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AKAP-Scaffolding Proteins and Regulation of Cardiac Physiology

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A kinase anchoring proteins (AKAPs) compose a growing list of diverse but functionally related proteins defined by their ability to bind to the regulatory subunit of protein kinase A. AKAPs perform an integral role in the spatiotemporal modulation of a multitude of cellular signaling pathways. This review highlights the extensive role of AKAPs in cardiac excitation/contraction coupling and cardiac physiology. The literature shows that particular AKAPs are involved in cardiac Ca^{2+} influx, release, reuptake, and myocyte repolarization. Studies have also suggested roles for AKAPs in cardiac remodeling. Transgenic studies show functional effects of AKAPs, not only in the cardiovascular system but in other organ systems as well.

AKAPs: Multiprotein Signal Integration Complexes

PKA signaling plays a prominent role in the modulation of cardiac function. Extensive research indicates that AKAPs, which bind and sequester PKA to specific subcellular locations, also nucleate multicomponent protein signaling complexes. Thus AKAPs are central mediators of cross talk and integration of cAMP/PKA signaling with other signaling pathways. Most known AKAPs bind to the type II regulatory subunit of PKA (RII); however, several "dual-AKAPs" also bind type I regulatory subunit (RI). A survey of some of the AKAPs found in the heart (Table 1) reveals that multiple signaling proteins, including kinases, protein phosphatases, and phosphodiesterases, can be found in complex with different AKAPs. The targeting of phosphodiesterases together with PKA is believed to enable finite control over local cAMP levels and thus the extent and duration of PKA activation (Refs. 30, 31, 79, 87; see also Refs. 4, 88). A comprehensive list of AKAPs is found in a recent review (51). Based on distinct complements of signaling molecules bound to each AKAP, the local spatial and temporal activation of PKA bound to each AKAP is likely to be unique. The ability of AKAPs to sequester discrete sets of signaling molecules to particular regions of the cell therefore allows for specificity and diversity of local cellular signaling dynamics (12).

Examples of signal integration and cross talk are seen with mAKAP and AKAP-Lbc. mAKAP is expressed in cardiac and brain tissue and is known to form a multi-enzyme complex that includes PKA, PDE4D3, Epac, Erk5, and PDK1 (57, 74, 77, 108). These components interact to form a local signal cascade that can positively or negatively modulate cAMP metabolism (30). Another example of signal integration is seen with AKAP-Lbc, which associates with Rho, PKC, and PKD, in addition to PKA. AKAP-Lbc demonstrates a Rho-specific guanine nucleotide exchange factor activity (GEF) at the COOH terminus (27, 29, 60). PKA-dependent phosphorylation of Ser1565 of AKAP-Lbc facilitates binding of an accessory protein (14-3-3), which, in turn, results in inhibition of GEF activity (26). Moreover, AKAP-Lbc positions PKC to phosphorylate and activate PKD (17). Thus AKAPs not only localize signaling peptides to particular locations in the cell but also provide a mechanism for a diverse array of signal integration.

AKAPs Bind PKA Regulatory Subunit Dimers

Canonical AKAPs bind the regulatory (R) subunits of the PKA holoenzyme via an amphipathic α-helix, typically 14–18 amino acids in length (20). A wide range of sequence variation is observed between the RII binding domains of individual proteins; therefore, AKAPs are typically defined as a functionally homologous family of proteins. A loose consensus motif (X[L,I,V]XX[L,I,V] [L,I,V]XX[L,I,V][L,I,V]XX[A,S][L,I,V]) has been proposed (103), but the critical feature of the A-kinase anchoring domain is a concentration of hydrophobic amino acids on one face of the helix that forms a "hydrophobic ridge." Introduction of a proline residue, with its rigid cyclic structure, introduces a "kink" that disrupts the amphipathic α-helix and eliminates binding, which highlights the importance of secondary structure in RII:AKAP interaction (19, 20). Binding to a general hydrophobic ridge rather than a sequence-specific motif permits high-affinity binding between diverse AKAPs and the RII subunit dimer (82). Despite the proposed consensus motif, "unconventional" RII binding proteins such as pericentrin are not predicted to contain the hallmark helix; however, they are believed to anchor RII in a manner still dependent on hydrophobic residues in the putative RII binding site (28).

