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mAKAP – A Master Scaffold for Cardiac Remodeling

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Abstract

Cardiac remodeling is regulated by an extensive intracellular signal transduction network. Each of the many signaling pathways in this network contributes uniquely to the control of cellular adaptation. In the last few years, it has become apparent that multimolecular signaling complexes or ‘signalosomes’ are important for fidelity in intracellular signaling and for mediating crosstalk between the different signaling pathways. These complexes integrate upstream signals and control downstream effectors. In the cardiac myocyte, the protein mAKAP β serves as a scaffold for a large signalosome that is responsive to cAMP, calcium, hypoxia, and mitogen-activated protein kinase signaling. The main function of mAKAP β signalosomes is to modulate stress-related gene expression regulated by the transcription factors NFATc, MEF2 and HIF-1 α and type II histone deacetylases that control pathological cardiac hypertrophy.

Keywords

mAKAP; nuclear envelope; signalosome; heart; remodeling

Myocyte hypertrophy is the primary response of the heart to stress (1). While in isolation myocyte hypertrophy can be compensatory for increased wall stress (LaPlace’s Law), this non-mitotic cell growth is typically accompanied in disease by changes in gene expression, ion fluxes and metabolism that can negatively impact cardiac contractility. In addition, pathological remodeling of the heart involves concomitant increased cell death and the development of myocardial interstitial fibrosis. Together, these adaptations contribute to both systolic and diastolic dysfunction that are present in different proportions depending upon the underlying disease (2). Pathological remodeling of the myocyte is regulated by a complex intracellular signaling network that includes mitogen-activated protein kinase

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(MAPK), cyclic nucleotide, Ca^{2+} , hypoxia, and phosphoinositide-dependent signaling pathways (3). Although much progress has been made in defining the components of this network, it is still unclear how the various member pathways act in concert to regulate overall cellular phenotype (4). The formation of multimolecular enzyme complexes by scaffold proteins is an important mechanism responsible for specificity and integration in intracellular signal transduction (5). Many signaling enzymes have broad substrate specificity or are present at low concentrations within the cell. The co-localization of an enzyme with its substrate by a scaffold protein can selectively enhance the modification of that substrate, providing specificity and efficacy beyond that intrinsic to the enzyme's active site (6). In addition, by binding a multivalent scaffold, a substrate may be co-regulated by the appropriate combination of enzymes responsible for determining specific downstream functions (7). Work over the last 15 years has established the scaffold protein muscle A-kinase anchoring protein β (mAKAP, AKAP6) as a critical component of the myocyte signaling network (8). As discussed below, mAKAP β signalosomes organize multiple signaling modules that modulate gene expression in the cardiac myocyte.

mAKAP was originally identified in a cDNA library screen for new cAMP-dependent protein kinase (PKA) regulatory-subunit (R-subunit) binding proteins, i.e. A-kinase anchoring proteins or AKAPs (9). mAKAP was initially named "AKAP100" for the size of the protein encoded by the original cDNA fragment (9). Subsequently, the full-length mRNA sequence for mAKAP α , the alternatively-spliced isoform of mAKAP expressed in neurons, was defined, revealing that wildtype mAKAP α is a 255 kDa scaffold (10). The sequence for mAKAP β , the 230 kDa alternatively-spliced isoform of mAKAP expressed in striated myocytes, was later obtained, showing that when expressed in heart or skeletal muscle, mAKAP is translated from an internal start site corresponding to mAKAP α residue Met-245 (11).

