Anchoring Proteins as Regulators of Signaling Pathways

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Abstract: **Spatial and temporal organization of signal transduction is coordinated through the segregation of signaling enzymes in selected cellular compartments. This highly evolved regulatory mechanism ensures the activation of selected enzymes only in the vicinity of their target proteins. In this context, cAMP-responsive triggering of protein kinase A is modulated by a family of scaffold proteins referred to as A-kinase anchoring proteins. A-kinase anchoring proteins form the core of multiprotein complexes and enable simultaneous but segregated cAMP signaling events to occur in defined cellular compartments. In this review we will focus on the description of A-kinase anchoring protein function in the regulation of cardiac physiopathology. (*Circ Res*. 2012; 111:482-492.)**

Key Words: AKAP ■ PKA ■ cAMP ■ cardiac disease

Spatial and temporal control of signal transduction events is frequently achieved by compartmentalization of intracellular effectors through adaptors or anchoring proteins. In particular, elements of the cAMP signaling cascade are localized in the cell via scaffold proteins referred to as A-kinase anchoring proteins (AKAPs).[1] cAMP is a second messenger involved in the regulation of different cellular events that occur in response to extracellular stimuli. Binding of an extracellular stimulus to a selective G-protein coupled receptor (GPCR) triggers the activation of a heterotrimeric Gs protein and its effector, the adenylyl cyclase (AC), which generates the second messenger cAMP. In turn, cAMP exerts its effects through the activation of 3 effectors: protein kinase A (PKA), the exchange protein directly activated by cAMP and the cyclic nucleotide-gated ion channels.[2,3] The primary effector of cAMP in the heart is PKA, a tetramer formed by 2 catalytic subunits that are inactivated by the binding of the 2 regulatory subunits. Binding of cAMP to the regulatory subunits induces the dissociation and the activation of the catalytic subunits, resulting in the phosphorylation of local substrates.[4]

Several studies have demonstrated that cAMP is not uniformly distributed throughout the cell.[5,6] Indeed, numerous imaging studies have shown that cAMP levels rise selectively in a specific cellular compartment in a stimulus-specific manner and do not diffuse from one compartment to the other, allowing fidelity of the response.[7] Spatially restricted activation of PKA is guaranteed by the binding of this kinase with AKAPs, a family of functionally related proteins that interact with the regulatory subunits of the PKA holoenzyme. The molecular feature of AKAPs is to possess a structurally conserved PKA anchoring domain, consisting of an amphipatic helix of 14 to 18 residues that selectively binds the dimerization and docking domain at the N-terminus of the PKA regulatory subunit dimer.[8–10] Although the vast majority of AKAPs bind the type II regulatory subunit of PKA, several AKAPs are referred to as dual-function anchoring proteins because they bind both the type I (RI) and the type II (RII) regulatory subunits of PKA.[11] More recently, type I PKA specific anchoring proteins have been described.[12–14] Several evidences have demonstrated that PKA-RI and PKA-RII isoforms are indeed anchored to specific subcellular sites via binding to these different AKAPs.[7]

AKAPs do not only position PKA inside the cell but they also ensure that this kinase is coupled to its upstream activators, including membrane receptors and ACs, and to signal termination enzymes, such as phosphodiesterases (PDE) and phosphatases.[15–17] In this way, AKAPs help to establish intracellular cAMP gradients, generated via activation of a specific GPCR and uniquely modulated by different subsets of PDEs, resulting in stimulus-specific activation and action of PKA.[7] AKAPs also coordinate signaling enzymes such as other kinases, GTPases, and regulatory proteins into multivalent transduction signalosomes. Thus, AKAPs provide the structural integrity for multiprotein complexes that often represent hubs for processing of multiple signals. A further layer of specificity proceeds through protein- or lipid-targeting domains on AKAPs that direct AKAP signaling complexes to intracellular membranes.[18] A concerted research effort over the past 20 years has identified more than 50 genes encoding distinct anchoring proteins.[19] Furthermore, numerous splice variants are transcribed from each gene in a cell type and tissue-specific manner.

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To narrow the focus of this article, we will restrict our discussion to the actions of compartmentalized cAMP signaling and AKAP function in the cardiovascular system. In the heart, several AKAPs play a critical role in modulating multiple signaling pathways at the basis of cardiac physiopathology (Table).[20] This review will especially focus on the importance of anchored-PKA in the regulation of cardiac cAMP compartmentation.

Non-standard Abbreviations and Acronyms

β-AR	β-adrenergic receptor
AC	adenylyl cyclase
AKAP	A-kinase anchoring protein
cAMP	cyclic AMP
GPCR	G protein-coupled receptor
GRK-2	G protein-coupled receptor kinase 2
HIF-1α	hypoxia-inducible factor 1α
LTCC	voltage-gated L-type Ca^{2+} channel
NFAT	nuclear factor of activated T cells
PDE	phosphodiesterase
PKA	protein kinase A
PLN	phospholamban
ROS	reactive oxygen species
RyR	ryanodine receptor
SERCA	sarcoplasmic reticular Ca^{2+}-adenosine triphosphatase
SR	sarcoplasmic reticulum