Crystallographic and NMR data have shown that R subunit dimers form an x-type, four-helix bundle

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containing a "hydrophobic groove" that is the binding site for AKAPs (58). Thus the "hydrophobic ridge" in the PKA anchoring domain of AKAPs fits the "hydrophobic groove" formed by R subunit dimers. Although a portion of the groove formed by RIα dimers contains a cavity that can accept bulky side chains, the corresponding sequence on RIIα dimers generates a relatively flatter hydrophobic surface. The difference in the hydrophobic grooves has been proposed as a mechanism that allows AKAP peptides to differentially interact with RI and RII dimers (58).

Highly localized dynamics of PKA are determined by the unique complement of signaling molecules associated with each AKAP. The affinity of individual AKAPs for RII differs, likely due in part to sequence variations within the PKA binding domain that correlate with differences in 3D structure of the R binding site (2, 111). For example, the 24-amino acid peptide called Ht31, which constitutes the RII binding domain of AKAP-Lbc, is used as an experimental tool to disrupt RII:AKAP interaction. Ht31 binds RII_α with much higher affinity (K_d = 2–10 nM) than RIα (K_d = 1,030–1,277 nM) (19, 46, 111). Differences in binding affinity between different R isoforms and other AKAPs have also been determined. Protein interaction can be measured via surface plasmon resonance analysis, and work by our laboratory and others has demonstrated that AKAP proteins can have varying affinities for specific R isoforms. S-AKAP84/D-AKAP-1, a "dual AKAP," binds both RI and RII with high (nM) affinity; in contrast, AKAP79 preferentially binds RII isoforms α and β (K_d = 1.5 and 4.5 nM, respectively) and binds RI with a K_d exceeding 1μM (46). AKAP95, mAKAP, and AKAP15/18 also preferentially bind RII isoforms, but variations such as a threefold difference in affinity for AKAP95 and RIIα vs. RIIβ suggest that isoform affinity is highly relevant in PKA anchoring (46, 111). These binding preferences between various AKAPs and R subunits may represent an important component of the mechanism by which isoforms of PKA are organized into highly localized, discrete, signaling microdomains.

Phosphorylation of the R subunit

To date, limited information is available on the functional significance of different affinities of different AKAPs for the PKA holoenzyme, as discussed above. PKA redistribution upon RII phosphorylation may be one mechanism by which dynamic changes in micro-PKA distribution occurs. For example, this could take place upon downregulation of the β-adrenergic signaling pathway in cardiac disease.

As cAMP levels increase, cAMP binds to cooperative sites on each R subunit, inducing a conformational change that releases the catalytic (C) subunits. The C subunit phosphorylates RII at Ser96, a residue within the PKA "inhibitory domain," which then decreases the affinity of RII for C (42, 85). In contrast, phosphorylated RII binds the A-kinase anchoring domain of AKAPs with higher affinity than unphosphorylated RII, as indicated by surface plasmon resonance studies (111) and, more recently, studies where RIIS96D and RIIS96A, mimicking phosphorylated and unphosphorylated RII, respectively, were expressed in isolated myocytes (71). Expression of RIIS96D also resulted in increased phosphorylation of PKA substrates and altered Ca^{2+} signaling (71). Increased PKA substrate phosphorylation suggests that a local conformational change that occurs upon Ser96 phosphorylation is communicated to the AKAP binding domain at the NH_2 terminal of the molecule.

Phosphorylation of RII at alternative sites can result in other downstream effects. For example, in quiescent cells, Yotiao localizes RIIα to the centrosome; however, as cells enter mitosis, RIIα is phosphorylated at Thr54 by cyclin B-p34cdc2 kinase (CDK1). Whereas Ser96 is located in the inhibitory domain of RII, Thr54 is adjacent to the dimerization/docking domain of RII. Phosphorylation at this latter site results in decreased affinity of RII for Yotiao and redistribution of PKA from the centrosome (16). Interestingly, CDK1-dependent phosphorylation of RIIα at Thr54 also increases the binding affinity of RIIα for AKAP95. This has the effect of recruiting RIIα for proper chromatin remodeling during mitosis (65). Thus phosphorylation of at least two sites on the RII subunit represents additional, distinct mechanisms for regulating localization of PKA in different microdomains within the cell, ultimately affecting local kinase function.

