A-kinase anchoring proteins: scaffolding proteins in the heart

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Anchoring of PKA Through A-Kinase Anchoring Proteins

PKA is a broad specificity tetrameric serine/threonine kinase consisting of two catalytic subunits maintained in an inactive filament and allows contraction to occur (24). Relaxation occurs when L-type Ca\(^{2+}\) channels inactivate and Ca\(^{2+}\) is removed from the cytosol. In humans, ~75% of cytosolic Ca\(^{2+}\) is transported back to the lumen of the sarcoplasmic reticulum by the ATP-dependent Ca\(^{2+}\)-pump, sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA2), whereas the remaining 25% is exported out of the cell by the Na\(^+\)/Ca\(^{2+}\) exchanger (Fig. 1) (4). The sympathetic nervous system enhances contractile force (inotropy), heart rate (chronotropy), and myocardial relaxation (lusitropy) through the release of the catecholamines norepinephrine and epinephrine, which stimulate β-adrenergic receptors (β-ARs) located on the sarcolemma of cardiomyocytes. Activated β-ARs enhance cAMP/PKA signaling, which directly or indirectly regulates the phosphorylation and activity of proteins controlling Ca\(^{2+}\) cycling and sarcromere contraction. In this respect, the phosphorylation of L-type Ca\(^{2+}\) channels and RyR2s increases their open probability and, as a consequence, Ca\(^{2+}\) mobilization from intracellular stores (48, 72). On the other hand, the phosphorylation of phospholamban (PLB), a regulatory transmembrane protein that inhibits SERCA2 activity, induces the dissociation of PLB from SERCA2 (39). This promotes SERCA2 activation and favors myocyte relaxation. Finally, the phosphorylation of sarcromeric proteins including cardiac troponin I (cTnI) and myosin-binding protein C (cMyBP-C) also promotes relaxation by decreasing myofilament Ca\(^{2+}\) responsiveness (14, 57, 62, 108).
state by association with a regulatory (R) subunit dimer. The binding of cAMP to the R subunits releases the active catalytic subunits, resulting in the phosphorylation of substrates within the consensus motif R-R-X-S/T-X (28). At the cellular level, it is now established that specificity of PKA action is achieved through subcellular targeting of the holoenzyme by A-kinase anchoring proteins (AKAPs) (113). AKAPs ensure correct spatial and temporal control of PKA action through the formation of multimolecular complexes that include specific upstream activators, select PKA substrates, and other signaling enzymes that participate in pathway cross talk (3). AKAPs contain a conserved anchoring domain for PKA that consists of an amphipathic helix of 14–18 residues that interacts with a dimerization and docking domain located in the NH2-terminus of the R-subunit dimer (35, 59). Although most of the AKAPs that have so far been identified bind the type II R subunit of PKA (RII), several dual-function anchoring proteins also bind the type I R subunit (RI) (44, 45). Subcellular compartmentalization of individual AKAP-PKA complexes occurs through specialized targeting domains located on each anchoring protein (106).

During the last decade it has become increasingly clear that AKAPs not only anchor PKA but also act as scaffolding proteins that coordinate the activity of many other signaling enzymes such as kinases, phosphatases, phosphodiesterases (PDEs), adenylyl cyclases, GTPases, and other regulatory proteins (3). Therefore, AKAPs assemble multienzyme signaling complexes that ensure the integration and processing of multiple signaling pathways (89). This review will highlight recent advances that have shed new light on the role of AKAPs in cardiac physiology and pathophysiology.

PKA Anchoring in the Heart

Over the last decade several studies have analyzed the implication of PKA anchoring through AKAPs in cardiac physiology. To address this issue, initial studies used peptides corresponding to the R-subunit-binding sequence of an anchoring protein known as AKAP-lymphoid blast crisis (AKAP-Lbc) to competitively inhibit the interaction between PKA and AKAPs in cardiomyocytes. The delivery of these peptides in primary cultures of cardiomyocytes or in rat hearts decreased PKA-dependent phosphorylation of RyR2, PLB, cTnI, and cMyBPC; inhibited cAMP-mediated regulation of L-type Ca21 currents; and altered the kinetics of cardiomyocyte contraction in response to β-adrenergic stimulation (26, 75). Similar results have been obtained by inhibiting PKA anchoring in cardiomyocytes using small molecule inhibitors (15).

In line with these findings, treatment of Langendorff-perfused mouse hearts with transactivator of transcription-conjugated anchoring disruptor peptides containing the PKA-binding region of AKAP10 strongly reduced the phosphorylation of cardiac PKA substrates, heart rate, and left ventricular developed pressure in response to isoproterenol stimulation (88). Collectively, these findings provide solid evidence for the importance of AKAPs in the compartmentalization of PKA and the regulation of cardiac function.

Over the last years, several AKAPs including AKAP18α (29), AKAP18β (68, 105), AKAP79 (98), AKAP250 (81), Yotiao (71), muscle AKAP (mAKAP) (56), dual-specific (D)-AKAP-1 and -2 (44, 45), AKAP95 (21), microtubule-associated protein 2 (97), Brefeldin A-inhibited guanine nucleotide-exchange protein 2 (64),...
ezrin (36), sphingosine kinase type 1-interacting protein (61, 96), gravin (81), synemin (95), myospryn (92), troponin T (109), and the phosphoinositide 3-kinase p110y (90) have been shown to be expressed in cardiac tissues (Table 1). In this context, it is now clear that in the heart, AKAP-based transduction complexes play a crucial role in coordinating signaling pathways that control physiological functions such as Ca\(^{2+}\) cycling, cardiac contractility, and action potential duration, as well as pathophysiological processes including arrhythmias, cardiomyocyte hypertrophy, heart failure, and the adaptive response to hypoxia.

