Basic Concepts in Heart Failure

A-Kinase Anchoring Proteins

Getting to the Heart of the Matter

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The heart is a sophisticated organ that continuously pumps blood to ensure that oxygen and nutrients reach the brain, other organs, and peripheral tissue. The right side of the heart pumps blood to the lungs, where oxygen is taken up and carbon dioxide is removed. The left side of the heart then pumps blood to the rest of the body, where oxygen and nutrients are delivered to tissues. This cycle is sustained by the repeated contraction of cardiomyocytes, specialized muscle cells that are designed to contract and respond rapidly to physiological stimuli.

Second messengers are small molecules that are mobilized or generated in response to extracellular stimuli. In the heart, Ca\(^{2+}\) and cAMP are the second messengers most frequently used by cardiac signaling pathways. They act at defined intracellular sites to initiate signaling events that control excitability, contraction, and gene expression. Occupancy of \(\beta\)-adrenergic receptors engages the cAMP-signaling pathway to activate protein kinases, guanine nucleotide exchange factors, and ion channels. Combinations of these cAMP-responsive enzymes often brought together in multiprotein complexes. The goal of the present review is to highlight recent progress on our understanding of how anchored signaling complexes influence cardiomyocyte physiology. We will focus on cardiomyocyte A-kinase anchoring proteins (AKAPs), a family of scaffolding proteins that compartmentalize cAMP- and Ca\(^{2+}\) responsive enzymes in proximity to preferred substrates such as ion channels, contractile proteins, Ca\(^{2+}\) pumps, and the transcriptional machinery.

A-Kinase Anchoring Proteins

Seminal experiments in the 1950s demonstrated that hormone-mediated stimulation of cAMP synthesis by different agonists induced distinctive physiological outputs, even within the same tissue. Thirty years later, it was shown that adrenergic stimulation selectively activated a pool of the cAMP-dependent protein kinase (PKA) associated with the particulate fraction of cardiomyocytes, whereas prostaglandin E\(_1\) stimulation activated a cytosolic pool of PKA in the same cells to induce different physiological effects. As more investigators pondered this concept, it became clear that cAMP was not distributed uniformly throughout the cell. This led to the hypothesis that the opposing actions of adenylyl cyclases and phosphodiesterases (PDEs) generate intracellular gradients and compartmentalized pools of cAMP. More recently, a genetically encoded fluorescence-based biosensor was used to measure microdomains of...
cAMP along sarcomeric Z lines in cardiomyocytes in response to adrenergic stimulation.17

Although it was accepted that cAMP levels were distributed unevenly within the cell, it remained unclear exactly how PKA was retained within different subcellular compartments.18 Soon, a variety of protein-protein interaction screens identified a set of molecules that interact with the R subunits of the PKA holoenzyme (a tetramer consisting of a regulatory [R] subunit dimer and 2 catalytic [C] subunits).19,20 These proteins were named AKAPs in recognition of their ability to anchor PKA at defined subcellular locations.21 A principal function of AKAPs is to position PKA and other cAMP-responsive enzymes in proximity to their substrates.6,22,23 However, AKAPs also serve to cluster regulatory enzymes with the cAMP synthesis machinery.24,25 For example, interactions between AKAP79/150 and the adenyl cyclase isoforms ACV or ACVI facilitate the preferential PKA phosphorylation of the enzyme to inhibit cAMP synthesis. Such a PKA-AKAP79/150-ACV complex forms a negative-feedback loop that generates pulses of cAMP production.24 This configuration may be particularly relevant in the pacemaker cells, because studies in ACV-knockout mice suggest that this adenylyl cyclase isoform participates in sympathetic and parasympathetic regulation of cardiac contractility.26,27