mAKAP is localized to the nuclear envelope both in neurons and striated cardiac and skeletal myocytes (Figure 1), the three cell types in which mAKAP is clearly expressed (10–12). mAKAP is not a transmembrane domain protein and contains three spectrin-like repeat regions (residues 772–1187) that confer its localization (10). Binding of mAKAP's third spectrin repeat (residues 1074–1187) by the outer nuclear membrane protein nesprin-1 α is both necessary and sufficient for mAKAP nuclear membrane localization, at least in myocytes and when expressed in heterologous cells (12). Nesprin-1 α may also be present on the inner nuclear envelope where it might bind A-type lamins and emerin. Interestingly, mutations in lamin A/C, emerin, and nesprin-1 α have been associated with Emery-Dreifuss muscular dystrophy, as well as other forms of cardiomyopathy (13–17). However, no disease-causing mutations have yet been identified in the human mAKAP gene, and mAKAP β knock-out in the mouse heart early in development does not induce cardiomyopathy (8). Besides binding nesprin-1 α , mAKAP β also binds phospholipase C ϵ (PLC ϵ) through mAKAP's first spectrin repeat, potentially strengthening its association with the nuclear envelope (18). There were early reports of mAKAP β being present on the sarcoplasmic reticulum (9, 19, 20), but these findings have been called into question due to technical issues including antibody specificity (10, 21).

Besides PKA, PLC ϵ and nesprin-1 α , mAKAP β binds a wide variety of proteins important for myocyte stress responses: adenylyl cyclase type 5 (AC5), exchange protein activated by cAMP-1 (Epac1), cAMP-specific phosphodiesterase type 4D3 (PDE4D3), MEK5 and ERK5 MAP-kinases, 3-phosphoinositide-dependent protein kinase-1 (PDK1), p90 ribosomal S6 kinases 3 (RSK3), protein kinase C ϵ (PKC ϵ), protein kinase D (PKD1, PKC μ), the protein phosphatases calcineurin (CaN) A β and PP2A, the type 2 ryanodine receptor (RyR2), the sodium/calcium exchanger NCX1, ubiquitin E3-ligases involved in HIF1 α regulation, and myopodin (11, 12, 18, 19, 21–31). Bound to mAKAP β , these signaling molecules co-regulate the transcription factors hypoxia-inducible factor 1 α (HIF1 α), myocyte enhancer factor-2 (MEF2), and nuclear factor of activated T-cell (NFATc) transcription factors, as well as type II histone deacetylases (Figure 2) (8, 25, 32, 33). Some of these molecules are bound directly and some indirectly, some constitutively and some in a regulated manner. Thus, it is likely that the composition of mAKAP β signalosomes depends upon the underlying state of the myocyte. As research continues on mAKAP β , the list of its binding partners grows, confirming its hypothesized role as an important orchestrator of signaling pathways required for remodeling. Most of what is known about mAKAP β is based upon work using cultured neonatal rat ventricular myocytes, in which mAKAP β was early on recognized to be required for the induction of hypertrophy by a variety of upstream receptors, including α - and β -adrenergic and cytokine receptors (22, 23). However, recently, we have published the phenotype of a conditional, cardiac-myocyte specific mAKAP β knock-out mouse confirming the centrality of mAKAP β to remodeling (8). In this review, we discuss the various upstream inputs, downstream effectors (outputs), and integrative circuitry within mAKAP β signalosomes and how they impact pathological remodeling of the heart.

mAKAP β – a prototypical A-kinase anchoring protein

Like most AKAPs, mAKAP contains an amphipathic helix (residues 2055–2072) responsible for binding PKA (10, 34). PKA is a heterotetramer of two R-subunits and two catalytic C-subunits, in the configuration C-R-R-C. Within the holoenzyme, the N-terminal docking and dimerization domains of the PKA R-subunits form a X-type, antiparallel four-helix bundle (35). This bundle contains a hydrophobic groove that accommodates the hydrophobic face of the AKAP amphipathic helix. mAKAP β binds selectively type II PKA (that contains RII subunits) with high affinity ($K_D = 119$ nM) (36). Interestingly, PKA-mAKAP β binding is increased 16-fold following RII α autophosphorylation (36), potentially affecting PKA-mAKAP β binding in states of altered β -adrenergic signaling. Besides mAKAP β , in the myocyte there are expressed over a dozen other AKAPs, each with its own distinct localization and sets of binding partners (8). Remarkably, mAKAP is one of the rarest AKAPs in the myocyte, such that loss of mAKAP does not even affect the localization of perinuclear PKA (M.S.K, unpublished observations). Despite the low level of expression of the scaffold, replacement in myocytes of endogenous mAKAP β with a full-length mAKAP β mutant that cannot bind PKA is sufficient to inhibit the induction of myocyte hypertrophy (22). Thus, mAKAP β signalosomes serve as an example of both how finely PKA signaling may be compartmentalized even on an individual organelle and how the level

of expression of a protein or a protein complex is not indicative of the functional significance of that protein.