**AKAPs and Ca\(^{2+}\) Cycling**

β-ARs, through the activation of PKA, regulate the activity of several proteins controlling the transport of Ca\(^{2+}\) across the sarcolemma and the release of Ca\(^{2+}\) from the sarcoplasmic reticulum including the L-type Ca\(^{2+}\) channel, RyR2, and SERCA2. It is now appreciated that AKAPs can associate with each of these Ca\(^{2+}\) transporters and favor their regulation by PKA. AKAP18α (also called AKAP15) is a membrane associated anchoring protein that directs PKA to the L-type Ca\(^{2+}\) channel through a direct interaction between a leucine zipper located in its COOH-terminal region and the cytoplasmic domain of the channel (29, 37, 46) (Fig. 1). Studies performed in heterologous expression systems show that overexpression of recombinant AKAP18α/15 reconstitutes cAMP-mediated regulation of the L-type Ca\(^{2+}\) channel (31). Similarly, functional experiments performed in primary cultures of cardiomyocytes show that disruption of PKA anchoring using competing peptides derived from the AKAP18α inhibits β-adrenergic regulation of the L-type Ca\(^{2+}\) channel. This suggests that the anchoring of PKA through AKAP18α might facilitate PKA-mediated regulation of the channel (47). However, later studies indicated that peptides derived from the RII-binding domain of AKAP18α can act as global disruptors of PKA anchoring inside cells (50), raising the possibility that anchoring proteins other than AKAP18α might ensure PKA-mediated phosphorylation of L-type Ca\(^{2+}\) channels in responses to β-AR activation.

In this respect, it has emerged that L-type Ca\(^{2+}\) channel can also form a complex with another anchoring protein named AKAP79/150 (AKAP79, human form; and AKAP150, murine form) (32, 83). In line with these findings, recent studies indicate that in cardiomyocytes, AKAP150 assembles a large signaling complex containing the β-AR, adenylyl cyclases 5 (AC5) and 6 (AC6), PKA, calcineurin, caveolin-3, and a

**Table 1. Cardiac AKAPs**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Alternative Names</th>
<th>Localization</th>
<th>Binding Partners</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKAP1</td>
<td>D-AKAP1, s-AKAP84, AKAP123, AKAP149</td>
<td>Mitochondria, nuclear envelope, endoplasmic reticulum</td>
<td>PKAI and I, PKCa, Src, RSK1, PKP2, PDK1, PDE5A, PLC, AMY-1</td>
<td>Regulation of cardiomyocyte hypertrophy</td>
</tr>
<tr>
<td>AKAP5</td>
<td>AKAP79, AKAP75, AKAP150</td>
<td>Plasma membrane, T tubules</td>
<td>PKAII, PKC, CaN, KCNQ2, L-type Ca(^{2+}) channel, β-AR, AC5 and -6, SAP97, caveolin-3</td>
<td>Regulation of cardiomyocyte hypertrophy</td>
</tr>
<tr>
<td>AKAP6</td>
<td>mAKAPβ</td>
<td>Nuclear envelope</td>
<td>PKAI, PDE4D3, AC5, RyR2, CaNaβ, PKP2, NFATc, ERK5, MEK5, Epac1, Rap1, HIF1α, VHL, Siah2, PKD1, PKR3, NCXC1, nesprin-1α, myopodin</td>
<td>Regulation of cardiomyocyte hypertrophy, regulation of HIF-1α stability during hypoxia, regulation of calcium cycling</td>
</tr>
<tr>
<td>AKAP7</td>
<td>AKAP15, AKAP18 (α, β, γ, δ)</td>
<td>Plasma membrane, endoplasmic reticulum</td>
<td>PKAII, L-type Ca(^{2+}) channel, phospholamban, PKP1, inhibitor 1</td>
<td>Regulation of calcium cycling</td>
</tr>
<tr>
<td>AKAP9</td>
<td>Yocto, AKAP350, AKAP450, CG-NAP, Hyperion</td>
<td>Plasma membrane, Golgi, centrosome</td>
<td>PKAII, PKP1, PKP2A, PKCz, PKN1, casein kinase 1, AC, PDE4D3, IP3-R, KCNQ1, CLIC</td>
<td>Regulation of cardiac repolarization</td>
</tr>
<tr>
<td>AKAP10</td>
<td>D-AKAP2</td>
<td>Outer mitochondrial membrane</td>
<td>PKAI and II, PDZK1, Rab4, Rab11</td>
<td>Regulation of cardiac rhythm</td>
</tr>
<tr>
<td>AKAP13</td>
<td>AKAP-Lbc, Ht31, Btx</td>
<td>Cytoskeleton</td>
<td>PKAI, Gu12, RhoA, 14-3-3, PKCβ, PKD, KSR1, Raf, MEK1/2, ERK1/2, PKNα, MICTK, MKK3, p38α, PKAII, PDE3B, Ras, PIK3R5, Bcr, PK3C6γ, Gβγ, p101, p84</td>
<td>Regulation of cardiomyocyte hypertrophy and heart development</td>
</tr>
<tr>
<td>PIK3γ</td>
<td>PI3Kγ</td>
<td>Unknown</td>
<td>Downregulation of β-ARs expression during heart failure</td>
<td></td>
</tr>
<tr>
<td>CMYA5</td>
<td>Myosprin</td>
<td>Sarcomere</td>
<td>PKAII, titin, calpain 3, disublin</td>
<td>Unknown</td>
</tr>
<tr>
<td>SYNM</td>
<td>Synemin</td>
<td>Intermediate filaments, Z-lines, intercalated discs, plasma membrane</td>
<td>PKAII, desmin, ezrin, talin, vinculin, vimentin, dystrobrevin, desminulin</td>
<td>Organization of the cytoskeleton</td>
</tr>
<tr>
<td>TNNT2</td>
<td>Troponin T</td>
<td>Sarcomere</td>
<td>PKAII, troponin I, troponin C, actin</td>
<td>Sarcomere contraction</td>
</tr>
</tbody>
</table>