Cardiac AKAPs

Cardiac Development

The heart is the first organ to form during embryogenesis and all subsequent events in the life of the organism are dependent on its function. Cardiac organogenesis is characterized by the precise temporal and region-specific regulation of cell proliferation, migration, death, and differentiation.[21,22] All these processes are finely tuned by a variety of signal transduction pathways. Among these, anchored cAMP signaling is essential for cardiomyocyte differentiation and heart morphogenesis. AKAP-Lbc (also referred to as AKAP13 or BRX) is a key regulator of these events and the deletion of this AKAP in the mouse results in a thin and enlarged myocardium that leads to an arrest in cardiac development and subsequent embryonic lethality.[23] This failure in cardiac formation is consistent with decreased activity of the small GTPase Rho, a direct target of AKAP-Lbc.[24] Reduced Rho function in turn correlates with a repressed activity of the myocyte enhancer factor-2, a transcription factor important for the proper regulation of cardiac gene expression.[25] AKAP-Lbc is thus a platform that links Rho signaling to an essential transcription program that drives cardiomyocyte development.[26]

Contractility

In the adult heart, cardiac contractility and relaxation are mediated by rapid changes in cytoplasmic Ca^{2+} concentration

Table. AKAPs in the Heart

Gene Name	Alternative Name	Function	Intracellular Localization	Signaling Partners
AKAP1	D-AKAP1, s-AKAP84, AKAP121, AKAP149	Hypertrophy	Mitochondria, nuclear envelope, endoplasmic reticulum	PKA RI, PKA RII, PKCα, Src, PP1, PP2A, PP2B, PTPD1, PDE7A, AMY-1, Lfc, RSK1
AKAP5	AKAP75, AKAP79, AKAP150	Contractility	Plasma membrane, T tubules	PKA RII, PKC, PP2B, LTCC, KCNQ2, β-AR, AC5, AC6, CAV3, SAP97
AKAP6	mAKAP	Hypertrophy, contractility, hypoxia	Nuclear envelope	PKA RII, PDE4D3, AC5, RyR2, CaNAβ, PP2A, NFATc, ERK5, MEK5, Epac1, Rap1, HIF1α, VHL, Siah2, PDK1, RSK3, NCX1, nesprin-1α, myopodin
AKAP7	AKAP15, AKAP18	Contractility	Plasma membrane, endoplasmic reticulum	PKA RII, LTCC, PLB, PP1, inhibitor1
AKAP9	Yotiao, AKAP350, AKAP450, CG-NAP, Hyperion	Cardiac repolarization	Plasma membrane, golgi, centrosome	PKA RII, PP1, PP2A, PKCε, PKN1, casein kinase 1, AC, PDE4D3, IP3-R, KCNQ1, CLIC
AKAP10	D-AKAP2	Cardiac rhythm	Mitochondria	PKA RI, PKA RII, PDZK1, Rab4, Rab11
AKAP12	Gravin, AKAP250, SSeCKS	β-AR signaling	Plasma membrane	PKA RII, β-AR, PKC, PDE4D, Src, PP2B
AKAP13	AKAP-Lbc, Ht31, BRX	Hypertrophy and development	Cytoskeleton	PKA RII, Gα12/13, RhoA, actin, 14-3-3, PKC, PKD, KSR1, Raf, MEK1/2, ERK1/2, PKNα
PDE4DIP	Myomegalin, MMGL, CMYA2	Contractility	Sarcomere	PKA, PDE4D
PIK3CG	p110γ	β-AR downregulation	Membrane	PKA RII, p101, p84/87, Ras, PDE3B, Bcr
SYNM	Synemin	Cytoskeletal organization	Plasma membrane, sarcomere	PKA RII, desmin, zyxin, talin, vinculin, vimentin, dystrobrevin, desmuslin, utrophin, α-actinin
TNNT2	Troponin T	Contractility	Sarcomere	PKA RII, troponin I, troponin C, actin

AKAP indicates A-kinase anchoring protein; PKA RI, type I regulatory subunit of protein kinase A; PP, protein phosphatase; PKA RII, type II regulatory subunit of protein kinase A; PDE, phosphodiesterases; LTCC, L-type Ca2+ channels; β-AR, β-adrenergic receptor; AC, adenylyl cyclase; CAV3, caveolin 3; RyR2, ryanodine receptor 2; NFAT, nuclear factor of activated T cells; Epac, exchange protein directly activated by cAMP; HIF1α, hypoxia-inducible factor-1; VHL, von Hippel-Lindau; Siah2, Seven in Absentia Homolog 2; PKN, protein kinase N; PTPD1, protein tyrosine phosphatase D1; AMY-1, associate of Myc-1; RSK, ribosomal S6 kinase; SAP97, synapse-associated protein 97; NCX1, sodium-calcium E changer-1; PLB, phospholamban; KSR1, kinase suppressor of Ras1.

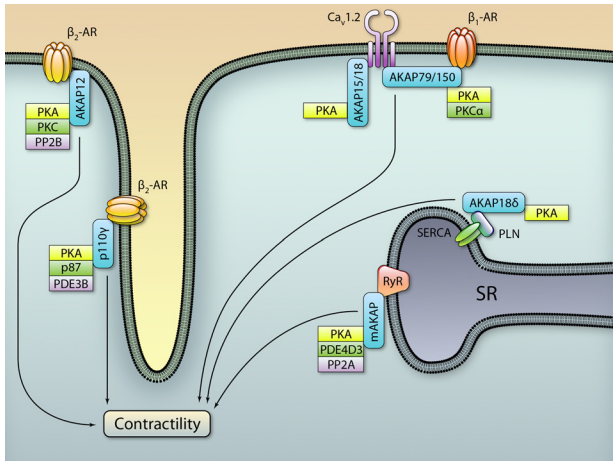

Figure 1. Regulation of cardiac contractility by A-kinase anchoring protein (AKAPs). Intracellular distribution of AKAP complexes involved in myocardial contractility modulation. PKA indicates protein kinase A; PP2B, protein phosphatase 2B; PDE, phosphodiesterase; SERCA PLN, sarcoplasmic reticular Ca^{2+}-adenosine triphosphatase phospholamban; SR, sarcoplasmic reticulum; AR, adrenergic receptor. (Illustration: Ben Smith.)