AKAPs and Cardiac Physiology

PKA enzymatic activity is extensively involved in normal cardiac myocyte function. It is widely known that perturbations of PKA activity, including decreased PKA phosphorylation of phospholamban, myosin binding protein C, and troponin I, accompany remodeling of the heart and heart failure (10, 45, 96, 104, 105, 112). The ryanodine receptor (RyR) Ca^{2+} release channel located in the sarcoplasmic reticulum and the α and β subunits of L-type Ca^{2+} channels are also

Table 1. Partial list of cardiac AKAPs and their heterogeneous binding partners

	Binding Partners	References
mAKAP	PKA, PP2A, PDE4D3	30, 31, 73
AKAP-Lbc	PKA, PKC, PKD, Rho	17, 29, 60
Yotiao	PKA, PP1	106
AKAP15/18	PKA, PP2B	91
AKAP15/18δ	PKA, PLN	68
Gravin	PKA, PKC, PDE4D, Src	80, 99, 107
Synemin	PKA, desmin, vimentin	41, 90
	Utrophin, vinculin	8, 98
	α-Actinin	5

phosphorylated by PKA. The functional effects resulting from PKA phosphorylation of RyR and the relative roles of different RyR phosphorylation sites are controversial. Recent conclusions from several investigators indicate that β-adrenergic stimulation has only a small effect on RyR phosphorylation, leading primarily to increased kinetics of Ca^{2+} release and affecting Ca^{2+} cycling (6, 40, 70). Others report that PKA phosphorylation of RyR does not change significantly in heart failure (6, 52). In contrast, Marks and colleagues identified a more significant role of PKA-dependent RyR phosphorylation. They report that in heart failure RyR hyperphosphorylation takes place, with a resultant increase in RyR channel leak (32, 73, 86). Further discussion of the role of mAKAP in the regulation of RyR channel phosphorylation is described at a later point in this review.

The functional importance of PKA targeting by AKAPs is supported by experiments in which the targeting of PKA is disrupted via expression of a competing peptide (Ht31) that binds RII dimers in cells or tissues. Under these conditions, a spectrum of changes as a result of disruption of PKA anchoring to AKAPs has been observed, including impaired forskolin-stimulated Cl^- current activity (61), decreased PKA potentiation of L-type current (37), decreased PKA phosphorylation of troponin I, phospholamban, and RyR, and altered contraction upon β-adrenergic-stimulation of cardiomyocytes (33) or hearts in vivo (75). Thus a substantial body of evidence highlights the involvement of multiple AKAPs in the heart.

Cardiac hypertrophy and AKAPs

Cardiac hypertrophy may be viewed as a graded adaptive response of the heart to systemic demands. Left unchecked, however, cardiac growth can lead to maladaptive remodeling of the heart that leads to poor performance and, eventually, failure. Recent studies have suggested that two AKAPs in particular (AKAP-Lbc and mAKAP) are involved in the hypertrophic response of the heart.

AKAP-Lbc. Chronic infusion of phenylephrine (PE) into mice increased cardiac weight index (ventricular weight/body weight) and levels of AKAP-Lbc mRNA from ventricular myocytes in vivo. Interestingly, downregulation of this AKAP, via RNA interference, inhibited the PE-stimulated activation of RhoA and cellular hypertrophy in rat neonatal cardiomyocytes (NCM). These investigators concluded that AKAP-Lbc is involved in the hypertrophic pathway activated by α_1-adrenergic receptors in rat NCM (3). The downstream effector(s) of activated RhoA in the hypertrophic response remains to be elucidated; however, putative pathways involved in participation of AKAP-Lbc in the hypertrophic process have been proposed (25). A recent report by Scott and colleagues (18) postulated that, by means of binding of PKD1, AKAP-Lbc plays a significant role in the agonist-stimulated hypertrophic response. These investigators showed that gene silencing of AKAP-Lbc blunted the hypertrophic response to phenylephrine in NCM. Agonist-stimulated hypertrophy could be reinstated by introduction of AKAP-Lbc constructs that retained