AC: adenylyl cyclase; AKAP: A-kinase anchoring protein; AKAP-Lbc: AKAP-lymphoid blast crisis; D-AKAP: dual-specific AKAP; mAKAP: muscle AKAP; s-AKAP: spermidin AKAP; AMY-1: apamin-like receptor; AR: breakpoint cluster region protein; Brx: breast cancer nuclear receptor-binding auxiliary protein; CaN: calcineurin; CN-GAP: centrosome and Golgi localized PKN-associated protein; CLIC: chloride intracellular channel; Epac1, exchange protein activated by cAMP 1; ERK, extracellular signal-regulated kinase; G012; α-subunit of the heterotrimeric G protein G012; Gβγ, βγ-subunit of G proteins; HIF-1α, hypoxia-inducible factor 1α; IP3-R, inositol 3,4,5-phosphate receptor; KCNQ2, gene encoding the KvLQT2 potassium channel; KSR1, kinase suppressor of Ras 1; Lbc, first locus; MEFK, mitogen-activated protein kinase; MKK3, mitogen-activated protein kinase 3; MICTK, mixed lineage related kinase; NCXC1, sodium-calcium exchanger; NFATc1, nuclear factor of activated T cells; PDE, phosphodiesterase; PKD1, phosphoinositide-dependent kinase-1; PDZK1, sodium-proton exchange regulatory factor; PI3K, phosphatidylinositol-3-kinase (PI3K) catalytic subunit; PIK3R5, PI3K regulatory subunit 5; PKC, protein kinase C; PKD, protein kinase D; PKN, protein kinase N; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A; PTEN, protein tyrosine phosphatase D1; KSR1, kinase suppressor of Ras 1; Rap1, Ras-related protein 1; RSK, ribosomal S6 kinase; RyR2, ryanodine receptor 2; SAP97, synapse-associated protein 97; Siah2, seven in absentia homolog 2; VHL, von Hippel-Lindau tumor suppressor.
subpopulation of L-type Ca\textsuperscript{2+} channels that were specifically phosphorylated in response to sympathetic stimulation (Fig. 1) (83). Surprisingly, the knockout of AKAP150 also inhibited PKA-mediated phosphorylation of RyR2 receptors and PLB (83). Based on these results, it was postulated that the AKAP150 complex, through the recruitment of AC5 and AC6, generates cAMP microdomains that promote PKA-dependent regulation of L-type Ca\textsuperscript{2+} channels, PLB, and RyR2 receptors located in caveolin-3-associated junctional regions of the sarcoplasmic reticulum adjacent to T tubules (Fig. 1) (41, 83).

The effects of PKA phosphorylation on RyR2 have been highly controversial, and a review of that literature is beyond the scope of this review (38, 102, 103). There is evidence that the targeting of PKA to RyR2 can be mediated by the muscle-selective anchoring protein mAKAP (56), which directly binds RyR2 via a leucine zipper motif, although whether RyR2-mAKAP complexes are present throughout the sarcoplasmic reticulum or only in a perinuclear compartment is unclear (Fig. 1) (54, 72). mAKAP is proposed to favor PKA-mediated phosphorylation of the channel and the consequent release of the inhibitory protein FK506-binding protein of 12 kDa (FKBP12.6) (72). This may increase RyR2 open probability, potentially enhancing the release of Ca\textsuperscript{2+} from the sarcoplasmic reticulum. Importantly, mAKAP can form a complex with the PKA-regulated PDE4D3. Phosphorylation of PDE4D3 by PKA enhances local cAMP hydrolysis and induces PKA deactivation (Fig. 1) (19, 20). This implies that the mAKAP complex permits a transient activation of PKA in response to β-AR stimulation. Finally, AKAP188 (42) was identified as the anchoring protein controlling ß-adrenergic regulation of SERCA2 activity (68). AKAP188 favors PKA-mediated phosphorylation of PLB and, as a consequence, the release of PLB from SERCA2 and Ca\textsuperscript{2+} reuptake (Fig. 1). Accordingly, the silencing of AKAP188 or the disruption of the interaction between AKAP186 and PLB in cardiomyocytes was shown to impair β-AR-induced Ca\textsuperscript{2+} reuptake from the cytosol into the sarcoplasmic reticulum and to inhibit cardiomyocyte relaxation (68). Interestingly, recent findings indicate that AKAP188 also recruits protein phosphatase 1 (PP1) and its negative regulator inhibitor-1 (I-1), favoring PKA-mediated phosphorylation of I-1 and the consequent inhibition of PP1 activity (105). Knowing that PP1 can inhibit SERCA2 activity via PLB dephosphorylation, it is plausible that AKAP188, by facilitating I-1-dependent PP1 regulation, might further promote cardiomyocyte relaxation.

**AKAPs and Myofiber Contractility**

It is well established that β-AR agonists promote PKA-dependent phosphorylation of two sarcomeric proteins involved in the regulation of actomyosin interactions: cTnI and cMyBP-C. The phosphorylation of cTnI and cMyBP-C by PKA is associated with a decrease in the Ca\textsuperscript{2+} responsiveness of myofilaments and an acceleration in the kinetics of crossbridge cycling, which attenuates myofilament force and favors myocyte relaxation (14, 57, 62, 108).

