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Pharmacological targeting of AKAP-directed compartmentalized cAMP signalling

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Abstract

The second messenger cyclic adenosine monophosphate (cAMP) can bind and activate protein kinase A (PKA). The cAMP/PKA system is ubiquitous and involved in a wide array of biological processes and therefore requires tight spatial and temporal regulation. Important components of the safeguard system are the A-kinase anchoring proteins (AKAPs), a heterogeneous family of scaffolding proteins defined by its ability to directly bind PKA. AKAPs tether PKA to specific subcellular compartments, and they bind further interaction partners to create local signalling hubs. The recent discovery of new AKAPs and advances in the field that shed light on the relevance of these hubs for human disease highlight unique opportunities for pharmacological modulation. This review exemplifies how interference with signalling, particularly cAMP signalling, at such hubs can reshape signalling responses and discusses how this could lead to novel pharmacological concepts for the treatment of disease with an unmet medical need such as cardiovascular disease and cancer.

1 Introduction

1.1 Compartmentalization of cAMP/PKA signalling

A-kinase anchoring proteins (AKAPs) comprise a family of around fifty proteins. AKAPs are distinguished by their ability to bind the serine/threonine protein kinase A (PKA) and to direct the AKAP-PKA complex to a specific cellular compartment (Fig. 1a) [1, 2]. A PKA holoenzyme consists of two regulatory (R) subunits that each binds one catalytic (C) subunit and thereby maintains the C subunits in an inactive state. The R subunits are dimers of either one of four different types, RIα, RIβ, RIIα or RIIβ; C subunits are Cα, Cβ, Cγ or PrKX. Various extracellular signals (e.g. hormones) activate a highly conserved and almost ubiquitous cascade: they stimulate their cognate G protein-coupled receptors (GPCR), which in turn activate the stimulatory G protein G_s. Active G_s stimulates adenylyl cyclases to synthesize the diffusible second messenger cAMP. cAMP binds to the R subunits of PKA, causing the release and thus activation of the C subunits. Free C subunits phosphorylate their nearby protein substrates (Fig. 1b) [3-7]. The phosphorylation can activate substrates such as the water channel aquaporin 2 [8] or inactivate proteins such as the small GTPase RhoA [9]. The tethering of PKA to cellular compartments by AKAPs increases the specificity of PKA signalling and facilitates specific cellular responses to each of the extracellular cues.

The only means to terminate cAMP signalling is cAMP degradation. Phosphodiesterases (PDEs) hydrolyse cAMP to 5'AMP. Depending on their intracellular location they establish cAMP gradients throughout the cells and thereby cAMP microdomains [10, 11]. Thus both PDEs and AKAPs define compartments and limit PKA activity. Substrates phosphorylated by PKA are dephosphorylated by protein phosphatases and return to their basal state (Fig. 1b) [12].

1.2 Canonical AKAPs

Cells express 10 to 15 different AKAPs. They not only act as physical constrainers of PKA but engage in direct interactions with further proteins, such as other protein kinases, phosphatases and PDEs organizing the interactors into distinct hubs at different cellular compartments, including nucleus, cytosol, mitochondria, plasma membrane, cytoskeleton and the Golgi (Fig. 1A) [2, 13-15]. Thereby, they spatially and temporally integrate signal transduction processes. Depending on its subcellular localization and specific protein interactors the same AKAP can regulate different pathways; WAVE-1 for example, is found at the actin cytoskeleton in neurons where it regulates actin crosslinking [16], or is involved in the formation of cel-

lular protrusions responsible for breast cancer cell migration and invasion [17], and at mitochondria in leukaemia cells where it regulates apoptosis [18]. Another example is Optic Atrophy 1 (Opa-1). In addition to its most recognized function in controlling mitochondrial fusion when localized at mitochondria [19, 20], it was shown to possess an AKAP function suggested to be involved in adrenoceptor-induced lipolysis when Opa-1 is located on lipid droplets [21]. However, criticism has been raised against this interpretation as Opa-1 may have been detected on the lipid droplets as a contaminant from mitochondria [22].

Canonical AKAPs interact *via* their structurally conserved A kinase-binding (AKB) domains with the dimerised N-terminal dimerization and docking (D/D) domains of regulatory subunits of PKA (amino acids 1-44 of R subunits; Tab. 1). The AKB domains are 14-25 amino acids in length and form amphipathic helices that dock with their hydrophobic face into a hydrophobic pocket formed of the D/D domain [23-26].

1.3 Non-Canonical AKAPs

Non-canonical AKAPs are defined by a different mode of interaction with R subunits of PKA. Examples are pericentrin/kendrin that binds to PKA through a unique 100 amino acids long motif [27-29], and RSK1. In its inactive form RSK1 interacts with RI subunits of PKA through a mechanism not involving a typical AKB domain as the interaction is insensitive to disruption with the global inhibitor of interactions between canonical AKAPs and PKA, Ht31 (see 2.1) [30, 31]. The binding between RI subunits of PKA and α/β tubulin [32] or between RIIα subunits and actin does also not require a canonical AKB domain [33]. The interaction of RIIα with actin was preserved in the presence of Ht31 and when two different mutations were introduced into the D/D domain. Substitutions of isoleucines 3 and 5 with serine or deletion of amino acids 2-5 allows dimerization of the R subunits but abolishes interaction with canonical AKAPs [34, 35], not with actin though [33].

