA, protein kinase A; PKC, protein kinase C; PP2B, protein phosphatase 2B; Stx3, syntaxin; NF-κB, nuclear factor-kappa light-chain enhancer of activated B cells; PK3, phosphoinositide 3; SRC, proto-oncogene tyrosine–protein kinase; Stx3, syntaxin 3; and RhoA, Ras homolog family member A.

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J Am Heart Assoc. 2020;9:e016615. DOI: 10.1161/JAHA.120.016615
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certain action. Usually, under pathophysiologic conditions, the global balance of signaling molecules, such as cAMP and PKA, is not affected. Rather imbalances within certain AKAP complexes are evident, thus making them valuable therapeutic targets. In what follows we briefly cover the involvement of different AKAPs in variable systems (Table 1), followed by a more detailed discussion of their functions in the cardiovascular system.

AKAPS AND CARDIOVASCULAR SYSTEM

Cardiac function is strongly impacted by the adrenergic system, mainly through the interaction of catecholamines, with β-ARs controlling both contractility and heart rate.48 Such distinct cellular events are mainly controlled via PKA followed by the secondary messenger cAMP, on stimulation of GPCRs (G protein-coupled receptors).49 Importantly, several reports showed that cAMP levels increase within specific cellular compartments, as a “local increase” in a stimulus-induced manner, assuring some level of specificity.50–52 Also, multiple AKAPs have been associated with the compartmentalization of cardiac signaling pathways. Of the >50 AKAPs characterized so far, several have been associated with cardiac development, contractility, cardiac morphology, and rhythm (Table 2).53–64

Cardiac Development

During cardiac development (Figure 1), AKAP-LBC (AKAP13) is postulated as a key regulator of cardiomyocyte differentiation and morphogenesis.65 The mechanism of this regulation of differentiation and morphogenesis is believed to be through producing a platform that links Ga12 and Rho signaling to an essential transcriptional program, as confirmed by the observed embryonic lethality upon deletion of AKAP13 in mice.66

Cardiac Contractility

Regarding cardiac contractility (Figure 2), normal contraction of the heart is controlled by a delicate regulation of cytosolic Ca²⁺ levels through a complex interplay between the ryanodine receptor, L-type calcium channels, SR (sarcoplasmic reticulum), and SERCA (sarcoplasmic/endoplasmic reticulum Ca²⁺-adenosine triphosphatase). Several AKAPs have been involved in facilitating the proper signal transduction within these complexes, such as AKAP79/150, AKAP12, AKAP15/18, mAKAP, and AKAP185. For instance, Ca²⁺ signaling on the surface of the cell was linked to AKAP15/18 ability to target the PKA to the C terminus of L-type voltage-gated calcium channel channels. Nuclear Ca²⁺ signaling is regulated through mAKAP acting on the nuclear membrane, whereas AKAP185 affects SR levels of Ca²⁺.66 mAKAP forms macromolecular complexes, composed of PKA, PDE4D3, adenylyl cyclase 5, protein phosphatase 2A, mitogen-activated protein kinase 5, ERK5 (extracellular signal–regulated kinase 5) Rap1, and Epac1.67–70 The strategic location of this complex on the nuclear membrane allows mAKAP to promote the opening of ryanodine receptor channels located on the nuclear envelope (through targeting

Table 2. Cardiac AKAP-Reported Functions

| AKAP       | Alternative Names | Effect                               | Mechanism                                                                 |
|------------|------------------|--------------------------------------|---------------------------------------------------------------------------|
| AKAP145    | D-AKAP, AKAP121  | Reduction of ROS in the heart        | Increase SOD2 mitochondrial expression                                    |
| AKAP575    | AKAP79, AKAP75, AKAP150 | Increase ROS, and inflammatory responses | Activation of PKC                                                         |
| AKAP674    | AKAP100, mAKAP   | Regulation of cardiomyocyte oxygen homeostasis | Enhances the transcriptional activation of HIF-1α-regulated genes         |
| AKAP775    | AKAP15, AKAP18   | Regulation of calcium handling        | Coordinates PKA phosphorylation of PLN                                    |
| AKAP976    | Yotiao           | Increases the slow outward potassium ion current | Targeting PKA and PP1 to hKCNQ1                                         |
| AKAP1057   | D-AKAP2          | Regulation of cardiac rhythm         | Unknown                                                                   |
| AKAP125,67,61 | Gravin, SSceKS  | Cardiac pathophysiology (to be defined) | Unknown                                                                   |
| AKAP135,62 | AKAP Lbc         | Mediates metabolic switch during the development of compensatory hypertrophy | Activation of PKD1                                                        |
| Synemin63  | SYNM             | Maintenance of normal ventricular function | Unknown                                                                   |
| Myosprin64 | CMYA5            | Modulates the clustering of RyR       | Unknown                                                                   |