Several studies have shown that the disruption of PKA anchoring in cardiomyocytes and in intact hearts inhibits PKA-mediated phosphorylation of cTnI and cMyBP-C (15, 26, 75, 88). Although these results demonstrated that cTnI and cMyBP-C phosphorylation requires AKAP-mediated PKA anchoring, the identity of the AKAP(s) mediating this function is still unknown. Over the last years several AKAPs have been shown to associate with the sarcomere. These include synemin, which can be recruited to the Z-disc (25, 95); myosyn, which localizes at the peripheral Z-disc/costameric region (92); cardiac troponin T (cTnT) (109); and myomagelin, which is proposed to regulate the phosphorylation of cMyBP-C (112). Future experiments will need to determine whether these AKAPs anchor PKA to modulate sarcomere contraction. Importantly, mutations associated with human cardiac diseases have been identified in genes encoding sarcomeric AKAPs. Indeed, the deletion of lysine-210 of cTnI is associated with familial-dilated cardiomyopathy (52). Interestingly, this mutation, which decreased phosphorylation of cMyBP-C and cTnI (101), impairs PKA anchoring to cTnI (109). On the other hand, a polymorphism at amino acid 2906 of myosyn, which replaces a lysine with an asparagine, is associated with left ventricular hypertrophy in patients with hypertension (79).

**AKAPs and Cardiac Repolarization**

The cardiac slowly activating, delayed rectifier K\textsuperscript{+} channel is responsible for the \( I_{ks} \) that mediates repolarization of the plasma membrane of cardiomyocytes and is critically important for the regulation of the cardiac action potential duration. It is composed of an α-subunit (KCNQ1) and a Rβ-subunit (KCNE1) (Fig. 2A) (82). In response to β-AR stimulation, PKA phosphorylates KCNQ1 on serine-27 located on its NH\textsubscript{2}-terminal region (71). This significantly increases the \( I_{ks} \), accelerates repolarization, and shortens ventricular action potential duration. The anchoring protein Yotiao has been shown to recruit PKA and PP1 to the carboxyl terminus of KNCQ1 to regulate the phosphorylation state of serine-27 and the activity of the ion channel (71) (Fig. 2A). Maximal activation of the \( I_{ks} \) by the sympathetic system occurs when anchored PKA also phosphorylates serine-43 on Yotiao (12). Recent findings indicate that Yotiao also recruits PDE4D3, which by locally degrading cAMP, regulates the extent of activation of anchored PKA (Fig. 2A) (110). Collectively, these findings indicate that Yotiao recruits a kinase-phosphatase-PDE complex that allows the fine-tuning of the \( I_{ks} \) channel activity.

**AKAPs and Cardiac Arrhythmias**

The common form of long QT syndrome (LQTS) is an inherited heart disease associated with delayed ventricular repolarization that is clinically identified by abnormal QT interval prolongation on the surface electrocardiogram (67). LQTS increases the probability of developing a malignant polymorphic ventricular arrhythmia called torsades de pointes that can result in sudden death (67). Most commonly, prolongation of the QT interval is produced by a reduction in the \( I_{ks} \) because of mutations in the genes encoding KCNQ1 and KCNE1. Interestingly, studies performed in the last few years indicate that LQTS can be associated with mutations that reduce the interaction between KCNQ1 and Yotiao (67, 82). Such inherited mutations have been identified both in the Yotiao-binding region of KCNQ1 (G589D) (27) and in the KCNQ1-binding region of Yotiao (S1570L) (Fig. 2). Such mutations have been shown to reduce the interaction between KCNQ1 and Yotiao (67, 82). Such mutations have been shown to reduce the interaction between KCNQ1 and Yotiao (67, 82). Such mutations have been shown to reduce the interaction between KCNQ1 and Yotiao (67, 82). Such mutations have been shown to reduce the interaction between KCNQ1 and Yotiao (67, 82).
ings suggest that altering the ability of the Yotiao signaling complex to regulate the \( I_{Ks} \) can cause LQTS.

Recent studies indicate that the dual-function anchoring protein D-AKAP2 is also involved in the regulation of the cardiac rhythm. An analysis of single nucleotide polymorphisms in a population of 6,500 healthy Europeans, Americans, and Asians identified a polymorphism at amino acid 646 of D-AKAP2, which replaces a key isoleucine residue within the RI-binding domain of D-AKAP-2 with valine (53). The D-AKAP2 Val-646 variant is associated with a shorter P-R interval (i.e., the time between the onset of atrial and ventricular depolarization) on the surface electrocardiogram (53).

Although biochemical studies revealed that the mutation of isoleucine 646 to valine enhances by threefold the affinity of D-AKAP2 for RI (6), it is currently unknown how this causes the phenotype observed in affected individuals. However, gene-trap mice expressing a COOH terminally truncated form of D-AKAP2 missing the entire RI-binding domain displayed higher susceptibility to sudden death because of cardiac arrhythmias, confirming the hypothesis that alteration of the RI-anchoring properties of D-AKAP-2 might affect cardiac rhythm (111).

AKAPs and Cardiac Hypertrophy

Ventricular myocyte hypertrophy is the primary compensatory mechanism whereby the myocardium reduces ventricular wall tension when submitted to stress because of myocardial infarction, hypertension, and congenital heart disease or neurohumoral activation. It is associated with a nonmitotic growth of cardiomyocytes, increased myofibrillar organization, and upregulation of specific subsets of “fetal” genes that are normally expressed during embryonic life (30, 43). The concomitant aberrant cardiac contractility, \( Ca^{2+} \) handling, and myocardial energetics are associated with maladaptive changes that include interstitial fibrosis and cardiomyocyte death and increase the risk of developing heart failure and malignant arrhythmia (7, 43). Increased in prevalence by risk factors such as smoking and obesity, heart failure is a syndrome that affects about six million Americans and has an annual incidence of 1% of senior citizens (94). Since the five-year survival rate after diagnosis is still very poor (lower than 50%), many efforts have been made during the last years to define the molecular mechanisms involved in this pathological process.

