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 multiprotein 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. PKA dependence of AKAP-Lbc 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 sequence-specific motif permits high-affinity binding to disrupt RII:AKAP interaction (19, 20). 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-X(L-V-X-L)^n-M) 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).

To date, limited information on the functional significance of AKAPs for the PKA regulatory subunit dimer (RII) has been obtained. Although a potential site for AKAPs for RII, the PKA regulatory subunit (R), is called Ht31, a “hydrophobic ridge” that can positively or negatively modulate cAMP signaling pathways is present (2, 111). For example, Ht31 is highly localized near a number of signaling proteins, including kinases, protein phosphatases, and phosphodiesterases, that can positively or negatively modulate cAMP signaling pathways. These proteins include 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.

Phosphorylation

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A wide range of M. Bond1,2 OOH terminus in the RII binding KD (17). Thus, pericentrin is highly homologous in the putative des to participate in a mechanism regulating the heart and heart failure (10, 45, 96, 104, 105, 112). The ryanodine receptor (RyR) Ca2+ release channel (2, 111). For example, the 24-amino acid peptide called H31, which constitutes the RII binding domain of AKAP-Lbc, is used as an experimental tool to disrupt RII-AKAP interaction. H31 binds RII, with much higher affinity (Kd = 2–10 nM) than RIIa (Kd = 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 RI isoforms α and β (Kd = 1.5 and 4.5 nM, respectively) and binds RI with a Kd exceeding 1μM (46). AKAP95, mAKAP, and AKAP15/18 also preferentially bind RI isoforms, but variations such as a threefold difference in affinity for AKAP95 and RIIb vs. RIIa result in decreased affinity of RII for Yotiao and redistribution of PKA from the centrosome (16). Interestingly, CDK1-dependent phosphorylation of RIIIs at Thr54 also increases the binding affinity of RIIb for AKAP95. This has the effect of recruiting RIIa 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 (15, 45, 96, 104, 105, 112). The rydnoide receptor (B2R) Ca2+ release channel located in the sarcoplasmic reticulum and the α and β subunits of L-type Ca2+ channels are also shown to be phosphorylated.

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

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<tbody>
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<td>PKA, PKC, PKD, Rhos</td>
</tr>
<tr>
<td>Yotiao</td>
<td>17, 29, 60</td>
</tr>
<tr>
<td>AKAP15/18</td>
<td>PKA, PP2B</td>
</tr>
<tr>
<td>AKAP5 is 185</td>
<td>PKA, PLN</td>
</tr>
<tr>
<td>Gravin</td>
<td>PKA, PKC, PDE4D, Src</td>
</tr>
<tr>
<td>Synemin</td>
<td>PKA, desmin, vimentin</td>
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"Donnell,1 M. Mann,2 M. Bond1,2 States of Physiology, School of Medicine, Indiana University, and Department of Molecular Biology, University of Padova, Italy. m_donnell@indiana.edu

1, 2, 4, 6, 8, 9, 39, 41, 90, 80, 99, 107, 81, 17, 29, 60, 91, 68, 80, 99, 107, 41, 90, 5

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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 Ca²⁺ release and affecting Ca²⁺ 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 (H831) 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, phosphothiolamban, 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 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 their ability to stimulate Ca²⁺ release and to drive nucleoas of PKD/HDAC5 that AKAP-Lbc response by enabling the “fetal protein” PKD1 to interact with mAKAP. This adrenergic- and PKA-stimulated NCM cardiomyocyte further showed activation and AKAPs involved in the hypertrophic response, involving activation factor NFA (5).

Cardiac EC and AKAPs
As indicated above, there is a crucial role for AKAPs in cardiac EC. These cells revealed extensive generation of calcium signaling transients. Multiple components of the cardiac EC, and, along with the modulation of mAKAP15/18, has been studied. mAKAP15/18 is named for slightly larger size than mAKAP15, but has multiple isoforms from 15 to 50 amino acids that it has been one of the larger isoforms (66, 101). mAKAP15/18 is immunoprecipitated by its ability to bind to Ca²⁺ entry and stimulate Ca²⁺ release. Studies have shown that mAKAP15/18 interacts with a subunit of the ryanodine receptor, a protein that is required for the regulation of Ca²⁺ release from the sarcoplasmic reticulum.

Table 2. AKAPs

<table>
<thead>
<tr>
<th>AKAP</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>AKAP10</td>
<td>(D-AKAP10)</td>
</tr>
<tr>
<td>D-AKAP4</td>
<td>(D-AKAP4)</td>
</tr>
<tr>
<td>AKAP5</td>
<td>(AKAP5)</td>
</tr>
<tr>
<td>mAKAP15/18</td>
<td>(mAKAP15/18)</td>
</tr>
</tbody>
</table>

**FIGURE 1.** AKAPs modulate cardiac excitation/contraction coupling. Different AKAPs are thought to be involved in the modulation of the cardiac action potential and Ca²⁺ 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.
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\(^{2+}\) influx. 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\(^{2+}\) channels

AKAP15/18 (or AKAP18) has multiple isoforms with molecular weights ranging from 15 to 50 kDa (35, 43, 44, 76, 101). The \(\alpha, \beta, \gamma\) 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 \(\alpha_1\) 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 \(\alpha_1\) and \(\beta_2\) 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 \(\alpha_1\) 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 \(\beta\)-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 in 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 \(\alpha_1\) 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 \(\alpha_1\) subunit, which was identified as the PKA phosphorylation site (56). Marks and colleagues reported that mutation of Ser1928 to alanine 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 \(\alpha_1\) subunit of L-type Ca\(^{2+}\) channels. The results 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).