AKAP indicates A-kinase anchoring protein, HIF-1α, hypoxia-inducible factor-1α; hKCNQ1, human potassium voltage-gated channel subfamily Q member 1; PKA, protein kinase A; PKC, protein kinase C; PKD1, polycystin 1; PLN, phospholamban; PP1, protein phosphatase 1; ROS, reactive oxygen species; RyR, ryanodine receptor; and SOD2, superoxide dismutase 2.
PKA to these channels using leucine/isoleucine zipper motifs within the mAKAP, impacting both Ca\textsuperscript{2+} rise and perinuclear flux to further increase cardiac function.\textsuperscript{66,71} Historically, mAKAP was thought to play a crucial role in cardiac contractility on the basis of studies identifying its localization on the SR.\textsuperscript{72,73} However, later studies alternatively have established that mAKAP is located on the nuclear membrane.

**Figure 1. Role of AKAP13 in cardiac function.**

GPCR signaling is directed by the type of ligand binding to this receptor. The nature of the ligand would favorably shift the equilibrium to a specific conformation of the receptor, which signals through a distinct G protein. For AKAP13 and GPCR signaling in the heart, it has been associated with 2 distinct G protein–signaling pathways (G\textsubscript{q12} and G\textsubscript{as}).

A. When stimulation of \( \beta \)-AR stabilizes its conformation to signal through G\textsubscript{q12}, AKAP13 signalosome is attracted near the receptor. AKAP13 interacts with G\textsubscript{q12} allowing Rho-GEF stimulation. Upon Rho-GEF stimulation, both SRF (Rho-GEF guanosine triphosphatase) and myocyte enhancer factor 2C (transcription factor) activities are increased. However, the mechanism of their activation depends on different regions of AKAP13. Activation of myocyte enhancer factor 2C depends on the carboxyl region of AKAP13 (BRX), whereas SRF activation depends on the GEF region of AKAP13. Collectively, this suggests that increased gene expression of factors associated with cardiac development may be partially dependent on AKAP13.\textsuperscript{65} B. AKAP13 has been reported in the cardiac hypertrophic signaling. Under states of increased hemodynamic load, it is usually accompanied by a favorable state of G\textsubscript{as} pathway signaling within the cardiomyocytes. AKAP13 signalosome releases PKD to phosphorylate HDAC5. Phosphorylation of HDAC5 activates MEF2, which drives the transcription of genes associated with cell growth, Ca\textsuperscript{2+} handling, and contraction. The aforementioned multistep process of PKD release starts with PKA activation as a response to the G\textsubscript{as} pathway. PKA further phosphorylates PKC. Phosphorylation of PKC activates PKD, leading to its dissociation from the signalosome.\textsuperscript{62} \( \alpha \textsubscript{12} \) indicates \( \alpha \)-subunit of G\textsubscript{q12} protein; \( \alpha \)-subunit of G\textsubscript{as} protein; \( \beta \)-AD, \( \beta \)-adrenergic receptor; AKAP, A-kinase anchoring protein; GPCR, G protein–coupled receptor; HDAC5, histone deacetylase 5; MEF2C, myocyte enhancer factor 2C; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; PM, plasma membrane; Rho-GEF, Rho guanine nucleotide exchange factor; and SRF, serum response factor.
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Figure 2. Role of AKAP complexes in cardiac contractility.
Cardiac contractility is controlled mainly by the delicate interplay between RyR, calcium channels (Ca\(^{2+}\), v1.2), SR, and SERCA. On electrical stimulation, the voltage-gated Ca\(^{2+}\) channels increase Ca\(^{2+}\) influx, leading to local areas of high Ca\(^{2+}\) (1). The local increase of Ca\(^{2+}\) activates the RyR channels located on the SR (2). The RyR channels activation increases Ca\(^{2+}\) efflux from the SR, causing a cytosolic (global) increased Ca\(^{2+}\) (3). The global increase in Ca\(^{2+}\) triggers muscle contraction cycle, as a result of Ca\(^{2+}\) binding to troponin (4). SERCA plays a major role in maintaining consistency in the contractile force between cardiac beats. As a response to high cytosolic levels of Ca\(^{2+}\), SERCA reuptakes Ca\(^{2+}\) back into the SR to return to baseline levels in the cytoplasm. SERCA activity is controlled by PLN, which, when phosphorylated by PKA, allows SERCA to reuptake Ca\(^{2+}\). AKAPs play a crucial role in each of these phases; AKAP15/18 facilitates Ca\(^{2+}\) influx through targeting PKA to the C terminus of Ca\(^{2+}\), v1.2 channels, AKAP79/150 binds PKA and PKC\(\alpha\) to Ca\(_{1,2}\) channels to facilitate their normal gating, and AKAP18\(\delta\) mediates PKA phosphorylation of PLN causing it to detach from SERCA, thus allowing the reuptake of Ca\(^{2+}\). AKAP indicates A-kinase anchoring protein; Ca\(^{2+}\), v1.2, calcium channel; Na\(^+\)/Ca\(^{2+}\) exchanger, sodium–calcium exchanger; PKA, protein kinase A; PKC, protein kinase C; PLN, phospholamban; PM, plasma membrane; RyR, ryanodine receptor; SERCA, sarco/endoplasmic reticulum Ca\(^{2+}\)-adenosine triphosphatase; and SR, sarcoplasmic reticulum.