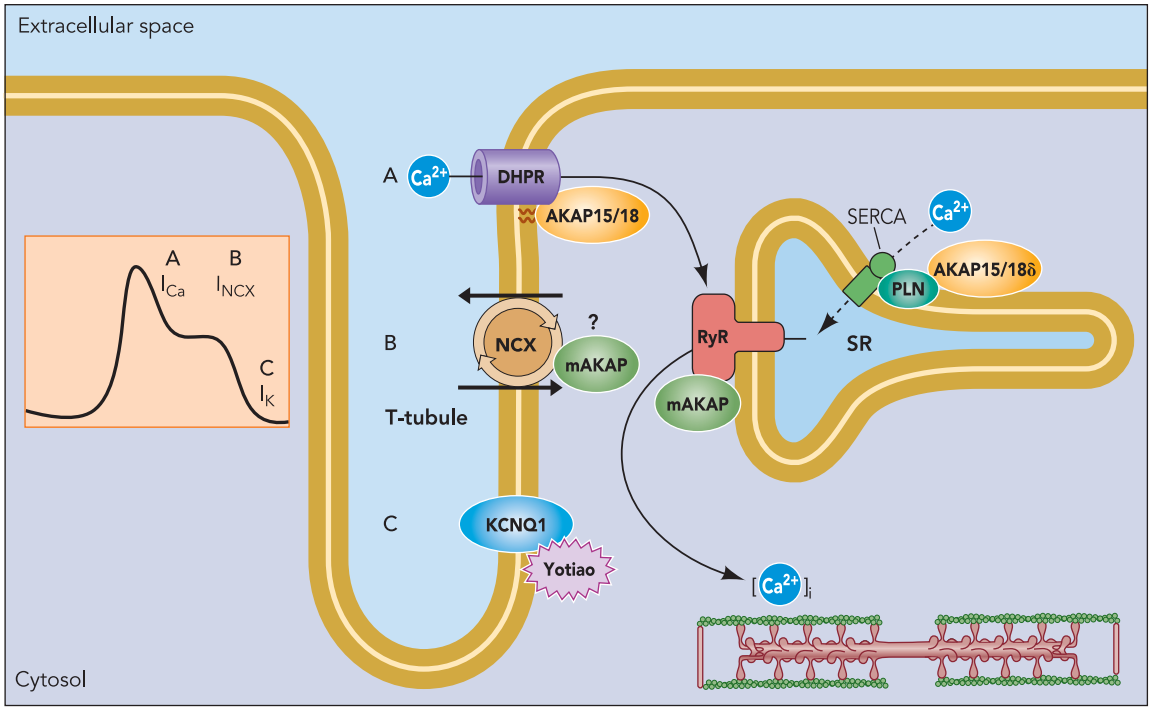

FIGURE 1. **AKAPs modulate cardiac excitation/contraction coupling**
Different AKAPs are thought to be involved in the modulation of the cardiac action potential and Ca^{2+} transient. The processes of calcium entry via L-type calcium channels, calcium release from the sarcoplasmic reticulum via ryanodine receptors, calcium re-uptake, and cardiac repolarization employ AKAPs.

their ability to bind PKD. Furthermore, exogenous expression of AKAP-Lbc in NCM increased agonist-driven nuclear PKD activity and export of histone deacetylase 5 (HDAC5). The investigators postulated that AKAP-Lbc participates in the hypertrophic response by enhancing the efficiency of activation of the "fetal program" involving upstream activity of PKD/HDAC5/MEF2 (18).

mAKAP. Two separate studies have shown that adrenergic- and cytokine-induced hypertrophy in cultured NCM can be decreased by downregulation of mAKAP (30, 83). The study by Kapiloff and colleagues further showed that adrenergic stimulation facilitated activation and nuclear localization of NFATc1. The investigators proposed that mAKAP participates in hypertrophic gene expression via a mechanism involving activation of the pro-hypertrophic transcription factor NFATc (83).

Cardiac EC coupling and AKAPs

As indicated above (33, 75), it is increasingly evident that AKAPs are intimately involved in modulation of cardiac EC coupling at many levels. Studies have revealed extensive participation of AKAPs in the generation of the cardiac action potential and Ca^{2+} transients. Multiple AKAPs associate with specific components of the cardiac EC coupling machinery and, along with other signaling proteins, facilitate modulation of the cardiac cycle.

AKAP15/18 and Ca^{2+} influx. AKAP15 (or AKAP18), named for slight differences in molecular weight following its discovery by two independent laboratories, has multiple isoforms with molecular weights ranging from 15 to 50 kDa (35, 43, 44, 76, 101). The α, δ, and γ isoforms have all been reported to be expressed in the heart (68, 101). AKAP15/18 colocalizes with and co-immunoprecipitates L-type Ca^{2+} channels (14, 43, 44). Lipid modification is involved in the localization of AKAP15/18 to the plasma membrane (35). AKAP15/18 interacts with the COOH-terminal domain of the α_1 subunit of skeletal L-type Ca^{2+} channel (48).