following the electric stimulation of the myocardium. During the excitation-contraction coupling, brief openings of sarcolemmal voltage-gated L-type Ca^{2+} channels (LTCCs) in response to an action potential generate local elevations in intracellular Ca^{2+}. This highly localized Ca^{2+} rise in turn activates closely apposed ryanodine receptors (RyRs) in the sarcoplasmic reticulum (SR), via a mechanism referred to as Ca^{2+}-induced Ca^{2+} release. This results in a substantial release of Ca^{2+} form the SR, thereby inducing a global increase in Ca^{2+} concentration that activates cardiac contractile proteins. LTCCs and RyRs are rapidly inactivated by Ca^{2+}-dependent mechanisms and allow the cardiac sarcoplasmic reticular Ca^{2+}-adenosine triphosphatase (SERCA2) pump to recover the released Ca^{2+} in the SR before the next heart beat.[27] Tight regulation of Ca^{2+} handling is thus required for proper force and rate of contraction of the heart. The sympathetic nervous system (SNS) is one of the major regulators of heart rate in response to exercise or emotional stress. SNS controls cardiac electric activity through the activation of β-adrenergic receptors (β-ARs) that modulate the function of selected ion channels via phosphorylation by PKA.[28] These Ca^{2+}-related signaling events are regulated by different combinations of AKAPs that finely modulate the PKA-dependent signaling (Figure 1). Displacement of PKA from AKAPs by PKA anchoring disruptor peptides results in altered phosphorylation of key players in excitation-contraction coupling, thus leading to compromised cardiac contractility.[29–32] Under this scenario, a pivotal role in regulating cAMP and Ca^{2+} transients is played by multiple AKAPs, including AKAP79/150, gravin, AKAP15/18, mAKAP, AKAP18δ and a group of sarcomeric AKAPs that have just recently been identified.

AKAP-βAR Complexes

Beta-adrenergic receptors (β-ARs) impact Ca^{2+} handling by increasing the force of contraction and by accelerating the rate of relaxation.[28] The effect of catecholamines on the heart is mainly mediated by β_1-ARs and β_2-ARs. Although both receptors are very similar in structure, they perform different functions. Whereas β_1-ARs couple only to Gs, agonist-bound β_2-ARs undergo sequential coupling to both Gs and Gi.[33] Functional differences between β_1-ARs and β_2-ARs can also be attributed to subtype-specific targeting to different cellular compartments.[34,35] Compartmentalization of β-ARs in different plasma membrane microdomains can explain subtype-specific signaling.[36–38] Likewise, different AKAPs organize distinct β-AR-containing signalosomes. Of note, AKAP79/150 (also referred to as AKAP5) is bound to the plasma membrane through a N-terminal polybasic targeting domain that binds phospholipids and a palmitoylation domain that specifically targets AKAP79/150 to lipid rafts, at the level of the synaptic junction.[39,40] The functional consequence of this targeting event is to confine PKA within lipid rafts.[41] In this compartment, AKAP79/150 organizes a complex containing PKA, β_1-AR, AC5/6, PP2B, $Ca_v1.2$, and caveolin 3 (CAV3) and controls a β_1-AR-stimulated microdomain of cAMP that impacts on Ca^{2+} transients. Accordingly, in cells lacking AKAP79/150, β-AR activation does not modulate intracellular Ca^{2+} signaling.[42] On the other hand, β_2-ARs bind both AKAP79/150 and another anchoring protein called gravin (also referred to as AKAP12 or AKAP250 or SSeCKS).[43] AKAP79/150 appears to function in switching signaling pathways of the receptor from AC to activation of the mitogen-activated protein kinase cascade. In contrast, gravin targets the receptor to the plasma membrane of cardiomyocyte-like H9c2 cells.[44,45] Within this context, gravin is bound to PKA, β_2-AR and PKC.[44,46–48] Perturbation of this signaling complex leads to disruption of β-AR internalization and resensitization, critical events in G-protein coupled receptors regulation.[47,49] Furthermore, although AKAP79/150 is essential to mediate the activation of the MAP kinase cascade on catecholamine stimulation, gravin is required for the ability of cells to recover from agonist-induced desensitization and recycling.[50] Collectively, these findings offer a compelling argument for the spatial activation and segregation of different adrenergic receptors by selective AKAP signaling complexes.

AKAP-LTCC Complexes

LTCCs are the primary source of Ca^{2+} influx to initiate excitation-contraction coupling.[28] From a molecular point of view, cardiac LTCCs include the pore-forming α1C subunit (also referred to as $Ca_v1.2$) and three auxiliary subunits (β, $\alpha_2\delta$ and γ) that are involved in trafficking $Ca_v1.2$ to the sarcolemma and in modulating the voltage dependence of channel gating.[51] Alterations in LTCC density or function have been implicated in a variety of cardiovascular diseases, including atrial fibrillation, ischemic heart disease and heart failure.[52] For these reasons, in cardiac physiology, LTCCs are regulated by a variety of neurotransmitters, hormones and cytokines. Of note, β-adrenergic system is a crucial regulator of LTCC-mediated Ca^{2+} homeostasis.[53] During the "fight or flight" response, stimulation of β-ARs increases LTCC currents through PKA-mediated phosphorylation of the channel itself ($Ca_v1.2$ or β subunit) or of its associated proteins.[54–56] The increase in Ca^{2+} currents induced by PKA activation is due to an enhancement of the open-state probability of the