Cardiac Hypertrophy

Cardiac hypertrophy, defined as an increase in cardiomyocyte size (adaptive remodeling of the heart) under stress or continuous stimulation, can develop into further pathophysiologic states, such as cardiac rhythm is potentially achieved through binding PKA and PKC\(\alpha\) to L-type voltage-gated calcium channels to facilitate their normal gateing, thus protecting against arrhythmias.\(^{79}\) Moreover, Yotiao (AKAP9) regulates normal cardiac rhythm by recruiting PKA and protein phosphatase-1 to the C-terminal subunit of the slowly activating delayed rectifier potassium channels, where these channels are responsible for conducting ionic currents.\(^{80}\) Finally, D-AKAP2 (AKAP10) was proposed to control pacemaker sensitivity to cholinergic stimulation.\(^{57}\) It is has been proposed that a coordinated interplay between these 3 AKAPs (AKAP79/150, Yotiao, and D-AKAP2) regulates heart rhythm in distinct yet interconnected pathways.

rather than SR and it acts on the nuclear ryanodine receptor.\(^{74,75}\) Considering the differences in the signaling between cytoplasmic and nuclear Ca\(^{2+}\) pools, mAKAP is primarily involved in gene expression regulation rather than cardiac contractility.\(^{76}\)

AKAP18\(\delta\) influences SERCA2 needed for reuptake of Ca\(^{2+}\) from the cytoplasm to assure consistency of cardiac contractile force within each beat. Briefly, SERCA2 activity is affected by calcium gradients, level of SERCA2 expression, and the levels of the SERCA2 suppressor PLN (phospholamban). In this manner, AKAP18\(\delta\) mediates PKA phosphorylation of serine 16 on PLN, dissociating it from SERCA2, which subsequently increases the uptake of Ca\(^{2+}\) into the SR and enhances cardiac function.\(^{55}\) Other AKAPs, such as synemin\(^{77}\) and myomegalin, have also been proposed to play a role in cardiac contractility because of their sarcomere intracellular localization.\(^{77,78}\)

In addition to the aforementioned function of AKAP79/150 in cardiac contractility, it has been also linked to cardiac rhythm regulation.\(^{79}\) This regulation of
as heart failure. Thus, the involvement of mAKAP, AKAP79/150, and AKAP-LBC was examined in mediating the pathologic state of cardiac hypertrophy. So far, mAKAP and AKAP79/150 activation has been associated with nuclear translocation of nuclear factor of activated cells, promoting the transcription of hypertrophic genes. In addition, mAKAP activates the mitogen-activated protein kinase pathway in a cAMP-dependent manner, which entails further activation of the prohypertrophic factor myocyte enhancer factor 2C. 