Functional analysis of AKAP action often takes advantage of reagents that can displace PKA from anchoring proteins. Detailed biochemical analyses have demonstrated that R-subunit dimerization is necessary for PKA interaction with AKAPs and that each anchoring protein contains a reciprocal binding sequence of 14 to 18 amino acids that forms an amphipathic helix.28,29 One of these proteins, initially called Ht31 but now known as AKAP-Lbc, contains an 18- amino acid sequence that can be used as a peptidase disruptor of RI-anchoring interactions inside cells.30 The utility of Ht31 peptide as a universal PKA-anchoring disruptor was initially demonstrated on perfusion into cultured hippocampal neurons to disrupt the location of PKA in relation to a key substrate, the AMPA-type glutamate receptor.30 Later, it was shown that perfusion of Ht31 peptide into cardiomyocytes uncoupled cAMP-dependent regulation of the L-type calcium channel.31 More recently, adenoviral gene transfer of Ht31 analogs into rat hearts has suggested that anchored pools of PKA contribute to β-adrenergic stimulation of cardiomyocyte contractility.32,33 Mislocalization of PKA alters the phosphorylation of key proteins that participate in this process, such as L-type calcium channels, the ryanodine receptors, phospholamban, and myofibrillar troponin I.34,35 Calcium imaging and echocardiogram analyses have concluded that disruption of PKA anchoring (1) affects cardiac contractility as measured by changes in the rate of calcium transients (2) increases the left ventricular ejection fraction and stroke volume.33 These studies provide a compelling rationale for more mechanistic studies to assess the contribution of individual AKAP complexes in the regulation of different aspects of cardiac function under normal and pathophysiological conditions.

**AKAPs Signal Cardiac Contractility**

As noted above, EC coupling is the process whereby an action potential triggers a myocyte to contract. This involves a transient rise in intracellular calcium that drives contraction (reviewed by Bers). From a signaling perspective, EC coupling can be considered to occur in 3 phases (Figures 1A through 1C). Phase 1 is initiated by brief openings of voltage-gated L-type Ca$^{2+}$ channels. Small amounts of Ca$^{2+}$ enter specialized regions of the ventricular myocyte where the junctional SR is close to the sarcolemma.36–38 In phase 2, this localized Ca$^{2+}$ influx triggers the synchronous activation of multiple ryanodine-sensitive Ca$^{2+}$ channels (RyRs) in the SR to produce a global Ca$^{2+}$ transient.39,40 The concomitant activation of Ca$^{2+}$-responsive contractile proteins such as cardiac troponin C initiates contraction. Phase 3 requires termination of SR Ca$^{2+}$ release and the transport of Ca$^{2+}$ back into the SR through the ATP-dependent Ca$^{2+}$ pump SERCA2 (sarcoplasmic/endoplasmic reticulum Ca$^{2+}$ pump 2), which decreases [Ca$^{2+}$], and begins myocyte relaxation. Recent evidence suggests that distinct AKAP complexes contribute to each phase of EC coupling by optimizing the phosphorylation of ion channels and contractile proteins.

The junction between the sarcolemma and the SR is decorated with local Ca$^{2+}$ signaling complexes that contain between 10 and 25 L-type Ca$^{2+}$ channels and approximately 100 to 200 RyRs. β-adrenergic agonists stimulate PKA to...
increase the amplitude of L-type Ca\(^{2+}\) currents to favor Ca\(^{2+}\)-influx. The AKAP79/150 family of anchoring proteins (human AKAP79, bovine AKAP75, and murine AKAP150) facilitates this process by directing the phosphorylation of L-type Ca\(^{2+}\) channel subunits (Figure 1A). Biochemical and electrophysiological studies initially showed that expression of recombinant AKAP79 enhanced the cAMP-dependent stimulation of L-type Cav1.2 currents. AKAP79/150 also associates with the calcium/calmodulin-dependent phosphatase PP2B and a variety of calcium/phospholipid-dependent protein kinase (PKC) isoforms. These additional AKAP79/150 binding partners are important in other aspects of cardiac myocyte function.

Recent studies suggest that AKAP79/150 has multiple regulatory roles in the cardiovascular system. For example, AKAP79/150-associated pools of PKC\(\alpha\) have been implicated in the induction of persistent Ca\(^{2+}\) sparklets, local Ca\(^{2+}\) signals produced by recurrent openings of L-type Ca\(^{2+}\) channels in arterial myocytes that enhance vascular tone (Figure 1A). Arterial myocytes isolated from AKAP150-knockout mice do not generate persistent Ca\(^{2+}\) sparklets. Furthermore, AKAP150 \(-/-\) mice are naturally hypertensive and do not develop angiotensin II–induced hypertension. Collectively, these results suggest that local modulation of L-type Ca\(^{2+}\) channels by AKAP150-targeted pools of PKA, PKC\(\alpha\), and PP2B may control different aspects of Ca\(^{2+}\)-influx that contribute to cardiac EC coupling, vascular tone, and changes in blood pressure.