mAKAP β is remarkable because it binds not only effectors for cAMP signaling, but also enzymes responsible for cAMP synthesis and degradation (30, 37). The synthesis of cAMP from ATP is catalyzed by adenylyl cyclases (AC), while cAMP metabolism to 5'AMP is catalyzed by phosphodiesterases (PDE). The differential association of ACs and PDEs with AKAPs contributes to cAMP compartmentation in cells, providing both for local activation of cAMP effectors and regulation of local cAMP levels by unique regulatory feedback and feedforward loops (38). mAKAP is capable of binding both AC2 and AC5, but AC5 appears to be the relevant mAKAP β -binding partner in the heart (30). The N-terminal, C1 and C2 domains of AC5 bind directly to a unique N-terminal site on mAKAP β (residues 275–340). AC5 activity is inhibited by PKA feedback phosphorylation that in cells is facilitated by mAKAP β complex formation (30). This negative feedback appears to be physiologically relevant to the maintenance of basal cAMP signaling. When the tethering of AC5 to mAKAP β is inhibited by a competitive peptide comprising the mAKAP AC5-binding domain, both the cAMP content and size of myocytes were increased in the absence of hypertrophic stimulus (30).

mAKAP was the first AKAP shown to bind a PDE (37). A site within mAKAP 1286–1831 binds the unique N-terminal domain of PDE4D3. Phosphorylation of PDE4D3 serine residues 13 and 54 results in increased binding to the scaffold and increased PDE catalytic activity, respectively (37, 39, 40). Because increased PDE4D3 activity accelerates cAMP degradation, PKA and PDE4D3 constitute a negative feedback loop that can modulate local cAMP levels and PKA activity (37). PDE4D3 bound to mAKAP serves not only as a PDE, but also as an adapter protein recruiting the MAPKs MEK5 and ERK5 and the cAMP-dependent, Rap1-guanine nucleotide exchange factor Epac1 to the scaffold (23). Although ERK5 binds to motifs on PDE4D3 initially described as ERK1/2 docking sites, ERK1/2 has not yet been detected in mAKAP complexes. Activation of MEK5 and ERK5 by upstream signals results in PDE4D3 phosphorylation on Ser-579, inhibiting the PDE and promoting cAMP accumulation and PKA activation (23, 41, 42). Epac1 is less sensitive to cAMP than PKA, such that very high cAMP levels results in the additional activation of mAKAP-associated Epac1. Through Rap1, Epac1 can inhibit ERK5 activity, thus preventing PDE4D3 inhibition by MAPK signaling, resulting presumably in maximal PDE4D3 activity due to concomitant PKA phosphorylation (23). As a result, Epac1, ERK5, and PDE4D3 constitute a third negative feedback loop that will attenuate cAMP levels in the vicinity of mAKAP complexes opposing cAMP elevation to extremely high levels.

Additional complexity is afforded by the binding of the serine-threonine phosphatase PP2A to the C-terminus of mAKAP (residues 2083–2319) (43). PP2A can catalyze the dephosphorylation of PDE4D3 Ser-54, thereby inhibiting the PDE in the absence of upstream stimulus. PP2A associated with mAKAP complexes contain B56 δ B subunits, which are PKA substrates. PKA phosphorylation enhances PP2A catalytic activity (44), such that phosphorylation of B56 δ by mAKAP-bound PKA increases PDE4D3 dephosphorylation, inhibiting the PDE. This presumably increases cAMP levels, constituting a positive feedforward loop for the initiation of cAMP signaling. Together with