Alternatively, AKAP-LBC (AKAP13) facilitates PKC/PKD coupling to inactivate histone deacetylase to induce cardiac hypertrophy (Figure 1). 

Together, it is evident from the accumulated studies that AKAPs play a major role in controlling many aspects of cardiac function, and that any alteration within their complexes may contribute to the development of cardiac disease. This notion is supported by the important roles reported on other AKAP signalosomes in developing cardiac hypertrophy. For instance, in heart failure there is upregulated gene expression of AKAP-LBC, AKAP18, and AKAP2, whereas the AKAP121 gene was downregulated.

Interestingly, AKAP12 (detailed in what follows) knockout ameliorated cardiac function in an animal heart failure model; however, the exact mechanism is not yet fully understood, and more research is needed to elucidate this observation.

Importantly, AKAPs may be involved in alterations of spatial localization of β-AR in heart failure. In failing hearts, β-AR signaling is redistributed from T tubules to cell crest. As a result, β-AR uncouples localized PKA pools, mediating a cell-wide rather than localized cAMP propagation. Such alteration in signaling further propagates the heart failure phenotype. As of now, roles of AKAPs in mediating such responses in cardiomyocytes are remain unknown.

β-AR dysregulation is associated with heart failure. Stimulated β-ARs couples with Gas and can subsequently switch to Gαi, regulating cAMP levels, PKA activity, and phosphorylation of several downstream pathways. Alterations in β-AR desensitization and resensitization play a major role in development of heart failure. Mechanisms of β-AR desensitization are well characterized, but few studies have investigated β-AR resensitization schemes. Notably, one study reported the importance of Ser-355/356 phosphorylation in regulating physiologic resensitization in neonatal cardiomyocytes. Another study using an integrated whole-cell response microfluidic system suggested the need for Src-regulated processes and dynamin (cytoskeleton-associated protein) for β-AR de/resensitization. Elevating AKAP12 levels reduced apoptosis of cardiomyocytes understimulated ischemia, through its action on dynamin-like receptor-1.

Accordingly, reports of failing human hearts showed diminished levels of AKAP12.

Collectively, AKAP complexes contain multiple proteins that play a significant role in regulating β-AR signaling and can be a valuable therapeutic target, as they contribute to mediating several cardiac functions. Thus, future studies should focus on the biochemical details of these protein networks and their involvement in both physiologic and pathophysiologic aspects.

**AKAP12 IS AN A-KINASE ANCHORING PROTEIN**

AKAP12 was first recognized as an autoantigen in serum from myasthenia gravis patients, hence the alias name (Gravin). It is also known as the Src-suppressed C kinase substrate, which is considered the rodent ortholog of the human identified Gravin; however, these terms are used interchangeably. This 250-kd multivalent AKAP interacts with PKA, along with several other molecules and signaling proteins such as PKC, β-AR, PDE4D, β-1,4-galactosyltransferase, non-receptor tyrosine kinase Src, and Ca2+/calmodulin. The AKAP12 signalosome plays a central role in organizing (GPCRs) to protein kinases and phosphatases. In particular, it targets PKA and other signaling molecules to the cell periphery near β-ARs, to regulate its resensitization and recycling.

It has been proposed that AKAP12 subcellular distribution is dynamic and affected by intracellular Ca2+ concentration, in addition to the level of PKC activation. This introduces the possibility of crosstalk between PKA, PKC, Ca2+/calmodulin, and potentially other additional molecules.

