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 fine 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, ERK1, and PKD1 (57, 74, 77, 108). These components interact to form a local signal cascade that can positively or negatively modulate cAMP metabolism (36). Another example of signal integration 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 [XL-LV][XL][XL-LV][XL-LV][XXX][XL-S][XL-LV] 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 α-type, four-helix bundle containing a 7-nm wide ridge for AKAP anchoring, suggesting a “hydrophobic lock” (30). Although AKAPs do not form dimers containing α-helical bundles, the concept of a hydrophobic ridge is still applicable. RII binding sites on each subunit interact with one mechanism of RII activity. The PKA distribution in the cardiovascular system but in other organ systems as well.
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 RIs dimers contains a cavity that can accept bulky side chains, the corresponding sequence on RIIs dimers generates a relatively flat hydrophobic surface. The difference in the hydrophobic grooves has been proposed as a mechanism that allows AKAP peptides to differentially interact with RI and RIIRDimer (58).

Highly conserved AKAPs 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 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 RIIs (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-AKAP1, a "dual AKAP," binds both RI and RII with high (nM) affinity; in contrast, AKAP79 preferentially binds RIIs and RI with much higher affinity (Kd = 1.5 and 4.5 nM, respectively) and binds RI with a Kd exceeding 1 hM (46). AKAP95, mAKAP, and AKAP15/18 also preferentially bind RIIs, but variations such as a difference in the hydrophobic grooves has been proposed as a mechanism that allows AKAP peptides to differentially interact with RI and RIIRDimer (58). 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) Ca2+ release channel located in the sarcoplasmic reticulum and the a and c beta subunits of type c Ca2+ channels are also shown to be substrates for PKA. PKA redistribution upon RII phosphorylation may be one mechanism by which dynamic changes in micro-PKA distribution occur. For example, this could take place upon downregulation of the beta-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 Ser93, 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 subunits and altered Ca2+ 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 NH2 terminal of the molecule.

Phosphorylation of RII at alternative sites can result in other downstream effects. For example, in quiescent cells, Yotiao localizes RIIs to the centrosome; however, as cells enter mitosis, RIIs 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 docking domain of AKAPs with higher affinity than unphosphorylated RII.

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

<table>
<thead>
<tr>
<th>Binding Partners</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAKAP</td>
<td>PKA, PKC, PKD, Rho</td>
</tr>
<tr>
<td>AKAP-Lbc</td>
<td>PKA, PKC, PKD, Rho</td>
</tr>
<tr>
<td>AKAP/15/18</td>
<td>PKA, P2B</td>
</tr>
<tr>
<td>AKAP/15/18</td>
<td>PKA, P2B</td>
</tr>
<tr>
<td>Gravin</td>
<td>PKA, PKC, PDE4D, Src</td>
</tr>
<tr>
<td>Synemin</td>
<td>PKA, deamin, vimentin</td>
</tr>
<tr>
<td>Physiol. Soc.</td>
<td>Uroporphyrin, vinculin</td>
</tr>
<tr>
<td>e-Actinin</td>
<td>5</td>
</tr>
</tbody>
</table>
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) posited 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 phosphorylate RyR 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 (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 Ca2+ 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.
graded adenylatecyclase. Left adrenergic stimulation leads to poor response by activating the β-adrenergic receptor. Recent studies have shown that adrenergic- and cytokine-induced hypertrophy in cultured cardiomyocytes can be decreased by downregulation of a- or b-adrenergic receptors (27). The downregulation of these receptors has been associated with the prevention of hypertrophy and cardiac dysfunction.

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 multiple levels. The studies have revealed extensive participation of AKAPs in the generation of the cardiac action potential and 

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\text{Table 2. AKAP knockouts/mutations and associated phenotypes}
$$

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKAP10 (D-AKAP2)</td>
<td>100</td>
</tr>
<tr>
<td>Cardiac cholinergic response, cardiac arrhythmia, Humm, 64/Vass</td>
<td></td>
</tr>
<tr>
<td>basal heart rate</td>
<td>81</td>
</tr>
<tr>
<td>D-AKAP1</td>
<td></td>
</tr>
<tr>
<td>Oocyte myosin defects, 1 female fertility</td>
<td>78</td>
</tr>
<tr>
<td>AKAP4</td>
<td></td>
</tr>
<tr>
<td>Sperm morphology, motility, and viability defects</td>
<td>67, 102</td>
</tr>
<tr>
<td>AKAP5 (AKAP150)</td>
<td></td>
</tr>
<tr>
<td>LTP defects, motor coordination, spatial memory</td>
<td></td>
</tr>
</tbody>
</table>

**References**

1. <http://physiologyonline.physiology.org/Downloaded from>
2. 10.220.32.247 on June 27, 2017 http://physiologyonline.physiology.org/
cardiac Na+-Ca2+ exchanger (NCX1), located on the plasma membrane and enriched in transverse tubules, plays a primary role in Ca2+ 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 NCX1 (38). Importantly, immunoprecipitation of NCX 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 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 investigations are needed to determine a potential role for mAKAP at the plasma membrane.