Cardiac hypertrophy can be induced by a variety of neurohumoral, paracrine, and autocrine stimuli, which activate several receptor families including G protein-coupled receptors, cytokine receptors, and growth factor tyrosine kinase receptors (5, 30). In this context, it is becoming increasingly clear that AKAPs can assemble multiprotein complexes that integrate hypertrophic pathways emanating from these receptors. In particular, recent studies have now identified anchoring proteins including mAKAP and AKAP-Lbc and D-AKAP1 that play a central role in organizing and modulating hypertrophic pathways activated by stress signals.

**mAKAP.** In cardiomyocytes, mAKAP\(\beta\) is localized to the nuclear envelope through an interaction with nesprin-1\(\alpha\) (87). mAKAP\(\beta\) assembles a large signaling complex that integrates hypertrophic signals initiated by \(\alpha_1\)-adrenergic receptors (\(\alpha_1\)-ARs) and \(\beta\)-ARs, endothelin-1 receptors, and gp130/leukemia inhibitor factor receptors (Fig. 3A) (19, 86). Over the last few years, the molecular mechanisms as well as the signaling pathways whereby mAKAP\(\beta\) mediates cardiomyocyte hypertrophy have been extensively investigated. It is now demonstrated that mAKAP\(\beta\) can recruit the phosphatase calcineurin A\(\beta\) (CaNA\(\beta\)) as well as the hypertrophic transcription factor nuclear factor of activated T cells c3 (NFATc3) (65). In response to adrenergic receptor activation, anchored CaNA\(\beta\)
CaNA
cular mechanisms controlling the activation of the pool of local Ca\textsuperscript{2+} completely understood but seem to require mobilization of receptor; IP\textsubscript{3}, inositol trisphosphate 1,4,5-trisphosphate.

This favors myocyte-specific enhancer-binding factor 2 (MEF2)-dependent hypertrophic gene transcription. LIF-R, leukemia inhibitor factor dephosphorylates and activates NFATc3 to promote hypertrophic gene transcription. Very high concentrations of cAMP (in ET1Rs, PKC\textgreek{e}) (115). This suggests that the anchoring of PLC\textepsilon binds phospholipase C pathway by 10.220.33.1 on July 6, 2017 http://ajpheart.physiology.org/ Downloaded from

dephosphorylates and activates NFATc3, which promotes the transcription of hypertrophic genes (Fig. 3A) (65). The molecular mechanisms controlling the activation of the pool of Ca\textsuperscript{2+} bound to the mAKAP\beta complex are currently not completely understood but seem to require mobilization of local Ca\textsuperscript{2+} stores. In this context, it has been shown that mAKAP favors PKA-induced phosphorylation of RyR2 (54), which, through the modulation of perinuclear Ca\textsuperscript{2+} release, could activate Ca\textsuperscript{2+} (3A). In line with this hypothesis, the deletion of the PKA anchoring domain from mAKAP\beta has been shown to suppress the mAKAP-mediated hypertrophic response (86). On the other hand, recent findings indicate that mAKAP\beta also binds phospholipase C\epsilon (PLC\epsilon) and that disruption of endogenous mAKAP\beta-PLC\epsilon complexes in rat neonatal ventricular myocytes inhibits endothelin 1-induced hypertropy (115). This suggests that the anchoring of PLC\epsilon to the nuclear envelope by mAKAP\beta controls hypertrophic remodeling. Therefore, it is also plausible that at the nuclear envelope, PLC\epsilon might promote the generation of inositol 1,4,5-trisphosphate, which through the mobilization of local Ca\textsuperscript{2+} stores, might promote the activation of CaNaB and NFATc3 bound to mAKAP\beta (Fig. 3A).

In cardiomyocytes, the dynamics of PKA activation within the mAKAP complex are tightly regulated by ACS (55) and the PDE4D3 (19, 20) that are directly bound to the anchoring protein. The mAKAP-bound ACS and upstream \beta-AR may be localized within transverse tubules adjacent to the nuclear envelope (22). In response to elevated cAMP levels, mAKAP-bound PKA phosphorylates both ACS and PDE4D3 (19, 20, 55). This induces the recruitment of 14-3-3, which inhibits the Rho-GEF activity of AKAP-lymphoid blast crisis (AKAP-Lbc) through Go\textsubscript{12}. GTP-bound RhoA is released from the AKAP-Lbc complex and promotes cardiomyocyte hypertrophy via a signaling pathway that remains to be elucidated. Activation AKAP-Lbc-anchored PKA promotes the phosphorylation of the anchoring protein on serine-1565. This induces the recruitment of 14-3-3, which inhibits active PKD phosphorylates histone deacetylase 5 (HDAC5), causing its export form the nucleus. This favors myocyte-specific enhancer-binding factor 2 (MEF2)-dependent hypertrophic gene transcription. LIF-R, leukemia inhibitor factor receptor; IP\textsubscript{3}, inositol trisphosphate 1,4,5-trisphosphate.