Upon evaluating AKAP12’s structure (Figure 3), domains responsible for its targeting to the cell periphery are believed to be the N terminal’s myristoylation sites along with its 3 polybasic domains located near the N terminus. However, the exact mechanism is still unclear. In particular, one study reported that the presence of the polybasic subunits was sufficient to only dissociate AKAP12 from the cell periphery in response to phorbol ester treatment, whereas the putative N-terminal myristoylation was required for AKAP12’s redistribution to intracellular vesicular compartments. Yet another study demonstrated that the presence of any 2 polybasic domains, as described in their constructs lacking the myristoylation sites, was sufficient for targeting AKAP12 to the cell periphery and subsequent regulation of β-AR resensitization.

Furthermore, 3 distinct isoforms of AKAP12 (α, β, or γ) have been identified so far and they share
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Figure 3. AKAP12 schematic topological map shows the known and potential protein–protein binding and phosphorylation domains.

2–7 coiled coils indicates 2–7 alpha-helices usually involved in gene expression; β2-AR indicates β2-adrenergic receptor; BD, binding domain; EGF, epidermal growth factor; GalTase, β-1,4-galactosyltransferase; PKA, protein kinase A; PKC, protein kinase C; SRC, non-receptor tyrosine kinase; and WSK, short motifs composed of 3 conserved residues found in a WXSXXK motif.

>95% amino acid sequence identity, with variability between the isoforms only shown to be within their N terminus. Thus, having 3 AKAP12 isoforms can potentially explain the distinct spatial distribution isoform profiles such that the myristoylation of an AKAP12α is necessary for targeting it to the endoplasmic reticulum. Notably, these isoforms are independent transcripts under the control of separate promoters, showing distinct spatiotemporal mRNA expression in different organs.

In summary, these 2 features of AKAP12—(1) the presence of many docking/binding domains for kinases/phosphatases and (2) its broad expression—drives its ability to influence cellular signaling pathways in many systems. Thus, these cellular signaling roles of AKAP12 are discussed in more detail, along with an emphasis on its cardiac capacities, in what follows.

AKAP12 SIGNALING IN CELLULAR SYSTEMS

AKAP12 is probably one of the most studied AKAPs in cell cycle regulation. It is known as a negative regulator of the G1/S-phase transition and important for the completion of cytokinesis through altering expression levels of cyclin D1 by either blocking its synthesis or scaffolding PKC to control the formation of actin–myosin rings. Accordingly, AKAP12 was intensively studied in the cancer field and reported as a metastasis suppressor in a variety of cancers, such as prostate and skin cancers. Metastasis suppression has been attributed to attenuating tumor-intrinsic PKC and Src pathways or reducing the secretion of tumor chemotacticants in the peritoneum. In contrast, a study examining the mechanism underlying glioblastoma multiform reported that patients with higher AKAP12 mRNA levels had overall worse survival rates. Although AKAP12 expression was shown to be downregulated in several cancers, including prostate, breast, gastric, skin, and colon cancers, in addition to hepatocellular carcinoma and melanoma, its upregulation may still be a risk factor in other types of cancer.

When examining the roles of AKAP12 in endothelial tissues, it has been proposed that vascular endothelial dysfunction, generated by lipopolysaccharide treatment, induces upregulation of AKAP12. Such upregulation of AKAP12 may improve endothelium-dependent relaxation through its binding to the atypical isoform of PKC (PKCζ), a well-known pathologic mediator of endothelial dysfunction. Upon binding, AKAP12 inhibits PKCζ-mediated reduction of the ERK5 pathway. Furthermore, depletion of AKAP12 was shown to increase pro-inflammatory proteins (tumor necrosis factor-α, interleukin-1β, and interleukin-6) and reactive oxygen species formation while reducing both endothelial nitric oxide synthase expression and ERK5 transcription. This suggests the potential participation of AKAP12 in altering cytoskeletal morphology during inflammation.
In addition, lipopolysaccharide-induced inflammatory response in astrocytes has revealed that the β1,4-galactosyltransferase-I–AKAP12 complex colocalizes in the perinuclear region at either basal levels or in the presence of lipopolysaccharide. The complex was directed to the trans-Golgi network, where astrocyte migration was inhibited during lipopolysaccharide treatment. This may be explained by inhibition of β1,4-galactosylation of integrin-β1, suggesting that AKAP12 integrates the various functions attributed to the galactosyltransferase I cytoplasmic domain.