channel, resulting from a shift in gating mode.[57] Regulation of LTCCs requires PKA targeting to the distal C terminus (DCT) of the channel. Truncation of $Ca_v1.2$ DCT abolishes the regulation of LTCCs by the β-AR/PKA pathway,[58] consistently with the finding of PKA phosphorylation sites at the distal C terminus of $Ca_v1.2$.[59–61] Several lines of evidence have emphasized the importance of AKAPs in targeting PKA in the vicinity of LTCCs.[62] In skeletal muscle and in cardiomyocytes, a low molecular weight AKAP, AKAP15/18 (also known as AKAP18α or AKAP7) has been identified as the anchoring protein that targets PKA to $Ca_v1.2$.[53,62–64] In higher detail, AKAP15/18 targets PKA to the C terminus of $Ca_v1.2$ through a modified leucine zipper motif located in its C-terminal region. Disruption of this interaction inhibits PKA-dependent enhancement of LTCC activity, both in skeletal muscle cells and in rat ventricular cardiomyocytes.[64,65] The C terminus of $Ca_v1.2$ undergoes proteolytic processing in vivo, giving rise to two isoforms that differ by truncation of the C terminus. The proteolytically cleaved DCT acts as a regulatory domain of LTCC normal function, by binding to the truncated channel and inhibiting its function.[66] Accordingly, mice expressing only truncated $Ca_v1.2$ develop severe cardiac hypertrophy and die perinatally. Deletion of the DCT disrupts the expression and localization of the AKAP15/18-PKA complex, resulting in an impaired regulation of LTCC function.[58]

Ca^{2+} signaling is regulated not only by AKAP15/18-PKA-$Ca_v1.2$ complex at the cell surface but also at the level of the sarcoplasmic reticulum. In this respect, two different AKAPs are involved: mAKAP and AKAP18δ.

mAKAP-RyR Complex

The muscle specific AKAP (mAKAP) is prominently expressed in cardiomyocytes and it is localized both at the sarcoplasmic reticulum, where it regulates Ca^{2+}-induced Ca^{2+} release,[67] and at the nuclear envelope, where it assembles a macromolecular complex integrating cAMP and Ca^{2+} signals.[68] Accordingly, it has been shown that a mAKAP–PKA–RyR complex is strategically located within the cell to modulate both SR-dependent cytoplasmic Ca^{2+} rise and the perinuclear Ca^{2+} fluxes.[67] mAKAP functions as a scaffold for a wide range of proteins including type II PKA,[68] PDE4D3,[16] AC5,[69] protein phosphatase 2A,[67] the MAP kinases MEK5 and ERK5, the small GTPase Rap1, and the cAMP-activated Rap1 exchange factor Epac1.[70] Within this macromolecular complex, mAKAP-mediated PKA phosphorylation of the RyR is considered to promote opening of this channel and to increase cardiac function.[71] Within this context, cAMP may increase Ca^{2+} fluxes via PKA-dependent phosphorylation of the RyR, in a manner tightly controlled by the PKA-activated PDE4D3 and protein phosphatase 2A-mediated dephosphorylation. Alternatively, recent studies report that PKA/PDE4D3-mediated control of RyR phosphorylation is irrelevant to normal cardiac function and sympathetic stimulation of the heart.[72–74]

AKAP18δ-SERCA2 Complex

SERCA2 controls Ca^{2+} reuptake into the sarcoplasmic reticulum, a rate-limiting step for cardiac relaxation. SERCA2 activity is regulated by numerous factors, including the cytoplasmic/SR Ca^{2+} gradient, the protein concentration of SERCA2, and the SR inhibitory protein phospholamban (PLN). Dephosphorylated PLN binds to SERCA2 and suppresses its activity, whereas phosphorylation of PLN on Ser16 by PKA dissociates PLN from SERCA2, increasing the Ca^{2+} reuptake into the SR. This PKA-mediated phosphorylation of PLN is strictly dependent on the function of AKAP18δ, a long splice variant of the AKAP18 gene.[75,76] Both the displacement of AKAP18δ from PLN or the silencing of AKAP18δ significantly reduce the PKA-dependent PLN phosphorylation after β-adrenergic stimulation, resulting in a decrease in Ca^{2+} reuptake into the sarcoplasmic reticulum. Alterations in the function of PLN-SERCA2 complex are linked to cardiac dysfunction.[77] Because AKAP18δ mediates PLN phosphorylation and subsequent increase in SERCA2 activity, modulation of AKAP18δ could represent a novel pharmacological target in the treatment of heart failure.[78]

Sarcomeric AKAPs

Several actin-associated (ezrin, gravin, WAVE-1, and AKAP79/150) and microtubule-associated (MAP2, AKAP350/450, hAKAP220, pericentrin, flagellar radial spoke protein 3) AKAPs have been described in different tissues.[79] In the heart, multiple evidences have demonstrated the crucial role of AKAPs in targeting PKA at the sarcomere.[80] In particular, 3 different AKAPs are involved in mediating PKA-dependent phosphorylation of sarcomeric proteins, crucial regulators of myocardial contractile function.