A number of studies have demonstrated that L-type Ca^{2+} currents are modulated by AKAP15/18-dependent mechanisms (FIGURE 1). PKA-mediated phosphorylation of the Ca^{2+} channel augments channel activity (24, 55, 110). PKA-dependent phosphorylation of both the $\alpha 1_C$ and $\beta 2_a$ subunits has been reported; however, the question of whether one or more of these sites is required for the increased Ca^{2+} flux through the channel in intact cardiac myocytes is still being debated, in part because of long-standing difficulties encountered in reconstituting the AKAP-dependent signaling complexes in heterologous expression systems. For example, although Hosey and colleagues (37) identified Ser1928 of the α_{1C} subunit as critical for PKA-mediated modulation of the channel, O'Rourke and colleagues reported that mutation of Ser1928 to an alanine did not significantly attenuate the β-adrenergic response (36). However, when AKAP15/18 is expressed in HEK293 cells, it targets PKA to the channel and enhances L-type Ca^{2+} channel activity in response to activation of PKA. This effect is lost on expression of the inactive AKAP15/18 mutant (35). Thus both measurements of Ca^{2+} currents and colocalization/co-immunoprecipitation studies indicate a role for AKAP15/18 in the modulation of Ca^{2+} influx (FIGURE 1) and, by extension, cytosolic Ca^{2+} concentration.

Interestingly, a report by Hosey and colleagues also suggested that AKAP79 can modulate activity of L-type Ca^{2+} channels (37). These investigators reported that, when α_{1C} subunit of L-type channels is expressed in HEK293 cells stably expressing wild-type AKAP79, L-type Ca^{2+} channels are phosphorylated upon activation of PKA. Ser1928 of α_{1C} was identified as the PKA phosphorylation site. PKA-dependent phosphorylation of Ser1928 did not increase in cells expressing an AKAP79 construct in which a proline residue disrupted the structure of the RII binding domain. The results suggested that AKAP79 can potentially substitute for AKAP15/18 in the facilitation of the phosphorylation of the α_{1C} subunit of L-type Ca^{2+} channels. The question of whether AKAP79 plays a physiological role in the modification of this channel in the heart remains to be determined. This particular AKAP has also been reported to regulate L-type Ca^{2+} channel trafficking independently of PKA, although to date this has only been observed in the brain (1).

mAKAP and Ca^{2+} release. mAKAP localizes to the junctional membrane of the sarcoplasmic reticulum and to the perinuclear region (56, 57, 73, 74, 108). In the heart, mAKAP forms a complex with type 2 ryanodine receptor (RyR2) and PKA (56, 108). Marks and colleagues reported that FKBP12.6, PP2A, and PP1 form a complex with RyR2 and that PKA-dependent phosphorylation of RyR2 decreased the amount of FKBP12.6 that co-immunoprecipitated with RyR2 (73). Dissociation of FKBP12.6 was predicted to increase the open probability of the Ca^{2+} release channel (13,

Table 2. AKAP knockouts/mutations and associated phenotypes

	Phenotype	References
AKAP10 (D-AKAP2)	↑ Cardiac cholinergic response, cardiac arrhythmia Human; 646Vassoc ↑ basal heart rate	100
D-AKAP1	Oocyte meiosis defects, ↓ female fertility	81
AKAP4	Sperm morphology, motility, and viability defects	78
AKAP5 (AKAP150)	LTP defects, motor coordination, spatial memory	67, 102

53). Recently, our laboratory demonstrated that overexpression of a phosphomimic of RII (with the phosphorylatable serine 96 substituted by an aspartate) increased the binding of RII for AKAPs and resulted in increased PKA phosphorylation of RyR2 (Ser 2809) in NCMs (71). Taken together, these studies suggest that localization of PKA to RyR2, represents an important mechanism for modulating the activity of RyR2 and, ultimately, the cardiac Ca^{2+} transient (FIGURE 1). As indicated previously in this review, the functional significance of mAKAP-dependent PKA phosphorylation of RyR2 continues to be actively investigated.