In line with these findings, AKAP12 has been associated with macrophage regulation during the convalescent stage of inflammation. In particular, this was shown by AKAP12 activating the M2 macrophages (anti-inflammatory type), which then induced a more compact extracellular matrix. This was further confirmed by the positive correlation between both CD206 (M2 marker) and AKAP12 expression in humans and animal models. Interestingly, a recent study investigating GPCR-mediated transactivation of epidermal growth factor receptor in epithelial cells showed that AKAP12 stabilized naked cuticle homolog 2 by facilitating PKA phosphorylation of Ser-223 on naked cuticle homolog 2. This further ensured efficient cell-surface delivery of transforming growth factor-α to increase epidermal growth factor receptor activation. Furthermore, AKAP12 knockout canceled baseline phosphorylation of the naked cuticle homolog 2 domain and reduced it in response to forskolin, VIP (vasoactive intestinal peptide), and prostaglandin estradiol, stimulating a potential constitutive phosphorylating capacity of AKAP12 within its domains. Considering the homeostatic capacity of epithelial cells within multiple organs, further studies are crucial to examine such constitutive activity.

Within the pulmonary smooth muscles, the main signaling pathways are governed by coupling of the G protein, either Gas or Gαq, to β-ARs. The Gαq pathway controls smooth muscle contraction and tone through activating phospholipase C and diacylglycerol, whereas the Gas pathway causes local elevation of cAMP, which further causes activation of PKA and Epac in airway smooth muscles to influence contraction/relaxation of these muscles. AKAP12 and AKAP5 are the main mediators of compartmentalizing phospholipase C, diacylglycerol, and PKA near the β2-ARs and muscarinic receptor 3. AKAP5 increases G protein–coupled receptor kinase 2 recruitment to the cell surface, causing β2-AR desensitization and internalization. AKAP12, however, is not involved in agonist-specific desensitization or the internalization of GPCRs. Instead, it is responsible for the dephosphorylation, resensitization, and recycling of these receptors from intracellular vesicles back to the periphery. Thus, a balance between these AKAPs in addition to AKAP78 (involved in β2-AR internalization) is needed for proper function of the airways.

In addition, both AKAP12 and AKAP5 have been proposed to be regulators of pulmonary endothelial barrier function, and AKAP12 is required for this cAMP-mediated barrier stabilization. However, a study examining the mechanism of cigarette smoke extract deterioration on airway endothelial function showed that mRNA expression levels of AKAP12 and AKAP5 were not affected, whereas a reduction in expression levels of another AKAP member (AKAP9) was noted and associated with reduced E-cadherin levels. Interestingly, another study linking hypoxia in endothelial cells, widely implicated in many pathologic conditions, has also been linked to elevated AKAP12 gene expression, which is in contrast to other members of the AKAP family (AKAP1, AKAP17A, AKAP79, and AKAP100). Moreover, an analysis of the cloned promoter of AKAP12 revealed a functional hypoxia-responsive domain with 2 binding sites for hypoxia-inducible factor 1.

Furthermore, AKAP12 gene expression has also been inversely associated with estrogen receptor gene expression, particularly Erβ. In Erβ-null granulosa cells, upregulation of AKAP12 genes was identified as a mechanism that reduced cAMP levels through combating the sequestration of PKA regulatory subunits. This was further supported in a study examining the agonist-dependent AKAP12 role in regulating cAMP levels. The study demonstrated that AKAP12 expression is crucial to allow (human embryonic kidney 293 cell line and human epidermoid carcinoma cell line) cells to recover their cAMP response to the agonist.

Overall, it is apparent that AKAP12 plays a critical role in regulating multiple signaling pathways, but further studies are necessary to elucidate its role under different conditions.