AKAP79 and Ca\(^{2+}\) release

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 mutations in the RyR2 channel and phosphorylation by PKA enhanced the formation of the complex and the RyR2 channel, contributing to the expression of PKA-dependent modulation of the channel. O’Rourke and colleagues reported that mutation of Ser1928 to an alanine in AKAP79 decreased the amount of AKAP79 that co-immunoprecipitated with RyR2 (73). Dissociation of FKBP12.6 was predicted to increase the open probability of the Ca\(^{2+}\) release channel (13,

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Table 2. AKAP knockouts/mutations and associated phenotypes

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac cholinergic response, cardiac arrhythmia Human</td>
<td>64/66Vass</td>
</tr>
<tr>
<td>ak</td>
<td>basal heart rate</td>
</tr>
<tr>
<td>Oocyte meiosis defects, I female fertility</td>
<td>78</td>
</tr>
<tr>
<td>LTP defects, motor coordination, spatial memory</td>
<td>67, 102</td>
</tr>
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</table>
53). Recently, our laboratory demonstrated that over-expression of a phosphomimetic of RII (with the phosphoacceptor serine 96 substituted by an aspartate) increased the binding of RII to 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\textsuperscript{2+} transient (84).

As indicated previously in this review, the functional significance of mAKAP-dependent PKA phosphorylation of RyR2 continues to be actively investigated in the cardiac muscle. mAKAP and Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange: mAKAP may participate in the modulation of Na\textsuperscript{+}/Ca\textsuperscript{2+} exchange. The cardiac Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger (NCX1), located on the plasma membrane and enriched in transverse tubules, plays a primary role in Ca\textsuperscript{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 (111). Whereas some investigators report effects of PKA-dependent phosphorylation of NCX1 (62, 89), others have not detected PKA-mediated modulation of NCX1 (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, Bakunin 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. Bakunin and colleagues reported that the complex also contained protein phosphatase 1 and 2A (PP1 and PP2A) and PKG, 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.

AKAP15/18 and SR Ca\textsuperscript{2+} re-uptake. A recent study by Klussmann and colleagues described a role for AKAP15/18 in modulation of Ca\textsuperscript{2+} re-uptake into the SR via SERCA in cardiomyocytes. Immunogold staining in neonatal heart tissue showed that SERCA2, phospholamban (PLN), and AKAP15/18 colocalized and that these three proteins were found to co-immunoprecipitate as a protein complex. Furthermore, disruption of the PLN-AKAP15/18 interaction via expression of a short peptide derived from PLN, disrupted the striated distribution of AKAP15/18, decreased isoproterenol-induced phosphorylation of PLN (Ser 16), and reduced Ca\textsuperscript{2+} re-uptake. Knockdown of AKAP15/18 abolished the effect of isoproterenol on Ca\textsuperscript{2+} re-uptake. The investigators concluded that AKAP15/18 plays a significant role in PKA-mediated phosphorylation of PLN and Ca\textsuperscript{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 \(\alpha\) subunit of the IK\textsubscript{a} channel (KCNQ1) via two cooperative domains on Yotiao itself: a 17-amino acid binding site at the amino terminus and a leucine zipper domain on KCNQ1 (22, 72). The channel responsible for the IK\textsubscript{a} current is composed of two subunits, a regulatory subunit (KCNQ1) and the \(\alpha\) subunit (KCNQ1) (92). \(\beta\)-Adrenergic receptor-mediated modulation of IK\textsubscript{a} is known to involve Yotiao (72). Yotiao anchors PKA near the channel, which in turn affects the function of IK\textsubscript{a} via phosphorylation of the \(\alpha\) subunit at Ser 27 (FIGURE 1). In CHO cells heterologously expressing IK\textsubscript{a} stimulation with cAMP resulted in phosphorylation of the \(\alpha\) subunit and augmentation of the IK\textsubscript{a} current (63, 64). Interestingly, PKA phosphorylates Yotiao itself (Ser 43). Mutation of Ser 43 in Yotiao to an Ala decreased the response of IK\textsubscript{a} to cAMP, therefore, Yotiao may also modulate IK\textsubscript{a} 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 arrhythmias and increase risk of sudden cardiac death (93). Some forms of congenital LQTS have been linked to mutations in either the \(\alpha\) or \(\beta\) subunit of the IK\textsubscript{a} current (63, 64). Interestingly, PKA phosphorylated the \(\alpha\) subunit of IK\textsubscript{a}. Computational analysis of each variant of PKA revealed that the AKAP2-bound complex of each variant of the \(\beta\) subunit of the LQTS associated with the \(\alpha\) subunit of the LQTS channel was associated with an altered phenotype of the LQTS channel. In a study conducted on a large number of patients, it was found that the LQTS patient group had a significantly increased percentage of patients with the LQTS genotype (34, 72). The mutant phenotype was observed after \(\beta\)-adrenergic stimulation, which promoted a prolonged QT-wave and T-wave abnormalities (72). The S1570L mutation was introduced into KCNQ1, the amount of Yotiao that co-immunoprecipitated with KCNQ1 was significantly decreased (22). Furthermore, PKA-dependent phosphorylation of the \(\alpha\) subunit in response to elevated cAMP was decreased in cells expressing the S1570L mutant subunit compared with wild type (21, 92). These findings suggest that heterozygous mice have protective potential, suggesting that LQTS and diabetes may be associated (22).