mAKAP and Na^+ Ca^{2+} exchange. mAKAP may participate in the modulation of Na^+-Ca^{2+} exchange. The cardiac Na^+-Ca^{2+} exchanger (NCX1), located on the plasma membrane and enriched in transverse tubules, plays a primary role in Ca^{2+} extrusion in the heart, as well as in other tissues (for review, see Refs. 7, 9). The intracellular loop of NCX1 can be phosphorylated by PKA (50), but the functional outcome of PKA phosphorylation of NCX1 remains to be clarified (113). Whereas some investigators report effects of PKA-dependent phosphorylation of NCX1 (62, 89), others have not detected PKA-mediated modulation of NCX (39). Interestingly, immunoprecipitation of NCX1 from rat ventricular cardiac lysates revealed that mAKAP, but not other AKAPs, was present in the immunoprecipitated complex (94). In this study, Ruknudin and colleagues showed that the RI subunit of PKA was also found in complex with mAKAP. These findings differ from results of surface plasmon resonance studies that reported no appreciable binding of RI to mAKAP (111). The reason for this inconsistency is unknown, but differences may be due to the methods and conditions used for the two studies. Ruknudin and colleagues reported that the complex also contained protein phosphatase 1 and 2A (PP1 and PP2A) and PKC, and was localized to the Z-line in rat cardiomyocytes. To date, no additional studies have described an mAKAP-NCX complex. As indicated earlier, the majority of studies demonstrate that mAKAP targets RII to the RyR at the junctional sarcoplasmic reticulum (SR) and nuclear membrane. Further investigation is needed to determine a potential role for mAKAP at the plasma membrane.

AKAP15/18δ and SR Ca^{2+} re-uptake. A recent study by Klussmann and colleagues described a role for AKAP15/18δ in modulation of Ca^{2+} re-uptake into the SR via SERCA in cardiomyocytes. Immunogold staining in neonatal heart tissue showed that SERCA2, phospholamban (PLN), and AKAP15/18δ colocalize and that these three proteins were found to co-immunoprecipitate as a protein complex. Furthermore, disruption of the PLN-AKAP15/18δ interaction, via expression of a short peptide derived from PLN, disrupted the striated distribution of AKAP15/18δ, decreased isoproterenol-induced phosphorylation of PLN (Ser 16), and reduced Ca^{2+} re-uptake. Knockdown of AKAP15/18δ abolished the effect of norepinephrine on Ca^{2+} re-uptake. The investigators concluded that AKAP15/18δ plays a significant role in PKA-mediated phosphorylation of PLN and Ca^{2+} re-uptake into cardiac SR (68) (FIGURE 1).

Yotiao and cardiac repolarization. Yotiao was initially identified in the brain, where it is involved in the regulation of NMDA receptors (66, 106). In cardiac tissue, Yotiao interacts with the α subunit of the IK_s channel (KCNQ1) via two cooperative domains on Yotiao itself: a 17-amino acid binding site at the amino terminus and a leucine zipper motif in the carboxy-terminus, which is likely to interact with a complimentary leucine zipper domain on KCNQ1 (22, 72). The channel responsible for the IK_s current is composed of two subunits, a regulatory subunit (KCNE1) and the α subunit (KCNQ1) (92). β-Adrenergic receptor-mediated modulation of IK_s is known to involve Yotiao (72). Yotiao anchors PKA near the channel, which in turn affects the function of IK_s via phosphorylation of the α subunit at Ser 27 (FIGURE 1). In CHO cells heterologously expressing IK_s, stimulation with cAMP resulted in phosphorylation of the α subunit and augmentation of the IK_s current (63, 64). Interestingly, PKA phosphorylates Yotiao itself (Ser 43). Mutation of Ser 43 in Yotiao to an Ala decreased the response of IK_s to cAMP; therefore, Yotiao may also modulate IK_s via allosteric mechanisms (21).

Long-QT syndrome (LQTS) is a cardiac disorder characterized by a prolonged repolarization of the cardiac action potential. This temporal lengthening of the action potential can promote arrhythmia and increase risk of sudden cardiac death (93). Some forms of congenital LQTS have been linked to mutations in either subunit of the slowly activating IK_s channel (reviewed in Refs. 84, 47). Some mutations have been shown to affect channel current and endosomal recycling of IK_s, whereas others disrupt the ability of IK_s to associate with the regulatory signaling complex nucleated by Yotiao (22, 95).