the negative feedback loops based upon AC5 phosphorylation and PDE4D3 regulation by PKA and ERK5, one would predict that cAMP levels at mAKAP β signalosomes would be tightly controlled by upstream β -adrenergic and MAPK signaling. Signaling upstream of AC5 and ERK5 will promote cAMP signaling that will be initially promoted by PP2A feedforward signaling, while PDE4D3 activation and AC5 inhibition by PKA and Epac1 negative feedback will constrain signaling. Interestingly, Rababa'h et al. recently demonstrated how mAKAP proteins containing non-synonymous polymorphisms differentially bound PKA and PDE4D3 (45). The potential for cAMP signaling to be differentially modulated by crosstalk between upstream signaling pathways or by human polymorphisms makes compelling further work in myocytes to show the relevance of this complicated signaling network.

mAKAP β and MAP-kinase-RSK3 Signaling

The recruitment of ERK5 by PDE4D3 to mAKAP β complexes was initially shown to be relevant to the local regulation of cAMP through the aforementioned feedback loops (23). However, we also recognized at the time that ERK5 was an important inducer of myocyte hypertrophy, preferentially inducing the growth in length (eccentric hypertrophy) of cultured myocytes, while also being important for concentric hypertrophy in vivo due to pressure overload (transverse aortic constriction in the mouse) (46, 47). Notably, inhibition by RNA interference (RNAi) of mAKAP β expression in cultured myocytes inhibited the eccentric growth induced by the interleukin-6-type cytokine leukemia inhibitory factor (LIF) (23). A potential effector for mAKAP β -bound ERK5 was MEF2 transcription factor, as discussed below. However, we also found that in both heart and brain, mAKAP bound PDK1, a kinase that together with ERKs (ERK1, 2 or 5) can activate the MAPK effector p90RSK, a kinase also associated with mAKAP (11, 48). Importantly, binding of PDK1 to mAKAP obviated the requirement for membrane association in RSK activation (11). Taken together, these data suggested that mAKAP β could orchestrate RSK activation in myocytes in response to upstream MAPK signaling.

p90RSK is a pleiotropic ERK effector that regulates many cellular processes, including cell proliferation, survival, migration, and invasion. RSK activity is increased in myocytes by most hypertrophic stimuli (49, 50). In addition, RSK activity was found to be increased in human end-stage dilated cardiomyopathy heart tissue (51). RSK family members contain 2 catalytic domains, an N-terminal kinase domain and a C-terminal kinase domain (49). The N-terminal kinase domain phosphorylates RSK substrates and is activated by sequential phosphorylation of the C-terminal and N-terminal kinase domain activation loops by ERK and PDK1, respectively, such that PDK1 phosphorylation of the N-terminal domain on Ser-218 is indicative of full activation of the enzyme. There are 4 mammalian RSK family members that are ubiquitously expressed, but only RSK3 binds mAKAP β (52). The unique N-terminal domain of RSK3 (1–30) binds directly mAKAP β residues 1694–1833, explaining the selective association of that isoform with the scaffold (52). Despite the fact that RSK3 is expressed less in myocytes than other RSK family members, we found that neonatal myocyte hypertrophy was attenuated by RSK3 RNAi, inactivation of the RSK3 N-terminal kinase domain, and disruption of RSK3 binding to mAKAP using an anchoring disruptor peptide (52). Importantly, we found that RSK3 expression in vivo was required for

the induction of cardiac hypertrophy by both pressure overload and catecholamine infusion, as well as for the heart failure associated with a mouse model for familial hypertrophic cardiomyopathy (α -tropomyosin Glu180Gly) (52, 53). The RSK3 substrates important for cardiac remodeling are not yet known, nor is it clear whether RSK3 phosphorylates substrates at or distant from the mAKAP β scaffold. However, the recognition that this specific RSK isoform is required for cardiac remodeling makes it a compelling candidate for therapeutic targeting.