Fig. 3. Regulation of cardiac hypertrophy by AKAP complexes. A: mAKAP assembles a multienzyme signaling complex at the outer nuclear membrane containing ACS, PKA, PDE4D3, PP2A, RyR2, calcineurin A\beta (CaNA\beta), nuclear factor of activated T cells 3 (NFATc3), exchange protein activated by cAMP 1 (Epac1), and ERK5. Activation of ACS by \beta-adrenergic stimulation generates cAMP, which in turn activates anchored PKA at submicromolar concentrations. In a negative feedback loop, activated PKA phosphorylates PDE4D3, leading to its activation and increased cAMP degradation, and ACS, leading to its inactivation and decreased cAMP synthesis. Anchored PKA also regulates the activity of PP2A, which promotes PDE4D3 dephosphorylation, and RyR2, which enhances Ca\textsuperscript{2+} mobilization from intracellular stores. This is proposed to induce the activation of CaNaB, which, in turn, dephosphorylates and activates NFATc3 to promote hypertrophic gene transcription. Very high concentrations of cAMP (in \muM) also stimulate Epac1. This in turn activates the GTPase Ras-related protein 1 (Rap1), which exerts an inhibitory effect on the MEK5-ERK5 pathway. In the absence of very high local cAMP, Epac1 is inactivated and the hypertrophic ERK5 pathway de-repressed. Stimulation of endothelin-1 receptors (ET\textsubscript{1}s) activates mAKAP\beta-bound PLC\epsilon, which, in turn, promotes cardiomyocyte hypertrophy via a signaling pathway that remains to be elucidated. B: activated ACSs and ET\textsubscript{1}s stimulate the Rho-guanine nucleotide exchange factor (GEF) activity of AKAP-lymphoid blast crisis (AKAP-Lbc) through Go\textsubscript{12}. GTP-bound RhoA is released from the AKAP-Lbc complex and promotes cardiomyocyte hypertrophy via a signaling pathway that remains to be elucidated. Activation AKAP-Lbc-anchored PKA promotes the phosphorylation of the anchoring protein on serine-1565. This induces the recruitment of 14-3-3, which inhibits the Rho-GEF activity of AKAP-Lbc. AKAP-Lbc also recruits PKC\eta and PKD. Upon stimulation by the Go\textsubscript{q}-phospholipase C pathway by \alpha\textgreek{e}-ARs and ET\textsubscript{1}s, PKC\eta becomes activated and phosphorylates PKD. Active PKD phosphorylates histone deacetylase 5 (HDAC5), causing its export form the nucleus. This favors myocyte-specific enhancer-binding factor 2 (MEF2)-dependent hypertrophic gene transcription. LIF-R, leukemia inhibitor factor receptor; IP\textsubscript{3}, inositol trisphosphate 1,4,5-trisphosphate.

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Interestingly, PDE4D3 can also act as a scaffold protein that recruits the cAMP-activated guanine nucleotide exchange factor (GEF) exchange protein activated by cAMP 1 (Eapc1), the prohypertrophic mitogen-activated protein kinase ERK5, and its upstream activator MEK5 (19) to the mAKAP complex (Fig. 3A). The elevation of local cAMP levels activates Eapc1 in the complex. This promotes the activation of Ras-related protein 1, which inhibits ERK5 signaling, reversing ERK5-mediated PDE4D3 inhibition and leading to enhanced cAMP degradation. In contrast, in the absence of very high cAMP levels, ERK5 signaling can promote hypertrophic gene transcription (Fig. 3A) (19). In the light of these findings, targeting the interactions between mAKAP and the associated prohypertrophic regulators using specific disruptors might offer a potential approach for inhibiting hypertrophic cardiac remodeling.

AKAP-Lbc. The anchoring protein AKAP-Lbc is mainly expressed in cardiac tissues and functions as a type II PKA anchoring protein as well as GEF for the small molecular weight GTPase RhoA (17, 60). RhoA activation is mediated by consecutive Dbl and pleckstrin homology domains that are located in the COOH-terminal half of the anchoring protein (17). In cardiomyocytes, AKAP-Lbc Rho-GEF activity is enhanced by α1-ARs via a pathway that requires the α- subunit of the heterotrimeric G protein G12 (Fig. 3B) (2, 17). On the other hand, AKAP-Lbc-bound PKA decreases the activation of RhoA by inhibiting the Rho-GEF activity of the scaffolding protein. Phosphorylation of serine-1565 on AKAP-Lbc by anchored PKA promotes the recruitment of 14-3-3, which, in turn, inhibits RhoA activation (Fig. 3B) (16).

Several lines of evidence now indicate that AKAP-Lbc is an important mediator of the hypertrophic responses induced by several G protein-coupled receptors including the α1-Ar, the angiotensin II type 1 receptor and the endothelin-1 receptor (2). Initial studies performed on isolated rat cardiomyocytes indicated that knockdown of AKAP-Lbc expression or inhibition of Gα12 activity in rat neonatal ventricular cardiomyocytes strongly reduces the ability of phenylephrine, angiotensin II, and endothelin I to induce RhoA activation and hypertrophic responses (Fig. 3B) (2). Potential effectors of the Gα12/13-AKAP-Lbc/RhoA pathway could include protein kinase N (76) and the p38 kinase (70), which have been recently shown to interact with AKAP-Lbc (8).

Importantly, AKAP-Lbc also coordinates the activation of protein kinase D (PKD). In this respect, it has been shown that AKAP-Lbc acts as a scaffold that recruits PKD and its upstream activator kinase protein kinase Cη (PKCη) (10). Stimulation of α1-ARs or endothelin-1 receptors promotes the activation of anchored PKCη, which, in turn, phosphorylates and activates AKAP-Lbc-bound PKD (Fig. 3B). Active PKD is released from the scaffold when serine-2737 within the PKD-binding site of AKAP-Lbc is phosphorylated by anchored PKA (10). Live-cell imaging experiments performed in primary cultures of neonatal cardiomyocytes indicate that AKAP-Lbc enhances nuclear PKD activity (11). Activated PKD promotes the phosphorylation and the nuclear export of the class II histone deacetylase 5. This favors myocyte-specific enhancer-binding factor 2-dependent transcriptional activation of hypertrophic genes (Fig. 3B) (11). Based on these findings, one could speculate that interfering with the ability of AKAP-Lbc to activate Rho and/or to interact with PKD might represent a potential interesting strategy to reduce cardiac hypertrophy.