Another AKAP also modulates cardiac L-type Ca\(^{2+}\) channels. Alternate splicing of the AKAP15/18 gene yields several isoforms that reside in distinct subcellular locations. The \(\alpha\)– and \(\beta\)-isoforms of AKAP15/18 are found at the plasma membrane in complex with L-type Ca\(^{2+}\) channels. Lipid modification of 3 residues in the extreme amino terminus of the anchoring protein is required for association with the inner face of the plasma membrane, whereas a leucine zipper–like motif in the carboxyl terminus interacts with the cytoplasmic domain of the L-type Ca\(^{2+}\) channel. This arrangement ensures that an anchored pool of type 4 PDE, which is believed to increase the amplitude of L-type Ca\(^{2+}\) currents, to favor Ca\(^{2+}\)-influx, may interact directly with cardiac L-type Ca\(^{2+}\) channels, because phospha-biding sites have been mapped to the cytoplasmic regions of the \(\alpha_{1.2}\) subunit of the channel. The formation of these more sophisticated multireceptor signaling complexes that are mediated by AKAP79/150 may permit the more precise transmission of second-messenger signals to this Ca\(^{2+}\) channel. Another advantage of the formation of more sophisticated AKAP signaling complexes in proximity to Ca\(^{2+}\) channels is that it may permit integration of cAMP and calcium/phospholipid signals at this intracellular locus.

The second phase of EC coupling begins when openings of L-type Ca\(^{2+}\) channels cause a local increase of [Ca\(^{2+}\)], from 100 nmol/L to approximately 10 to 20 \(\mu\)mol/L, which activates nearby RyRs to release Ca\(^{2+}\) from the SR (Figure 1B). Planar bilayer work suggests that phosphorylation of the RyR by PKA increases the sensitivity of the channel to Ca\(^{2+}\) and accelerates the kinetics of adaptation. In the intact cell, PKA is recruited to the RyR complex by the muscle-selective anchoring protein (mAKAP). mAKAP is a multivalent anchoring protein that brings PKA and the cAMP-metabolizing enzyme phosphodiesterase PDE4D3 to the RyR. The clustering of a cAMP-metabolizing enzyme with PKA limits the availability of cAMP to ensure that kinase activation is transient (Figure 1B). Furthermore, the phosphorylation state of the RyR is constantly monitored by the mAKAP-associated phosphatase PP2A. Loss of anchored PDE4D3 and hyperactivation of the anchored PKA may correlate with the onset of “leaky” channels found in certain models of exercise-induced cardiac arrhythmias and heart failure. However, an equally plausible explanation is that compensatory changes in calcium content of the SR may make the RyR leaky during arrhythmias. These latter events could be critical in determining whether abnormal calcium waves and sparks are propagated.

The third phase of EC coupling involves active transport of Ca\(^{2+}\) back into the lumen of the SR to initiate myocyte relaxation (Figure 1C). The SERCA2 pump and its modulator protein, phospholamban, control this process. Mono-meric phospholamban is a 52–amino acid protein that represses SERCA2. However, \(\beta\)-adrenergic stimulation favors PKA phosphorylation of phospholamban. This promotes its pentamerization and the derepression of SERCA2. Precise regulation of this system is achieved in part because all of the components are organized into a supramolecular complex that consists of the SERCA2, phospholamban, PKA, and a high-molecular-weight isoform of AKAP15/18. Elegant biochemical and imaging studies show that disruption of PKA interaction with the AKAP15/18 isoform interferes with the phosphorylation of phospholamban (Figure 1C). This prevents the release of phospholamban from SERCA2 to blunt Ca\(^{2+}\) reuptake and myocyte relaxation. Reversible control of the cAMP response may be provided by an anchored pool of type 4 PDE, which is believed to associate with AKAP15/18.