mAKAP β and Phosphatidylinositol Signaling

The cAMP effector Epac1 activates Rap1 at mAKAP β complexes affecting ERK5 signaling (23). In addition, Epac1-Rap1 activates PLC ϵ , a phospholipase whose Ras association domains directly bind the first spectrin repeat-like domain of mAKAP β (18). Like mAKAP β , PLC ϵ was required for neonatal myocyte hypertrophy, whether inhibited by RNAi or by displacement from mAKAP β by expression of competitive binding peptides. In an elegant paper by the Smrcka laboratory, mAKAP β -bound PLC ϵ has been shown to regulate PKC ϵ and PKD activation through a novel phosphatidylinositol-4-phosphate (PI4P) pathway in which PLC ϵ selectively converts perinuclear PI4P to diacylglycerol and inositol-1,4-bisphosphate (31). PKD1 phosphorylates type II histone deacetylases (HDACs 4/5/7/9) inducing their nuclear export and de-repressing hypertrophic gene expression (54, 55). Smrcka and colleagues found that PLC ϵ was required for pressure overload-induced PKD activation, type II HDAC phosphorylation and hypertrophy in vivo (31). Subsequently, we have confirmed that mAKAP β too is required in vivo for PKD activation and HDAC4 phosphorylation in response to pressure overload (8). Remarkably, mAKAP β can form a ternary complex with PKD and HDAC4. Together, these results show how local cAMP signaling may ultimately affect the regulation of cardiac gene expression.

mAKAP β and Calcium signaling

Besides cAMP, phosphoinositide and MAP-kinase signaling, mAKAP β contributes to the orchestration of Ca²⁺-dependent signaling transduction. The second binding partner for mAKAP β identified was the ryanodine receptor Ca²⁺ release channel (RyR2) responsible for Ca²⁺-induced Ca²⁺ release from intracellular stores (19, 21). RyR2 is best known for its role in excitation-contraction coupling, in which bulk Ca²⁺ is released to induce sarcomeric contraction. PKA phosphorylation can potentiate RyR2 currents (56–58), although the importance of PKA-catalyzed RyR2 phosphorylation to excitation-contraction coupling is highly controversial (59, 60). We have found that a small fraction of RyR2, presumably located at perinuclear dyads (61), can be immunoprecipitated with mAKAP β and nesprin-1 α antibodies (12, 21). We suggest that mAKAP β brings together elements of the excitation-contraction coupling machinery and signaling molecules important for regulating nuclear events germane to pathological remodeling. Thus, mAKAP β complexes may provide one mechanism for matching contractility to the induction of hypertrophy. We found that β -adrenergic stimulation of primary myocyte cultures results in increased PKA phosphorylation of mAKAP β -associated RyR2 (22). It is, therefore, plausible that PKA-catalyzed RyR2 phosphorylation could potentiate local Ca²⁺ release within the vicinity of mAKAP β signalosomes during states of elevated sympathetic stimulation.

While it is unlikely that the few mAKAP β -associated RyR2s could affect overall contractility, a potential target for increased perinuclear Ca²⁺ may be the Ca²⁺/calmodulin-dependent phosphatase (CaN) that can bind the scaffold. There are three isoforms of the catalytic subunit for CaN (α, β, γ), but only CaNA β -mAKAP β complexes have been detected in myocytes (33). Remarkably, CaNA β is the CaNA isoform important for the induction of cardiac hypertrophy in vivo, as well as for myocyte survival after ischemia (62, 63). CaNA β binds directly to a unique site within mAKAP β (residues 1286–1345) (22, 33). CaNA β binding to mAKAP β is enhanced in cells by adrenergic stimulation and directly by Ca²⁺/calmodulin (33). Notably, CaNA β -mAKAP β binding was required for α -adrenergic-induced neonatal myocyte hypertrophy in vitro (33).