Whereas a number of point mutations in the α subunit of IK_s have been associated with development of LQTS, at least two (G598D and S1570L) directly disrupt the ability of the Yotiao signaling complex to associate with the α subunit of IK_s. Computational analysis has demonstrated that the G598D mutation in KCNQ1 disrupts the targeting of the Yotiao-PKA-PP1 complex to the channel subunit. A study conducted in Finland identified this mutation in >50% of LQTS patients (34, 72). The mutant phenotype was observed after β-adrenergic stimulation, which promoted a prolonged QT-wave and T-wave abnormalities (72). When the S1507L was introduced into KCNQ1, the amount of Yotiao that co-immunoprecipitated with KCNQ1 was significantly decreased (22). Furthermore, PKA-dependent phosphorylation of the α subunit in response to elevated cAMP was decreased in cells expressing the S1570L mutant subunit compared with

wild type (21, 22). Computational analysis indicated that heterozygous and homozygous S1570L mutant mice have prolonged duration of the cardiac action potential, suggesting a link between development of LQTS and disruption of the Yotiao scaffolding protein complex (22).

AKAP Mutations and Knockouts

Members of the diverse AKAP family participate in a wide array of signaling processes. Perturbations of the signaling complexes nucleated by different AKAPs can potentially disrupt cellular homeostasis. Considering the multiple roles of AKAPs and the vital nature of cAMP-dependent signaling, deficiencies in these proteins are likely to be associated with human disease; however, interestingly, to date, few AKAPs have been definitively linked to human disease. There is also a lack of published studies that describe ramifications of a loss of function of particular AKAP family members.

Single nucleotide polymorphism in D-AKAP2

D-AKAP2 (AKAP10) is a dual-function AKAP associated with mitochondria, membrane, and cytosolic cell fractions. Analysis of ~6,500 single nucleotide polymorphism (SNPs) in a population of "healthy' European-Americans revealed a polymorphism at *amino acid 646* of D-AKAP2, in which an Ile residue was replaced with a Val. This SNP is located within the PKA binding domain of the AKAP. Biochemical analysis of each variant demonstrated that the Ile-646 D-AKAP2 bound RIα with threefold lower affinity than the Val-646 variant. Interestingly, binding of both variants to RII remained unaffected. The Val-646 variant was associated with a shorter cardiac P-R interval than that of Ile-646 (54). The investigators suggested that the cardiac phenotype resulted from altered localization of RIα from the sarcolemma. In a separate study, Conklin and colleagues (100) examined the Val-646 polymorphism in a cohort of 122 patients. Patients with the Val-646 SNP had an elevated heart rate but low heart rate variability, which is considered a risk factor for sudden cardiac death (11, 38, 59, 100). Interestingly, the study by Conklin and colleagues, conducted on patients who already had coronary heart disease, found no correlation between the SNP occurrence and other factors, such as age, that were highlighted in the study by Braun and colleagues (54). Clearly, additional studies are needed to elucidate the role of this AKAP in cardiac function and disease.

Gene trapping, a technique used to insert a deleterious fragment into the mouse genome (for review, see Ref. 97), was employed by Conklin and colleagues to disrupt a COOH-terminal 51-amino acid section of D-AKAP2 that contains the A-kinase anchoring domain. The resulting phenotype displayed severe functional abnormalities in the cardiovascular and nervous systems, including changes in heart rate, baroreceptor function, and abnormal conduction at the sinoatrial and atrioventricular nodes. These mice exhibited arrhythmias characterized by extended P-P and P-R intervals. These factors suggest a potential risk of sudden cardiac death in organisms lacking a complete, functional D-AKAP2 (100).

"The AKAP family has rapidly expanded in number and diversity over the last two decades, yet limited progress has been made in defining the role of these proteins using mouse knockout models."

AKAP knockout studies

To better define the role of individual AKAPs in the whole organism, efforts have been made to generate knockout mice. Table 2 summarizes the phenotypes observed in a selection of AKAP knockout studies. Selective knockout of particular AKAPs have functional outcomes not only in the cardiovascular system but in multiple organ systems. Transgenic studies have also shown that examination of the effects of AKAP knockout in mice can be a complex undertaking. For example, loss of AKAP95, a nuclear protein that recruits RII to chromatin during mitosis, resulted in no observable phenotype; however, crossing AKAP95-null mice with mice lacking the chaperone protein fidgetin resulted in poor survival in neo- and postnatal periods, cleft palate, and respiratory distress (23, 109).

In a study designed to examine the role of mAKAPα in the brain, knockout of mAKAPα, which is 244 amino acids longer than the β isoform, resulted in significant postnatal lethality, low body weight, and craniofacial defects. Interestingly, loss of mAKAPα, the preferred isoform expressed in brain, induced expression of the normally heart-specific β isoform in the brains of mAKAPα knockout mice (77). Thus the effects of total knockout of mAKAP or of loss of its β isoform, a significant A-kinase anchoring protein in the heart, remain unresolved.