**AKAP12 ROLE IN CARDIOVASCULAR SYSTEM**

In contrast to the extensive studies involving AKAP12’s role in cancer research, there is little knowledge of its role in the cardiovascular system. AKAP12 is highly expressed in the heart and functions to scaffold its signalosome complex near β2-AR to influence resensitization and sequestration events. Usually, scaffold proteins physically and transiently tether their complexes. However, AKAP12/β2-AR interaction is unique as it was found to be strengthened upon stimulation of β2-AR and remains intact even during the internalization of these receptors. Considering that cardiac function
is mainly influenced by β-Ars, and that AKAP12 directly binds to β2-AR, it is logical to assume that AKAP12 plays a role in regulating cardiac function.

To date, few studies have investigated AKAP12’s involvement in cardiac pathophysiology, with the majority of studies indicating that AKAP12 downregulation improves cardiac function. For example, downregulation of AKAP12 reduced ventricular remodeling after chronic myocardial infarction (MI) surgery in transgenic mice overexpressing thioredoxin-1. This is believed to be associated with AKAP12’s regulation of redox-sensitive transcription factor hypoxia-inducible factor-α. Hypoxia-inducible factor-α is a critical expression regulator of both vascular endothelial growth factor and endothelial nitric oxide synthase, where AKAP12 destabilizes hypoxia-inducible factor-α by enhancing its proteosomal degradation. Thus, the thioredoxin-1 cardioprotective effects of overexpression are partially attributed to reduced oxidative stress–mediated stabilization of hypoxia-inducible factor-α, which thereby increases vascular endothelial growth factor and endothelial nitric oxide levels.

Knowing that cardiac hypertrophy and fibrosis are strongly associated with increased mortality in diabetic patients, the role of vascular endothelial growth factor, endothelial nitric oxide, and heat shock proteins in relation to AKAP12 were studied in the diabetic milieu of post-myocardial infarction (MI) surgery. Surprisingly, the study confirmed that heat shock protein A12B attenuated AKAP12 action, mediated cardioprotection through upregulating (thioredoxin-1), and suggested AKAP12

**Figure 4. Proposed AKAP12 signaling downstream of β2-AR.**

AKAP12 scaffolds multiple kinases, in addition to polybasic domains, which control its interactions and signaling. Under the unstimulated β2-AR state, AKAP12 is found mainly within the cytoplasm. However, when β2-AR is stimulated, the polybasic domains found within the AKAP12 signalosome targets the complex to the cell membrane. This colocalization of the signalosome facilitates a stable binding of β2-AR and AKAP12. Activation of PKA is associated with increased levels of cAMP in its proximity. β2-AR stimulation induces local increases of cAMP. AKAP12 is important for PKA activation through scaffolding and collocating PKA near areas of high cAMP levels. The active form of PKA phosphorylates multiple downstream molecules, such as CREB (cellular transcription factor), SRC (tyrosine kinase), and GRK (G protein kinases), all playing a role in downstream signaling of β2-AR. Importantly, PKA phosphorylates AKAP12, which stabilizes the interaction between AKAP12 and the β2-AR, even during post-internalization of the receptor. On the other hand, phosphorylation of AKAP12 by PKC decreases AKAP12–PKC scaffolding and induces translocation of the AKAP12/PKA/β2-AR complex to the perinuclear space. AKAP indicates A-kinase anchoring protein; β2-AR, β2-adrenergic receptor; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CREB, cellular transcription factor; EPAC, exchange protein directly activated by cAMP; ERK, extracellular signal-regulated kinase; GRK, G protein kinase; MEK, mitogen-activated protein kinase; PDE4, phosphodiesterase-4; PKA, protein kinase A; PKC, protein kinase C; PM, plasma membrane; Raf, rapidly accelerated fibrosarcoma; and SRC, tyrosine kinase.
involvement in the downregulation of thioredoxin-interacting protein. In addition, on the basis of previous reports of heat shock protein A12B binding directly to a specific domain within AKAP12 (829–860 amino acid residues) located between the N and C terminus,101 cardioprotection may be induced through genetic modifications causing AKAP12 downregulation. Taking into account that AKAP12 downregulation affected the cycling of the β2-AR, this observation likely occurs through reduced recruitment of proteins involved in receptor desensitization (G protein–coupled receptor kinase 2 and β-arrestin).28