mAKAP β and Gene Expression

Among its many substrates, CaN is responsible for the activation of NFATc and MEF2 transcription factors. The NFATc transcription factor family includes four CaN-dependent isoforms that are all expressed in myocytes and that can contribute to the induction of myocyte hypertrophy (64). In general, NFATc family members are retained in the cytoplasm when heavily phosphorylated on the multiple serine-rich motifs within the N-terminal regulatory domain. NFATc translocates into the nucleus when these motifs are dephosphorylated by CaN. Multiple NFATc family members can bind mAKAP β , and binding to mAKAP β was required for CaN-dependent dephosphorylation of NFATc3 in myocytes (33). Accordingly, mAKAP β expression was also required for NFAT nuclear translocation and transcriptional activity in vitro (22, 33). These results correlate with our recent observation that NFAT-dependent gene expression in vivo was attenuated by mAKAP β cardiac-myocyte specific knock-out following transverse aortic constriction (8).

Like NFATc2 and NFATc3, MEF2D is a transcription factor required for cardiac hypertrophy in vivo (65–67). MEF2 family members contain a conserved DNA binding domain that includes both a MADS box and a MEF2 homology domain (68). We found that the DNA-binding domain of MEF2D bound directly to an N-terminal domain of mAKAP (27, 65). CaN and MEF2D are important not only in the heart, but also in skeletal muscle (69–73). Interference with MEF2-mAKAP β binding blunted MEF2 transcriptional activity and the expression of endogenous MEF2 target genes in C2C12 skeletal myoblasts (27). In addition, disruption of MEF2-mAKAP complexes attenuated the differentiation of C2C12 myoblasts into myotubes, as evidenced by decreased cell fusion and expression of differentiation markers (27). Remarkably, CaN-MEF2 binding is mAKAP β -dependent in cardiac myocytes (32). Accordingly, disruption of CaN-mAKAP β binding inhibited both MEF2 transcriptional activity in C2C12 cells and cardiac myocyte hypertrophy (32). Like NFATc2, MEF2D de-phosphorylation in vivo in response to pressure overload was attenuated following mAKAP β conditional knock-out, correlating with the decreased expression MEF2-target genes, including the expression of atrial natriuretic factor (8).

The regulation of NFATc, MEF2 and HDAC4 by mAKAP β in vivo during pressure overload shows the importance of mAKAP β to stress-regulated gene expression (8). The published reports show how, at mAKAP β , NFATc and MEF2 are regulated by CaN, while HDAC4 is regulated by PKD (31–33). It is probable, however, that mAKAP β facilitates the

modulation of these gene regulatory proteins by other signaling enzymes. For example, it is possible that mAKAP β -associated ERK5 phosphorylates MEF2, activating the transcription factor (74). In addition, PKA can phosphorylate MEF2, affecting its DNA-binding affinity (75). On the other hand, the Olson group has proposed that PKA phosphorylation of HDAC4 can inhibit MEF2 activity through the generation of a novel HDAC4 proteolytic fragment (76). How the activities of the many mAKAP β binding partners are ultimately integrated to control gene expression will require further investigation both in vitro and in vivo.

Other mAKAP β binding partners

There are other binding partners for mAKAP β for whom the significance of docking to the scaffold remains poorly characterized, including myopodin and NCX1 (28, 29). HIF-1 α , a transcription factor that regulates systemic responses to hypoxia, also binds mAKAP β (25). Under normoxic conditions, the abundance of HIF-1 α in the cell is kept low by ubiquitin-mediated proteasomal degradation. HIF-1 α is hydroxylated by a family of oxygen-sensitive dioxygenases called prolyl hydroxylases (PHD1, PHD2, and PHD3) (77). Hydroxylated HIF-1 α is subsequently recognized by the von Hippel–Lindau protein (pVHL), which recruits the Elongin C ubiquitin ligase complex to ubiquitinate HIF-1 α and to promote its proteasome-dependent degradation (78). Under hypoxic conditions, PHDs are inactivated, HIF-1 α degradation is decreased and HIF-1 α accumulates in the nucleus, where it can dimerize with HIF-1 β to promote the transcription of target genes. We found that mAKAP β can assemble a signaling complex containing HIF-1 α , PHD, pVHL and the E3 ligase Siah2 (seven in absentia homolog 2) in cultured neonatal myocytes (25). Under normoxic conditions, mAKAP β -anchored PHD and pVHL favor HIF-1 α ubiquitination and degradation (25). Under hypoxic conditions, however, Siah2 activation induces proteasomal degradation of bound PHD, favoring HIF-1 α accumulation (25). Whether these events are relevant in vivo remain to be proven, although one might expect from these findings that mAKAP β knock-out would affect cardiac myocyte survival after ischemia-reperfusion.