Recent findings indicate that AKAP-Lbc can assemble a signaling complex containing the scaffold kinase suppressor of Ras 1, the Ras effector Raf, and MEK1/2 that coordinates the activation of ERK1/2 in HEK-293 cells (107). Given the importance of ERK1/2 in regulating cardiomyocyte hypertrophy in vivo, it will be crucial to define the cardiac role of the AKAP-Lbc-ERK1/2 pathway.

In mice, the expression of AKAP-Lbc is strongly induced in the heart at the onset of cardiac hypertrophy in response to several hemodynamic or neurohumoral stresses such as chronic infusion of phenylephrine (2), angiotensin II, and thoracic aortic constriction (A. Appert-Collin and D. Diviani, unpublished observations). Similarly, AKAP-Lbc mRNA is increased in heart samples obtained from patients with hypertrophic cardiomyopathy (11). This raises the hypothesis that AKAP-Lbc might play a role in the pathological cardiac remodeling process in vivo. AKAP-Lbc knockout mice die in utero between 8.5 and 10.5 days post because of defects in heart tube formation (74). While this argued for a role of AKAP-Lbc in cardiac development, it precluded the possibility to analyze the role of the anchoring protein in the process of cardiac hypertrophy and heart failure. Future experiments using tissue-specific and/or inducible knockout models will certainly circumvent this problem and bring new answers on the implication of AKAP-Lbc in this heart pathology.

D-AKAPI. D-AKAPI (45), also called AKAP121 (9), and its shorter splice variant AKAP84 are mitochondrial proteins that are widely expressed in different tissues including heart ventricles (97). Recent functional studies performed in rat neonatal ventricular myocytes indicate that D-AKAPI negatively regulates cardiomyocytes hypertrophy (1). Silencing of D-AKAPI promotes an increase in cardiomyocyte size, whereas its overexpression inhibits isoproterenol-induced hypertrophy (1). Interestingly, it appears that this anchoring protein can inhibit dephosphorylation and nuclear translocation of the prohypertrophic transcription factor NFATc3 possibly via the binding and the inhibition of the phosphatase calcineurin (1). On the other hand, it remains unclear whether D-AKAPI-anchored PKA regulates hypertrophy of cardiomyocytes (1).

Consistent with a role of D-AKAPI in inhibiting hypertrophy, the induction of hypertrophy in rats by aortic banding strongly reduces the ventricular expression of D-AKAPI (91). Interestingly, the displacement of D-AKAPI from mitochondria by competitive peptides increases the mitochondrial levels of reactive oxygen species, promotes mitochondrial dysfunction, and induces cardiomyocyte apoptosis in rat hearts (91). Interestingly, anchored PKA has been previously shown to increase the activity of the 18-kDa subunit of complex I (NDUFS4) (85) and to inhibit the activity of the proapoptotic protein Bad (40). Therefore, one could speculate that the mitochondrial defects observed in response to D-AKAPI uncoupling might be due to an impaired regulation of these PKA substrates. Collectively, these findings raise the hypothesis that the downregulation of D-AKAPI in response to cardiac stress might contribute to the development of cardiac dysfunction.
**AKAPs and Heart Failure**

The downregulation of β-AR expression during congestive heart failure contributes to the depressed heart contractility characterizing congestive heart failure (66, 93). It is now shown that phosphatidylinositol 3-kinase-γ (PI3Kγ) assembles a newly identified signaling complex containing PKA and PDE3B that might regulate β-AR expression at the surface of cardiomyocytes (90). In physiological conditions, the β-AR/cAMP pathway leads to the activation of PI3Kγ-anchored PKA, which, in turn, phosphorylates PI3Kγ to inhibit its activity (90). In heart failure, PI3Kγ is upregulated and escapes PKA-mediated inhibition (90). Activated PI3Kγ reduces cell surface expression of β-ARs most likely through its interaction with the β-AR kinase 1 (77) and the adaptor protein complex 2 (78), two key regulators of receptor internalization. These findings indicate that altering the expression of PI3Kγ in the myocardium can compromise heart function and contribute to heart failure.

**AKAPs and Hypoxia**

Myocardial oxygen levels need to be maintained within narrow levels to sustain cardiac function. During ischemic insult, in response to conditions of reduced oxygen supply (termed hypoxia), cardiomyocytes mobilize the hypoxia-inducible factor 1α (HIF-1α), a transcriptional complex that promotes a wide range of cellular responses necessary to adapt to reduced oxygen (99). Transcriptional responses activated by HIF-1α control cell survival, oxygen transport, energy metabolism, and angiogenesis (99). Under normoxic conditions, HIF-1α is hydroxylated on two specific proline residues by the prolyl hydroxylase domain proteins (PHDs) and subsequently recognized and ubiquitinated by the von Hippel-Lindau protein (51, 73). Ubiquitinated HIF-1α is targeted to the proteasome for degradation. On the other hand, when oxygen concentration falls, the enzymatic activity of PHD proteins is inhibited. Moreover, PHD proteins are ubiquitinated by an E3 ligase named “seven in absentia homolog 2 (Siah2)” and targeted for proteasomal degradation (80). This inhibits HIF-1α degradation and allows the protein to accumulate in the nucleus where it promotes gene transcription required for the adaptive response to hypoxia. In line with this finding, the delivery of exogenous HIF-1α improves heart function after myocardial infarction (104), whereas cardiac overexpression of HIF-1α reduces infarct size and favors the formation of capillaries (58).