Synemin is the first intermediate filament protein shown to bind PKA RII and to localize a pool of PKA, allowing local substrate phosphorylation within the myocyte cytoskeleton. Intermediate filament-targeted PKA could phosphorylate substrates found at the Z-line or regulate intermediate filament structure. Synemin is overexpressed in failing hearts: this correlates with an increase in PKA targeting to sites undergoing molecular remodeling.[81]

Cardiac troponin T has been recently characterized as a novel dual-specificity AKAP able to dock PKA at the thin filaments in proximity of its main sarcomeric substrates.[82] Within the myocardial contraction machinery, PKA phosphorylates cardiac myosin binding protein C and this event results in enhanced cardiac contractility due to the rearrangement of the myosin crossbridges and thick filament structure.[83] This configuration ensures that PKA is tethered near its substrate thanks to the recently characterized dual AKAP myomegalin (MMGL). Myomegalin is a PDE4D-interacting protein[84] involved in assembling a cAMP/PKA/PDE signaling module at the sarcomere.[85] The translocation of myomegalin to the sarcomere is therefore compatible with a mechanism that would lead to increased β-adrenergic-stimulated phosphorylation of cardiac myosin binding protein C and cTnI, thus enhancing cardiac contraction as well as cardioprotection.[86,87]

Cardiac Rhythm and Arrhythmias

Cardiac contractility and rhythm respond rapidly to physical activity and emotional stress to meet the changes in the metabolic needs of the organism. The sympathetic nervous system is the main player of this response and acts by enhancing the current amplitude of the slowly activating delayed rectifier I_{Ks} potassium channel (also referred to as

HERG).[88] I_{Ks} channel is composed by the pore-forming α-subunit KCNQ1 that conducts the ionic current and the auxiliary β-subunit KCNE1 that controls the biophysical properties of the channel.[89,90] I_{Ks} channels are regulated by the sympathetic nervous system via the β-AR/cAMP/PKA pathway. High cAMP levels cause an increase in the I_{Ks} amplitude and a slowdown in the current decay during deactivation.[88] This cAMP-mediated regulation of the channel is controlled by the scaffold protein Yotiao (also referred to as AKAP9), which recruits PKA and the protein phosphatase 1 to the C-terminal domain of the KCNQ1 subunit.[91,92] PKA-dependent functional regulation of I_{Ks} channels is lost when the binding site for Yotiao on the KCNQ1 subunit is mutated (KCNQ1-G589D).[91,93] Mutations in both subunits of the I_{Ks} channel are associated with at least 2 heritable arrhythmic syndromes, referred to as catecholaminergic polymorphic ventricular tachycardia[94] and long-QT syndrome.[95] Variants of long-QT syndrome have been shown to be caused by mutations in both the I_{Ks} channel α (KCNQ1, LQT1) and β (KCNE1, LQT5) subunits.[96,97] Recently, a cohort of patients with genotype-negative long-QT syndrome have been described to carry a missense mutation in Yotiao (S1570L). The S1570L mutation is in the binding domain of Yotiao for KCNQ1. Disruption of the Yotiao/KCNQ1 interaction reduces the PKA-mediated phosphorylation on KCNQ1 amino terminus (Ser27) and eliminates the functional response of I_{Ks} channel to cAMP.[98] The interaction between Yotiao and KCNQ1 is thus essentially required for the maintenance of a normal heart rhythm.

Recent evidences suggest that AKAP79/150 is also involved in heart rhythm regulation. In physiological conditions, AKAP79/150 coordinates the binding of PKA and PKCα to $Ca_v1.2$ and facilitates the coordinated opening and closing of the channel.[99,100] A gain of function mutation (G406R) in a cytoplasmic loop of $Ca_v1.2$ correlates with an abnormal coupling with AKAP79/150, eventually leading to LQT8, a disease also known as Timothy syndrome.[101] This occurs through a mechanism whereby AKAP79/150 functions like a subunit of $Ca_v1.2$ that stabilizes the open conformation of the channel. Ablation of this anchoring protein restores normal gating of $Ca_v1.2$ and protects the heart from arrhythmias.[101]

Besides Yotiao and AKAP79/150, heart rhythm modulation involves D-AKAP2 (also referred to as AKAP10). D-AKAP2 controls the sensitivity of pacemaker cells to cholinergic stimulation, both in mouse embryonic stem cell-derived cardiomyocytes and in vivo, in mouse hearts. Accordingly, D-AKAP2–deficient mice display heart rhythm abnormalities, eventually leading to premature death from arrhythmia.[102] Interestingly, a human polymorphism (I646V) affecting the affinity of D-AKAP2 for the regulatory subunit RI of PKA has been described. This variant correlates with increased basal heart rate and decreased heart rate variability, 2 events that are indicative of high risk of sudden cardiac death.[102] Thus, heart rhythm regulation relies on the coordinated action of Yotiao, AKAP79/150, and D-AKAP2. Furthermore, a growing body of evidence indicates that arrhythmogenesis can also be linked to mitochondrial function.[103]

Oxidative Stress (Mitochondria, Hypoxia)