AKAP Mutations and Long-QT Syndrome

Members of the AKAP family display a wide array of signaling functions and are potentially distributed to a variety of signaling proteins. Clearly, however, interactions can be definitively limited by a lack of functional evidence.

Single nucleotide polymorphisms (SNPs) in the myosin-binding protein C (MYBPC3) gene have been associated with long-QT syndrome (16). SNPs in the β-1 integrin (ITGB1) gene have also been associated with long-QT syndrome (15). A recent study by Ruknudin and colleagues described a role for the AKAP2-mediated modulation of the beta-1 integrin (ITGB1) gene. The authors demonstrated that the G598D mutation in ITGB1 was replaced with an A598T mutation and that this mutation was associated with a loss of function. A recent study by Ruknudin and colleagues described a role for the AKAP2-mediated modulation of the beta-1 integrin (ITGB1) gene. The authors demonstrated that the G598D mutation in ITGB1 was replaced with an A598T mutation and that this mutation was associated with a loss of function.
AKAP95 knockout mice (77). Thus the knock out of mAKAP or of loss of its isoform, resulted in significant postnatal lethality, low body weight, and craniofacial defects. Interestingly, loss of mAKAP, the preferred isoform expressed in brain, induced expression of the normally heart-specific β isoform in the brains of mAKAP 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 sperm morphology, motility, and viability (78). Although the phenotypic knockout of AKAP (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 PII isoform of AKAP20 (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."

**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 (AKAP20) is a dual-function AKAP associated with mitochondria, membrane, and cytosolic cell fractions. Analysis of more than 6,500 single nucleotide polymorphisms (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 RII with a 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 RII 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 no loss 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).

**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 AKAP5, a nuclear protein that recruits RII to chromatin during mitosis, resulted in no observable phenotype; however, crossing AKAP5-null mice with mice lacking the chaperone protein hsp70 resulted in poor survival in neonate and postnatal periods, cleft palate, and respiratory distress (25, 109).

In a study designed to examine the role of mAKAPs in the brain, knockout of mAKAPs, which is 244 amino acids longer than the β isoform, resulted in significant postnatal lethality, low body weight, and craniofacial defects. Interestingly, loss of mAKAPs, the preferred isoform expressed in brain, induced expression of the normally heart-specific β isoform in the brains of mAKAP 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.

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**A-PP1 complex**

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**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 (AKAP20) is a dual-function AKAP associated with mitochondria, membrane, and cytosolic cell fractions. Analysis of more than 6,500 single nucleotide polymorphisms (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 RII with a 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 RII 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 no loss 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.

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Reports from other investigators show more straightforward effects of other AKAP knockouts. Homozygous strains of mice deficient in D-AKAP1 exhibited disrupted sperm morphology, motility, and viability (78). Although the phenotypic knockout of AKAP (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 PII isoform of AKAP20 (gravin) from the nervous system resulted in no observable phenotype (15).

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).
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 specific function has been elucidated, with a phenotype associated with disruption of AKAP function and human pathology. AKAPs in general are involved in the regulation of various cellular processes involving PKA activity. Since AKAPs in general accommodate disrupted PKA targeting, 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. Alternatively, future studies should include manipulations of functional domains on individual AKAPs. The earlier studies utilizing oligopeptide constructs to effect broad and global disruption of AKAPs, and prevention progression into heart failure (48, 68, 69). In addition, studies can be designed to incorporate cell- and organ-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.

**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 AKAP18/15 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 (48, 69, 68).

**AKAPs in general**

The earlier studies utilizing oligopeptide constructs (HG3, AKAP6, 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 knockouts should continue to provide valuable insights, the future of AKAP research should be one of "increased specificity" and "increased context", 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. For example, loss of AKAP function could uncover subtle effects on PKA activity, at times of disease, which could be genedependent and prevent progression into heart failure. In addition, studies can be designed to incorporate cell- and organ-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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include genetic insights on the future in part, by the small molecule to c-teract. we include various studies on the above, for AKAP-LC.

In addition, cell- and organ protein expression will enable us to understand the relationships within the cell. 

The protein kinase A anchoring protein mAKAP is a component of the 

In conclusion, genetic studies, cell- and organ protein expression, and pharmacological strategies will enable us to better understand the role of AKAP-LC in cardiac disease.
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