Reports from other investigators show more straightforward effects of other AKAP knockouts. Homozygous strains of mice deficient in D-AKAP1 exhibited disrupted oocyte meiosis and decreased female fertility and litter size (81). Loss of AKAP4 (AKAP82), a sperm-specific AKAP, resulted in altered sperm morphology, motility, and viability (78). Although the phenotypic knockout of AKAP5 (AKAP75/79) was not analyzed, knock-in of a mutant protein on this mouse background that lacked the PKA-anchoring domain affected neural function in the hippocampus, suggesting a role for this AKAP in long-term memory (67). In contrast, loss of the P1 isoform of AKAP250 (gravin) from the nervous system resulted in no observable phenotype (15).

The AKAP family has rapidly expanded in number and diversity over the last two decades, yet limited progress has been made in defining the role of these proteins using mouse knockout models. Even when null mice are successfully generated, differences between mice and humans can render interpretation of these phenotypes difficult. SNP analysis of normal human populations vs. those at risk for conditions such as cardiac disease is a promising tool through which the functional significance of AKAP-mediated signaling may be examined. To date, D-AKAP2 is the only AKAP, of which we are aware, with a phenotype-associated polymorphism within the PKA binding domain. It is possible that the vital nature of PKA anchoring to growth and development precludes introduction of deleterious SNPs in populations that have been analyzed to date. For example, loss of AKAP function could compromise survival of carriers of critical mutations. Given the number of AKAPs that sequester PKA to similar areas within the cell, it is reasonable to assume that redundancy is built into the PKA-anchoring system. The isoform switch observed following knockout of mAKAPα is an example of an adaptation that arises to accommodate disrupted PKA targeting. Future studies focusing on the role of AKAP SNPs and isoforms in the context of disease should elucidate a link between disrupted AKAP function and human pathology.

Future Directions

AKAPs in the heart

Studies to date show that AKAPs play an integral role in cardiac function. Two cardiac AKAPs are currently implicated in agonist-induced cardiac myocyte hypertrophy. It would be very interesting to explore whether the same AKAPs are involved in other paradigms of hypertrophy (e.g., aortic banding, etc.). Certain AKAPs are also of interest for development of therapeutic strategies. The delta isoform of AKAP15/18 has been proposed as a therapeutic target for modulating Ca^{2+} reuptake, and thus cardiac relaxation, in patients with heart failure (68). Such strategies may be combined with existing therapies to enhance cardiac function and prevent progression into heart failure (49, 68, 69).

AKAPs in general

The earlier studies utilizing oligopeptide constructs (Ht31, AKAP-*is*, etc) to effect broad and global disruption of the distribution of PKA have identified a multitude of cellular processes involving PKA activity. Since this disruption is global for all AKAPs and thus nonselective, the studies have not manipulated specific PKA-AKAP interactions, and interpretation of results have always included this caveat. The studies that followed, employing RNA interference-mediated downregulation of specific AKAPs in cells, as well as generation of AKAP knockout mice, have explored and revealed functional roles of particular AKAPs at the cellular and organismal level. AKAPs can have diverse roles, well beyond anchoring of PKA. Moreover, AKAPs may behave in a modular fashion, wherein peptide domains execute particular functions, or as signal integration complexes. Wholesale knockdown or downregulation of AKAPs may not differentiate between modular and integrative functions of AKAPs. Which direction should future inquiries into AKAP biology take? We would suggest that, whereas studies utilizing RNAi and gene knockout should continue to provide valuable insights, the future of AKAP research should be one of "increased specificity." Effective future approaches could include genetic manipulations of functional domains on individual AKAPs. Alternatively, future studies should include design and development of peptides or small molecules that bind with high affinity and specificity to certain domains on individual AKAPs. These tactics could then be employed to selectively examine various functional domains on the AKAP. Examples of the above-mentioned strategies have been presented for AKAP-Lbc, AKAP15/18δ, and AKAP15/18α (18, 48, 68). In addition, studies can be designed to incorporate cell- and organ system-specific modifications of AKAP protein expression. These combined strategies will enable researchers to both tease out and piece together the functional roles of this fascinating and diverse group of proteins. ■

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