**AKAP Signaling Under Pathophysiological Conditions**

Adverse genetic profiles and unfavorable environmental factors contribute to the origin of many cardiac disorders.
Because AKAPs orchestrate signaling events that modulate cardiac contractility, it appears reasonable that defects in anchoring protein genes or pathophysiological changes in AKAP signaling complexes may underlie certain heart diseases. Several recent reports now support this notion.

Sudden death due to arrhythmia kills more than 400,000 people a year in the United States and many more throughout the world. As a result, there is a concerted international effort to identify genes that regulate heart rhythm. Several independent findings point toward a role for dual-function anchoring proteins (d-AKAP-2) in heart rhythm control. A genetic screen of single-nucleotide polymorphisms in DNA pools of 6,500 age-stratified healthy European, Asian, and American individuals detected an amino acid change from Ile to Val at position 646 in d-AKAP-2 (AKAP10). Individuals harboring the d-AKAP-2 646Val variant often exhibited a decrease in the length of the PR interval on ECGs (the time from the onset of atrial depolarization to the beginning of ventricular depolarization). In vitro biochemical analyses of the d-AKAP-2 646Val variant suggested that this amino acid substitution in the PKA-anchoring domain of the protein enhanced its affinity for the type 1 PKA holoenzyme 3-fold. Although a mechanistic connection between these 2 observations remains unclear, d-AKAP-2 mutant mice expressing a truncated form of d-AKAP-2 that are unable to anchor PKA develop cardiac arrhythmias and die prematurely. Furthermore, cultured myocytes isolated from these mice were reported to display an increased contractile response to cholinergic signals.

Long-QT syndrome is a rare congenital heart condition that can result in sudden death as a consequence of exercise- or excitement-induced arrhythmia. This condition occurs because of the delayed repolarization of myocyte membranes. This prolongs the duration of the ventricular action potential and thus is diagnosed as a lengthening of the QT interval. Myocyte repolarization requires the movement of potassium ions out of the cell. Consequently, the most common type of long-QT syndrome (LQT1) is associated with mutations in the KCNQ1 subunit of a potassium channel. The anchoring protein Yotiao associates with a variety of ion channels, including the KCNQ1 subunit responsible for $I_{Ks}$ currents that shape cardiac action potential duration in response to $\beta$-adrenergic agonists. The primary function of Yotiao is to facilitate phosphorylation of Ser27 on KCNQ1 to modulate ion channel activity; however, anchored PKA also phosphorylates serine 43 on Yotiao to enhance cAMP-dependent activation of $I_{Ks}$. Inherited mutations in another region of Yotiao (S1570L) that reduce the interaction between KCNQ1 and Yotiao suppress cAMP-induced phosphorylation of the $I_{Ks}$ channel. The functional consequence of the S1570L mutation in mice is delayed repolarization of the ventricular action potential, a recognized symptom of LQT1 (Figure 2A). Taken together, these findings suggest that spatial organization of the cAMP-signaling enzymes by Yotiao is important to orchestrate ion channel activity during myocyte repolarization. More recent evidence suggests that the phosphodiesterase PDE4D3, a negative regulator of anchored PKA, is also present in the Yotiao signaling complex (Figure 2A). Electrophysiological studies have shown that pharmacological inhibition of anchored PDE activity enhances cAMP-dependent stimulation of $I_{Ks}$. In this context, the recruitment of a PDE4D3 constrains the local availability of cAMP to more precisely regulate the anchored pool of PKA.

Another means to vary the modulation of signaling scaffolds may be to induce time-dependent changes in the composition or amount of AKAP complexes. There have been recent reports suggesting that temporal changes in AKAP complexes can occur, either in response to sudden physiochemical changes in the environment or more gradually as a cellular adaptation to stress. For example, activation of $\beta$-adrenergic receptors during periods of cardiac stress initially improves cardiac output by increasing heart rate and contractility; however, chronic mobilization of the same signaling pathway ultimately harms the heart.