mAKAP β – a conductor of the remodeling symphony

The above discussion shows how multiple signaling pathways known to be important for cardiac hypertrophy and pathological remodeling are modulated by the binding of key signaling intermediates to the mAKAP β scaffold. We recently published the characterization of the cardiac myocyte-specific, conditional mAKAP knock-out mouse, showing the relevance of mAKAP β signalosomes in vivo (8). mAKAP β was required in cardiac myocytes for the induction of cardiac hypertrophy by transverse aortic constriction and isoproterenol infusion. Most remarkable, however, was the prevention of pathological remodeling, including myocardial apoptosis and interstitial fibrosis, and the preservation of cardiac function in the face of long-term pressure overload, together resulting in a significant increase in mouse survival (8). As best we can tell, these results established mAKAP β as the first scaffold whose ablation confers a survival benefit in heart disease. Importantly, mAKAP β did not appear to be necessary for either the development or maintenance of normal adult cardiac function, as the use of a Nkx2-5-directed cre deleter line did not result in an overt phenotype by six months of age (8). Although mAKAP β

knock-out did attenuate the physiological hypertrophy induced by forced exercise (swimming), these results suggest that the targeting of mAKAP β complexes in disease should be further investigated.

Various strategies for targeting mAKAP β complexes in humans may be envisioned, including siRNA knock-down of the scaffold. However, our relatively detailed understanding of the structure and function of mAKAP β signalosomes provides us with additional approaches to targeting these pathways. For example, the expression of peptides targeting key protein-protein interactions involving mAKAP β has already been shown to be effective in vitro, including anchoring disruptor peptides targeting mAKAP β -CaNA β , mAKAP β -MEF2D, mAKAP β -PLC ϵ , and mAKAP β -RSK3 binding (18, 27, 32, 52). A leading cause of death, heart failure is a disease that incurs 50% mortality within 5 years of diagnosis despite modern therapy, at a cost of over \$30 billion/year in the USA alone (79). Many candidates for potential targeting in cardiac disease are pleiotropic, complicating the development of drugs with sufficient specificity in vivo. The specific targeting of mAKAP β signalosomes provides an opportunity to consider the targeting of relatively rare protein-protein interactions that appear to be dedicated to pathological cardiac remodeling and whose ablation may be promoted without significant side-effects.

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Figure 1. mAKAP β – A Perinuclear Scaffold

Top: Mouse heart sections (left ventricle) stained for with mAKAP antibody (gray scale panels and green), Hoechst nuclear stain (blue), and wheat germ agglutinin (red, shown in enlarged control image only). Lower left panels are from control, mAKAP knock-out mice. Bar = 20 μ m. **Middle:** Adult rat myocyte stained with antibodies to mAKAP (green) and actinin (red). **Bottom:** mAKAP domain structure. Direct binding partners whose sites have been finely mapped in mAKAP β are shown. mAKAP β starts at residue 245 of mAKAP α . Therefore, all binding sites are numbered per mAKAP α . Images are from Kritzer, et al. (8).

Figure 2. mAKAP β Signaling Modules

mAKAP β binds multiple signaling enzymes and gene regulatory proteins. Modules may be defined that involve cAMP, Ca²⁺, hypoxic, phosphatidylinositol and MAPK signaling. See text for details. In this figure, the mAKAP β scaffold is presented as a yellow globe sitting on a grey base representing nesprin-1 α , on which are assembled the various signaling molecules. Gold cylinders represent nuclear pore complexes inserted in the nuclear envelope.