Mitochondria constitute a major generator of cellular energy and their activity is controlled by normal cellular homeostasis. A key aspect of mitochondrial function is the dynamic balance of fusion and fission, events that alter mitochondrial morphology and activity.[104] Control of mitochondrial dynamics is evolutionary conserved and its deregulation is implicated in pathological conditions, including cardiovascular disorders such as dilated cardiomyopathy, myocardial infarction, and heart failure.[105–107] The cAMP/PKA pathway has been recently found to regulate mitochondrial respiration, dynamics, and cellular apoptosis.[108] Localization of PKA in proximity to mitochondrial substrates ensures efficient propagation of cAMP signals from the plasma membrane to this target organelle. cAMP signals are carried to mitochondria by a set of mitochondrial AKAPs that regulate mitochondrial function through the organization of signalosomes in this cellular compartment.[109]

mAKAP

Reduced oxygen levels, referred to as hypoxia, affect mitochondrial function by increasing glycolysis and lactate production. At the molecular level, hypoxia stabilizes hypoxia-inducible factor 1α (HIF-1α), which controls transcription of a wide range of genes, including factors implicated in the regulation of mitochondrial energy metabolism.[110,111] Under normoxic conditions, the levels of HIF-1α are kept low through its ubiquitin-mediated proteasomal degradation.[112] This multiprotein signaling complex is compartmentalized inside the cell by mAKAP. mAKAP sequesters HIF-1α at the perinuclear membrane, thereby minimizing the translocation distance to its site of action in the nucleus. Furthermore, mAKAP assembles and compartmentalizes components of the protein ubiquitin machinery that determine the bidirectional control of HIF-1α stability.[113] During normoxia, mAKAP clusters HIF-1α with negative regulatory factors, like prolyl hydroxylase domains and Von Hippel Lindau, that enhance the efficiency of its degradation. Under hypoxic conditions, positive regulatory factors, including the ubiquitin E3 ligase seven in absentia homolog 2, bind to mAKAP and favor the stabilization of HIF-1α. The expression of HIF-1α target genes protects the heart from oxygen-deprivation injury that occurs under pathological stresses, and, in this condition, mAKAP favors the enhancement of the hypoxic response. Displacement of mAKAP from perinuclear membranes of cardiomyocytes alters the stability of HIF-1α and the transcription of genes associated with hypoxia.

AKAP121

Under hypoxic conditions, HIF-1α availability is controlled by the ubiquitin E3 ligase seven in absentia homolog 2.[114] Seven in absentia homolog 2 is normally bound to mitochondrial AKAP121 and the expression levels of this ubiquitin E3 ligase are induced during hypoxia, thereby causing a degradation of AKAP121 and an attenuation of the cAMP/PKA signaling at the mitochondria.[115] In normal physiology, AKAP121 regulates mitochondrial morphology by serving as a docking site for PKA at the mitochondrial membrane. Within this compartment, PKA phosphorylates and inhibits the mechanoenzyme dynamin-related protein 1, resulting in an inhibition of mitochondrial fission. Deregulation of

AKAP121, that occurs on increased seven in absentia homolog 2 expression in ischemia-induced cardiomyocyte cell death, alleviates dynamin-related protein 1 inhibition, resulting in mitochondria fission.[116]

AKAP-dependent activation of the cAMP-PKA signaling at the mitochondria also controls oxidative stress, mainly caused by reactive oxygen species (ROS) production. These cAMP-mediated effects are mainly associated with PKA-dependent phosphorylation of complex I subunits[117] that results in an enhanced functional capacity of the mitochondrial respiratory chain and in a reduced ROS production.[118] In cardiac physiology, AKAP121 tethers PKA at the mitochondria and is thus involved in the control of ROS production, thereby protecting the cardiomyocyte from oxidative stress. Deregulated ROS production within the cardiomyocyte may contribute to the development of cardiac dysfunction. Indeed, displacement of AKAP121 from mitochondria by competitive peptides increases ROS levels and promotes cardiomyocyte death. Furthermore, in response to pressure-overload, AKAP121 protein expression is downregulated, thus resulting in mitochondrial stress, increased ROS production and cell death.[119] All these evidences suggest that deregulated mitochondrial cAMP signaling could contribute to the development of cardiac dysfunction.

Hypertrophy

Cardiac hypertrophy is an adaptive remodeling process of the myocardium that occurs in response to various cardiac stresses. It is associated with an increase in cardiomyocyte size, a qualitative and quantitative change in the expression levels of contractile proteins and an activation of fetal cardiac genes.[120,121] Because hypertrophy can ultimately progress to ventricular dilation, contractile dysfunction, and heart failure, significant efforts have been made to investigate the molecular players at the basis of this pathological process. Cardiomyocyte hypertrophy is controlled by membrane receptors that trigger multiple networks of intracellular mediators, which in turn transmit the hypertrophic signal to the nucleus.[122] An emerging concept in the field of signal transduction is the existence of hubs where multiple signaling pathways converge and share common molecules, thereby facilitating crosstalk between pathways. In this respect, mAKAP, AKAP-Lbc, and AKAP79/150 are attractive candidates that could coordinate hypertrophic signals elicited from multiple stress stimuli (Figure 2).

mAKAP

In addition to its role in regulating cardiac contractility and oxidative stress, mAKAP is also implicated in cardiac hypertrophy. Within this scenario, mAKAP assembles a perinuclear macromolecular complex that regulates gene transcription in response to multiple hypertrophic stimuli. This mAKAP complex includes at least 3 enzymes that are involved in the hypertrophic responses: the mitogen activated kinase ERK5,[70] the Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin Aβ[123] and the epsilon isoform of PLC.[124] cAMP-dependent-triggering of the MAP kinase signaling activates the prohypertrophic transcription factor myocyte enhancer factor-2c and its regulated genes.[70] On the other hand, Ca^{2+}-induced activation of