**mAKAP and Na+-Ca2+ exchange.** mAKAP may participate in the modulation of Na+-Ca2+ exchange. The cardiac Na+-Ca2+ exchanger (NCX1), located on the plasma membrane and enriched in transverse tubules, plays a primary role in Ca2+ 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 NCX1 (38). Importantly, 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 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 investigations are needed to determine a potential role for mAKAP at the plasma membrane.

**ACAP2 and Na+-Ca2+ re-uptake.** A recent study by Klussmann and colleagues described a role for ACAP1 (38) in modulation of Ca2+ re-uptake into the SR via SERCA in cardiomyocytes. Immunogold staining in neonatal heart tissue showed that SERCA2, phospholamban (PLN), and ACAP1/180 colocalized and that these three proteins were found to co-immunoprecipitate as a protein complex. Furthermore, disruption of the PLN-ACAP1/180 interaction via expression of a short peptide derived from PLN, disrupted the striated distribution of ACAP1/180 and decreased isoproterenol-induced phosphorylation of PLN (Ser 16), and reduced Ca2+ re-uptake. Knockdown of ACAP1/180 abolished the effect of isoproterenol on Ca2+ re-uptake. The investigators concluded that ACAP1/180 plays a significant role in PKA-mediated phosphorylation of PLN and Ca2+ 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 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 current is composed of two subunits, a regulatory subunit (KCNQ1) and the α subunit (KCNQ1) (92). β-Adrenergic receptor-mediated modulation of IK is known to involve Yotiao (72). Yotiao anchors PKA near the channel, which in turn affects the function of IK via phosphorylation of the α subunit at Ser 2809 (Fig. 1). In CHO cells heterologously expressing IK, stimulation with cAMP resulted in phosphorylation of the α subunit and augmentation of the IK current (63, 64). Interestingly, PKA phosphorylates Yotiao itself (Ser 43). Mutation of Ser 43 in Yotiao to an Ala decreased the response of IK to cAMP, therefore, Yotiao may also modulate IK 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 the β subunit of the IKs channel subunit (KCNQ1) (92). Interestingly, some mutations in the β subunit of the IKs channel have been associated with the cardiac phenotype, whereas others disrupt the ability of the Yotiao-PKA-PP1 complex to associate with the regulatory signaling complex nucleated by Yotiao (22, 95).

Whereas a number of point mutations in the s subunit of IK have been associated with development of LQTS, at least two (G588D and S1570L) directly disrupt the ability of the Yotiao-signaling complex to associate with the α subunit of IK. Computational analysis has demonstrated that the G588D 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 S1570L 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). These findings suggest that heterozygous mice have a reduced potential for sudden cardiac death due to LQTS and disruption of the Yotiao signaling complex (22).
A-PP1 complex
thening of the
Yotiao was ini-
cAMP resulted
72). The chan-
cycling of IKs,
ains on Yotiao
ardiac disorder
PKA phospho-
erased in cells
etor-mediated
omplimentary
FIGURE 1

S patients (34,

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Conklin and colleagues (100) examined the Val-646 polymorphism in a cohort of 122 patients. Patients
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AKAP Mutations and Knockouts

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Single nucleotide polymorphism in D-AKAP2

D-AKAP2 (AKAP10) is a dual-function AKAP associat-
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conducted on patients who already had coronary
abnormalities in the cardiovascular and nervous sys-
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 sud-
den 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 func-
tional 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
fido
genuin resulted in poor survival in neon- and postnatal
periods, cleft palate, and respiratory distress (23, 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 sig-
nificant postnatal lethality, low body weight, and
craniofacial defects. Interestingly, loss of mAKAPs,
the preferred isoform expressed in brain, induced expres-
sion of the normally heart-specific β isoform in
the brains of mAKAPs knockout mice (77). Thus the
expectations 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 straight-
forward effects of other AKAP knockouts. Homozygous
strains of mice deficient in D-AKAP1 exhibited disrupt-
ed oocyte meiosis and decreased female fertility and lit-
ter size (81). Loss of AKAP4 (AKAP82), a sperm-specific
AKAP, resulted in altered sperm morphology, motility, and viability (78). Although the phenotypic knockout of
AKAPs (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 PI iso-
form 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 mAKAP 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 Ca²⁺ reuptake, and thus cardiac relaxation, in patients with heart failure (48). 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 (HG3, AKAP-α4, 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 has 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 in 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 studies 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, AKAP15/18, and AKAP15/18 (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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