Recent findings indicate that mAKAP assembles a signaling complex containing HIF-1α, PHD, von Hippel-Lindau protein, and Siah2 (Fig. 4) (114). This positions HIF-1α in proximity of its upstream regulators as well as to its site of action inside the nucleus. In this configuration, under normoxic conditions, negative regulators associated with the mAKAP complex favor HIF-1α degradation (Fig. 4 A) (114). On the other hand, during hypoxia, the activation of Siah2 within the mAKAP complex promotes HIF-1α stabilization, allowing the transcription factor to induce transcription (Fig. 4 B) (114). Therefore, mAKAP assembles a macromolecular complex that can favor degradation or stabilization of HIF-1α in cardiomyocytes in response to variations of oxygen concentrations. In this context, mAKAP could play an important role in cardiomyocyte protection during cardiac ischemia, when coronary blood flow is impaired.

**Fig. 4.** The role of mAKAP in regulating hypoxia-inducible factor 1α (HIF1α)-mediated transcription during normoxia and hypoxia. A: mAKAP assembles a signaling complex containing HIF-1α, prolyl hydroxylase domain protein (PHD), von Hippel-Lindau protein (pVHL), and seven in absentia homolog 2 (Siah2). Under normoxic conditions, HIF-1α is hydroxylated on proline residues (pro) by PHD, ubiquitinated (ub) by pVHL, and subsequently degraded by the proteasome. B: under hypoxic conditions, PHD is ubiquitinated by Siah2 and degraded. This favors HIF-1α stability, which accumulates in the nucleus to initiate transcriptional responses required to adapt to reduced oxygen. This could favor transcriptional responses controlling the induction of glycolysis, mitochondrial respiration, and cell survival during ischemia.
reduced or interrupted. By coordinating the molecular pathways that control HIF-1α stabilization in cardiomyocytes, mAKAP might favor HIF-1α-mediated transcriptional responses, controlling the induction of glycolysis (which maximizes ATP production under hypoxic conditions), the efficiency of mitochondrial respiration, and cell survival during ischemia (100).

**AKAPs as Potential Therapeutic Targets**

Over the last decade it has become increasingly clear that AKAP-based signaling complexes play a central role in regulating physiological and pathological cardiac events. It will be interesting to explore whether inhibiting the signaling properties of individual AKAP signaling complexes using drugs that target unique protein-protein interactions can represent a potential approach for limiting cardiac pathological processes. Such a therapeutic strategy might offer an advantage over classical therapeutic approaches since it would allow the selective inhibition of defined cellular responses.

Anchoring proteins including mAKAP, AKAP-Lbc, and AKAP188 could represent interesting therapeutic targets for the treatment of cardiac hypertrophy and heart failure. In particular, peptides disrupting AKAP-mediated protein-protein interactions could be used to inhibit the ability of mAKAP and AKAP-Lbc to coordinate the activation of prohypertrophic kinases (ERK5, PKD) (11, 19), phosphatases (CaNAβ) (65), and GTPases (RhoA) (2) that play a central role in activating key transcription factors such as NFAT and myocyte-specific enhancer-binding factor 2 that initiate the remodeling process leading to cardiac hypertrophy.

On the other hand, peptides have been developed that interfere with the interaction between PLB and AKAP188 (68) and that efficiently impair PKA-mediated phosphorylation of PLB (68). These molecular disruptors might be used as potential therapeutic agents to prevent chronic β-AR-mediated activation of SERCA2 in the failing human heart (69), which is suspected to cause excessive energy consumption and to induce cardiomyocyte dysfunction. However, the use of disruptor peptides in vivo might have some limitations because of their poor stability and tissue absorption. Poor delivery should be overcome by the use of cell-penetrating sequences such as the transactivator of transcription peptide and polyarginine tails or conjugated with lipid-derived groups such as stearate (49). On the other hand, poor stability may be overcome by the use of peptidomimetics [i.e., peptides with structural modifications in the original sequence giving protection against exo- and endoproteases without affecting the structural and functional properties of the peptide (34)].

One alternative approach to target specific interaction within AKAP-based complexes would be the use of small molecule disruptors, which could be identified by combining rational design and screening approaches (15, 33). Such compounds could be designed to target-specific binding surfaces on AKAPs. This approach has been successfully used to disrupt the interaction between AKAPs and PKA in cardiomyocytes and to enhance the contractility of intact hearts (15). Based on this and on the above considerations, targeting selected protein-protein interactions within AKAP complexes might represent a potential strategy for the treatment of chronic heart failure.

**Conclusions**

The present review illustrates the central role that AKAPs play in organizing signaling pathways that control cardiac contractility and rhythm as well as the pathological response of heart to stress. While in past years the function of AKAP-based signaling complexes has been mainly studied in isolated cardiomyocytes, a growing number of recent studies now suggest that AKAPs organize physiologically relevant signaling events also in vivo. The development of cardiac-specific knockout and knockin animal models will offer an invaluable tool for studying the role of AKAP complexes and for dissecting the implication of individual protein-protein interactions in heart physiology as well as in cardiac dysfunctions such as arrhythmias, cardiac hypertrophy, and heart failure. The combined application of these approaches with proteomic and in vivo imaging techniques will determine how this family of anchoring proteins controls the spatial dynamics and temporal organization of signaling pathways in healthy and diseased hearts. On the other hand, future clinical investigations and genome-wide association studies will be instrumental to determine whether mutations in AKAP genes are associated with known cardiac diseases. Finally, a better structural characterization of AKAP signaling complexes will be necessary to precisely delineate the interacting surfaces within AKAP complexes. This information will be crucial to design and develop new drugs that selectively interfere with AKAP-mediated pathological processes in the heart.

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