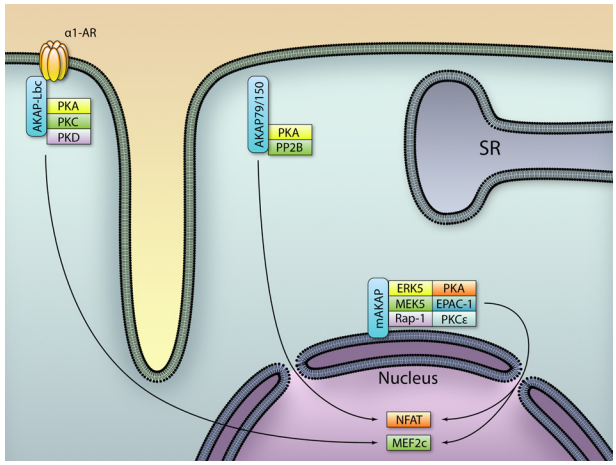

Figure 2. Regulation of cardiac hypertrophy by A-kinase anchoring protein (AKAPs). Intracellular localization of AKAP complexes controlling hypertrophic signaling pathways. PKA indicates protein kinase A; PKD, protein kinase D; PP2B, protein phosphatase 2B; SR, sarcoplasmic reticulum; NFAT, nuclear factor of activated T cells; MEF2, myocyte enhancer factor-2. (Illustration: Ben Smith.)

the mAKAP-associated calcineurin Aβ results in a dephosphorylation and in a nuclear translocation of the transcription factor nuclear factor of activated T cell (NFATc) that promotes the transcription of hypertrophic genes.[125,126] The control of hypertrophic gene expression by the epsilon isoform of PLC implicates both the myocyte enhancer factor- and NFAT-dependent transcription.[124] Whereas ERK-mediated hypertrophy is triggered by cytokine receptors[70] and calcineurin Aβ is activated through the β-AR/cAMP/PKA/RyR2 mediated Ca^{2+} release,[123] the epsilon isoform of PLC integrates multiple upstream signaling pathways that regulate hypertrophy, including endothelin-, norepinephrine-, insulin-like growth factor-1- and isoproterenol-activated signaling.[124] The crucial role of mAKAP in the hypertrophic process has been further demonstrated by the reduction of cardiac hypertrophy on the peptide-mediated displacement of mAKAP from the nuclear envelope.[70,124]

AKAP-Lbc

Several lines of evidence indicate that α-adrenergic transmission, through the activation of heterotrimeric G proteins Gq and G12/13, triggers the GTPase RhoA and its signaling cascade that controls the transcription of genes involved in cardiomyocyte hypertrophy.[127] At the cellular level, the activation of small GTPases is controlled by guanine nucleotide exchange factors that facilitate GDP-GTP exchange and the activation of the enzyme. Recent works have identified AKAP-Lbc not only as an AKAP that scaffolds PKA, PKC, and PKD,[128] but also as a guanine nucleotide exchange factor for the small GTPase RhoA.[24] AKAP-Lbc is activated in response to agonists that stimulate the α1-AR-G12/13 signaling pathway[129] and is inactivated via anchored PKA-mediated phosphorylation and subsequent recruitment of the regulatory protein 14-3-3, which prevents AKAP-Lbc from being able to activate Rho.[130] Thus, suppression of the Rho-specific exchange factor AKAP-Lbc correlates with a negative modulation of the hypertrophic signaling in response to GPCR-Gq/G12/13 stimulation. Furthermore, prolonged α-adrenergic

stimulation results in an upregulation of AKAP-Lbc protein levels, thereby directing the hypertrophic signal to the transcriptional machinery.[26] In more detail, AKAP-Lbc facilitates the activation of PKD that inactivates the histone deacetylase HDAC5, thereby favoring myocyte enhancer factor-2–dependent transcription and the onset of cardiac hypertrophy.[131] Therefore, AKAP-Lbc may provide a platform for crosstalk between PKD and Rho signaling pathways, in the context of cardiac hypertrophy.

AKAP79/150

Cardiac hypertrophy is also controlled by the calcium dependent Ser/Thr phosphatase calcineurin (CaN or PP2B) and the downstream transcriptional effectors, including NFAT. Indeed, hyperactivation of the CaN/NFAT pathway in cardiomyocytes of transgenic mice results in profound hypertrophy that rapidly progresses to heart failure.[132,133] Several studies have demonstrated the positive effect of the inhibition of the CaN/NFAT signaling pathway in the treatment of cardiac hypertrophy.[134–136] AKAP79/150 has a CaN-binding domain and is one of the endogenous inhibitors of CaN in the brain.[15] Cardiac-restricted transgenic mice overexpressing the CaN inhibitory domain of AKAP79/150 display inhibited CaN activity that is associated with attenuated cardiac hypertrophy in response to catecholamine stimulation and pressure overload.[137] These findings suggest a primary role for AKAP-mediated control of CaN in the hypertrophic response, even if the precise role of AKAP79/150 in this context still remains to be fully understood.

Heart Failure

Heart failure is a complex and multifactorial disease, characterized by the inability of the heart to pump sufficient blood to meet the metabolic needs of the body and represents a leading cause of mortality worldwide. Heart failure can result from aberrant signaling events that normally regulate myocardial function. In addition, altered gene expression is a peculiar feature of the failing heart. Gene expression profiles have pointed out a large scale of rearrangement in the AKAP-PKA signaling modules during end-stage heart failure. For instance, the expression of AKAP-Lbc, AKAP18δ, AKAP2, and SPHKAP was found upregulated, whereas AKAP121 levels were diminished in the failing human heart.[138,139] An example of altered cAMP compartmentation in the failing human myocardium is given by increased protein levels of AKAP18δ. The enhanced association of PKA RIIα with AKAP18δ may result in abnormal calcium reabsorption in the sarcoplasmic reticulum, ultimately leading to altered myocardial contractility.[78]