Elevated catecholamines in the heart evoke transcriptional activation of the myocyte enhancer factor pathway to induce a cellular response known as pathological myocardial hypertrophy. This initiates a developmental gene reprogramming paradigm known as the fetal gene response. A clearer picture of how individual steps in this hypertrophic signaling pathway are linked is beginning to emerge. The anchoring protein AKAP-Lbc is one of the genes upregulated in hypertrophic myocytes and functions as a scaffolding protein for PKA and PKC to mediate activation of a third enzyme, protein kinase D (PKD1; Figure 3A). A combination of cellular and live-cell imaging approaches supports a model in which AKAP-Lbc facilitates activation of protein kinase D, which in turn phosphorylates the histone deacetylase HDAC5 to promote its nuclear export. Finally, the concomitant reduction in nuclear histone deacetylase activity favors myocyte enhancer factor-2 transcription and the onset of cardiac hypertrophy. Although most of the study was conducted in neonatal rat cardiomyocytes, further support for this concept was provided by analysis of human heart tissue samples obtained after death from individuals who exhibited hypertrophic cardiomyopathy, which showed that AKAP-Lbc mRNA increased 2.0 ± 0.5-fold over normal age-matched patient control subjects. Because myocardial hypertrophy is extremely common, affecting 14% to 18% of the general adult population, further investigation of the AKAP-Lbc/
PKD/HDAC5 pathway is warranted to establish whether AKAP-Lbc is a valid biomarker for hypertrophic cardiomyopathy.

AKAP79/150 may also be involved in pathophysiological changes of gene expression that occur in vascular smooth muscle during the development of hypertension. In these cells, activation of PKCα induces persistent Ca²⁺ sparklets, producing an increase in local Ca²⁺ influx that activates nearby AKAP150-targeted PP2B. On activation, PP2B dephosphorylates NFATc3, which allows this transcription factor to translocate into the nucleus of arterial myocytes, where it modulates gene expression (Figure 3B). AKAP79/150-associated phosphatase PP2B and NFATc3 activity levels are low in arterial smooth muscle under physiological conditions because of low levels of persistent Ca²⁺ sparklet activity; however, in hypertension, elevated PKCα activity increases persistent Ca²⁺ sparklet activity, thereby increasing PP2B activity and consequently the NFATc3 nuclear import rate, which leads to high nuclear accumulation of this transcription factor. This in turn leads to downregulation of the b1 subunit of the large-conductance, Ca²⁺-activated K⁺ channel and the Kv2.1 channel subunit in arterial myocytes. When these findings are considered in light of evidence that abnormalities in the AKAP79/150-associated PKC contribute to the onset of hypertension, it appears reasonable to speculate that an anchored pool of PP2B may work via a distinct mechanism to counteract this process.

Like all organs, the heart needs a constant supply of oxygen-rich blood. A system of arteries and veins supplies the myocardium with oxygen-rich blood and then returns oxygen-depleted blood to the right atrium. As a result, the concentration of cellular oxygen is maintained within a narrow range (termed “normoxia”). A key cellular response to a state of reduced oxygen tension (hypoxia) involves the induction of genes by the transcription factor known as hypoxia-inducible factor 1α (HIF-1α). Under normoxic conditions, HIF-1α is kept low through its ubiquitin-mediated proteasomal degradation. The half-life of HIF-1α under normoxic conditions has been calculated to be approximately 5 minutes. However, if the oxygen supply suddenly drops, myocytes enter a hypoxic state, and the continual destruction of HIF-1α halts instantly (Figure 3C). This allows the protein to form a stable heterodimeric complex with the HIF-1β subunit to initiate transcription of proangiogenic, metabolic, and antiapoptotic genes that promote cell survival during hypoxia.
cardiac contractility. However, recent evidence suggests that the anchoring proteins themselves may play a more active role in the regulation of cardiac contractility than merely functioning as scaffolding proteins. For example, Yotiao binding to $I_{Ks}$ channels is believed to evoke allosteric changes that increase the magnitude of $I_{Ks}$ currents, and interaction with AKAP15/18 is believed to enhance the phospholamban rate of multimerization. Finally, anchoring proteins such as AKAP-Lbc and mAKAP interface with the protein acetylation and ubiquitination machinery, respectively, to evoke changes in transcriptional activation pathways. These latter observations imply that AKAPs not only contribute to the bidirectional control of second-messenger phosphorylation events but also shape broader aspects of cardiovascular physiology and pathophysiology.

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References


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