In efforts to better understand the mechanism of AKAP12 in β2-AR signaling pathways, AKAP12-t/t mice were examined after acute infusion with the nonspecific β-AR agonist isoproterenol.58,59 In contrast to a study fully ablated AKAP12,60 AKAP12-t/t only lack the critical region required for β2-AR, PKA, or PKC binding.58,59 This reduces the probability of influence of the upregulated compensatory mechanisms, as in full-knockout AKAP12 gene models. Acute β-AR stimulation of AKAP12-t/t delineated enhanced contractility and basal activity. The proposed mechanism of increased basal activity was because of increased cMyBPC Ser-273 phosphorylation by heat shock protein 20, although increased cardiac contractility could be associated with altering PKA phosphorylating capacity as well as activity of PDE4D.58 Also, it was suggested that AKAP12-t/t muscle exhibited increased myofilament responsiveness to Ca2+.59 supporting the concept of AKAP12 being more than just a scaffold protein.

An additional study speculated that AKAP12 ablation aggravated heart failure induced post–angiotensin II infusion by promoting oxidative stress, apoptosis, cardiac fibrosis, and inflammatory response.60 In that study, the authors suggested that the mechanism of increased cardiac fibrosis was mainly through increased levels of transforming growth factor-β1 and collagen, as well as through activation of the SMAD2/3 pathway.60 In addition to these findings, it has been reported recently that AKAP12-t/t mice exhibit a delayed high-fat-diet–induced hyperlipidemia and atherosclerosis.61 It is believed that such resistance to hyperlipidemia and atherosclerosis development is associated with reduced gene expression of sterol regulatory element-binding protein 2. Further reducing gene expression of liver 3-hydroxy-3-methyl-glutaryl-CoA reductase and low-density lipoprotein receptor.51 Consistent with these findings, Choi et al reported that overexpression of AKAP12 is involved in sterol regulatory element-binding protein 2 activation in a SCAP-dependent manner.59,127 probably through enhancing [3H]-cholesterol efflux to extracellular acceptors. These findings further argue in favor of the AKAP12-t/t cardioprotective phenotype from a pathophysiologic risk perspective (heart failure development as a result of hyperlipidemia and atherosclerosis).

Finally, it is noteworthy that none of these studies addresses the expression levels of other AKAPs. This is important because several AKAPs are present in the heart. Thus, observed alterations that were accredited to AKAP12 may have been influenced by other AKAPs, especially AKAP5, as AKAP5 also binds to β2-AR and is capable of switching the coupling of β2-AR from the Goα to the Gai pathway.128 In addition, AKAP5 can form not only homodimers but also a higher order supermolecular homo-oligomeric complex, thus influencing the stability of protein–protein interactions and signaling loops affecting the AKAP12 signalosome.9 Therefore, these aspects stress the importance of taking into consideration the AKAP5 expression levels in future studies. Together, on the basis of the aforementioned data, AKAP12’s role in the modulating cardiac function seems clearer. Thus, ongoing research of AKAP12’s overexpression and how this overexpression impacts the cardiovascular system may resolve some of these issues. In conclusion, AKAPs mediate the kinase signaling pathways needed for optimal cellular responses and alterations in their levels; either upregulation or downregulation explains the development or mitigation of different pathologic states. AKAP12 cellular signaling roles are established clearly in some systems (Figure 4).99,102,129 However, the implication of this finding in cardiovascular diseases is still speculative, especially in the area of heart failure progression. Further understanding of AKAP12 binding domains and signaling pathways may be the initial step in developing specific peptide-targeting drugs.

ARTICLE INFORMATION

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Acknowledgments

This work was done in partial fulfillment of the requirements for a PhD in pharmacology in the Department of Pharmacological and Pharmaceutical Sciences at the College of Pharmacy, University of Houston (H.Q.).

Sources of Funding

This study was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health (award number R15 HL141963 to B.K.M.; the American Heart Association (award number 18AIREA 33960175 to B.K.M.); and the Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation (to B.K.M.). The funders had no role in the preparation or decision to publish this work.

Disclosures

None.

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J Am Heart Assoc. 2020;9:e016155. DOI: 10.1161/JAHA.120.016155