Another distinctive feature of failing hearts is a chronic activation of the β-AR signaling pathway that initially compensates for contractile dysfunction but then progresses to deterioration of cardiac structure and function. At the molecular level, β-ARs are downregulated and desensitized through the action of a complex signaling module that includes PKA, G protein-coupled receptor kinase 2 (GRK2), and β-arrestin.[140] Tight control of cellular cAMP levels is thus required for normal myocardial contractility. The catalytic subunit of phosphoinositide-3 kinase gamma (p110γ) is an AKAP that controls cAMP levels.[141] p110γ tethers PKA in the vicinity of its negative

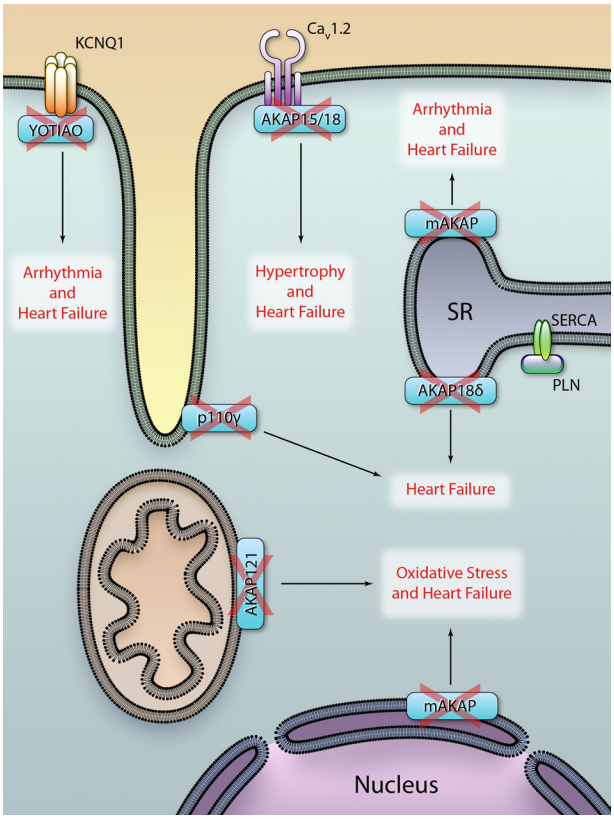

Figure 3. Disruption of A-kinase anchoring protein (AKAP)-protein kinase A (PKA) signaling underlies different cardiac diseases that finally lead to heart failure. Broken AKAPs correspond to disruption of the scaffolding function. SR, sarcoplasmic reticulum; PLN, phospholamban; SERCA, sarcoplasmic reticular Ca^{2+}-adenosine triphosphatase. PKA indicates protein kinase A. (Illustration: Ben Smith.)

modulator PDE3B, thereby constituting a feedback module that negatively controls cardiac contractility. Moreover, in physiological conditions, the β-AR pathway activates PKA, which in turn phosphorylates and inhibits the lipid kinase activity of the PKA-bound p110γ. In pressure overload-induced heart failure, p110γ is upregulated and escapes PKA-mediated inhibition. Activated p110γ reduces cell surface expression of β-ARs, thereby contributing to the development of heart failure.[142] Genetic and pharmacological inhibition of p110γ activity renormalizes β-AR density and improves contractility in failing hearts, thus establishing p110γ as a potential target for the treatment of heart failure.[141] Importantly, the spatial localization of β-ARs also plays a critical role in cardiac physiology and in the development of heart failure.[140] Indeed, redistribution of β_2-AR signaling from the T-tubules to the cell crest in failing cardiomyocytes results in uncoupling of the β_2-AR from the localized pools of PKA that are responsible for the compartmentation of the β_2-AR–cAMP signaling.[143] This results in a cell-wide cAMP propagation on β_2-AR activation in failing cells that is similar to the patterns observed for β_1-ARs, thus contributing to the heart failure phenotype. These findings, together with a still required more complete analysis of the AKAP function in failing cardiomyocytes, will provide a deeper understanding of this cardiac disease and will facilitate the development of new therapeutic strategies.

Conclusion

Evidence accumulated in decades of studies on AKAP and their partners clearly indicate that alteration of such complexes represents a key contributing factor for cardiac diseases (Figure 3). Manipulation of protein–protein interaction at AKAPs is thus emerging as a promising therapeutic strategy. Proof of concept studies show that small molecules can in principle act to pharmacologically modulate AKAP-based signaling complexes.[29] However, the limited number of such attempts has only scratched the surface of a vast potential of pharmacological intervention. Complexes at cardiac anchoring proteins can encompass 10, 20, or more components where each interaction is in principle amenable to pharmacological modulation. Our knowledge of the biological and chemical properties of these protein–protein complexes is only at its infancy. To better define targets of therapeutic interest, future work has to focus on the biochemical details and the pathophysiological meaning of such protein–protein interactions. First, 3-dimensional structures of protein–protein interactions are necessary to define how and where these interactions occur.[9,10] Native mass spectroscopy, small angle X ray scattering and cryo-electron microscopy have recently proven to be valuable tools suitable to tackle this issue.[144,145] Second, the role of such interactions in relevant disease conditions needs a detailed validation. Genetic modeling of the disruption of selected AKAP complexes in knock-in mice will likely provide conclusive proofs for the therapeutic value of such interventions. Third, new biochemical assays that simplify the search for disrupting moieties are required to select small molecules of pharmacological interest. Finally, it is tempting to speculate that the identification of small molecules that act on spatial and temporal restricted signaling will eventually prove to be more effective treatments for different aspects of heart failure, especially because our current therapeutic arsenal of drugs is still inadequate to combat this global health problem.

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Disclosures

None.

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