Recently, neurochondrin (also known as Norbin), a neuronal cytoplasmic protein involved in neurite outgrowth and initially described to play a role in the long-term potentiation in the hippocampus was identified as a non-canonical AKAP [36]. Surprisingly, the binding motif is discontinuous. Two short, distinct segments of neurochondrin mediate the binding. The core-binding region of the first segment comprises the amino acid sequence KTRRR, the second the sequence WQRNP. Neither of these sequences resembles canonical AKB domains with regard to the ability to form amphipathic helices. Neurochondrin binds directly with nanomolar affinity the D/D domain of RIIα subunits of PKA. It does not bind RIIβ or RI subunits, and

is therefore the first RIIα-specific AKAP. Neurochondrin associates with glutamate receptors, mGluR5 [37] which are phosphorylated by PKA at serine 870 [38]. It is tempting to speculate that neurochondrin itself facilitates this phosphorylation and thereby the consequent recruitment of $G_{q/11}$ to the receptor which increases neuronal excitability.

2 Pharmacological tools to interfere with AKAP-dependent protein-protein interactions

2.1 Peptides, peptidomimetics and small molecules

In order to elucidate functions of interactions of AKAPs with PKA and other binding partners inhibitors have been developed. Three different approaches have been used to achieve this, with peptides, peptidomimetics and small molecules (Fig. 2). Most efforts have been directed towards the development of inhibitors of AKAP-PKA interactions [14, 39-49]. Initially, synthetic peptides of 14-25 amino acids in length have been derived from the AKB domains of canonical AKAPs. They prevent AKAP-PKA interactions by binding with nanomolar affinity the D/D domain of R subunits of PKA and thereby block effectively their interaction with canonical AKAPs. Non-selective inhibitory peptides such as Ht31, derived from AKAP-Lbc (AKAP13) or AKAP18δ-derived peptides were used for globally uncoupling PKA from AKAPs and thereby to elucidate functions of compartmentalised PKA in defined biological processes such as vasopressin-mediated water reabsorption or cardiac myocyte contractility [50-53]. Follow up peptides were designed to preferentially or specifically interfere with interactions of RI or RII subunits of PKA with AKAPs [39, 40, 43, 54-56]. An example of a peptide that was developed using a combination of bioinformatics and peptide array screening and which preferentially binds RI subunits of PKA is RIAD [54].

Since peptides possess low bioavailability and membrane permeation ability as well as a short half-life, their use for cell and animal studies is limited, and peptides are also not generally considered starting points for drug development programs even though several peptide-based drugs have reached the market [57, 58]. As alternatives to peptides, different peptidomimetics were developed that also uncouple PKA from AKAPs through blocking the D/D domain of R subunits of PKA. The peptide RIAD was modified by insertion of non-natural amino acids in order to improve stability; rendered membrane-permeant, it reduced cAMP levels in HIV-infected cultured T cells [59, 60]. In NOD/SCID/IL2γ null mice infected with HIV-1, the molecule interfered with a cAMP/PKA type I pathway and inhibited HIV-1 replication, which was associated with stabilization of CD4 cells [61].

Recently, RIAD and the AKB domains of AKAP220 (AKAP11) and small membrane AKAP (smAKAP) were used as templates for the generation of stapled peptides. The insertion of pairs of non-natural amino acids in positions non-fundamental for PKA binding, and subsequent formation of bonds between the two amino acids generates “hydrocarbon staples” that stabilise the peptide, increase its resistance to proteases and extend its half-life. While the resulting three peptides, STAD1-3 interact with all R subunits *in vitro*, in cells STAD-2 and STAD-3 interact with RII subunits, but STAD-1 only weakly, suggesting that the STAD peptides are RII-selective in a cellular context [43]. STAD-2 has antimalarial properties *in vitro*. It enters Plasmodium-infected red blood cells and mediates lysis of the cells with an $IC_{50} \approx 1$ µM [62]. Recently, the technology led to the RI-STapled Anchoring Disruptor (RI-STAD). This molecule selectively binds regulatory RI subunits of PKA with high affinity. It is cell-permeant and inhibits phosphorylation events in cells that depend on PKA holoenzyme containing RI subunits [56].

Another strategy was the construction of terpyridine-based molecules as α-helix mimics of the AKB domain of AKAP18δ. A terpyridine core presents chemical groups resembling the ones exposed by the AKB of AKAP18δ at similar position and angles, so constituting a peptide analogue built on a chemical structure independent from amino acids [42]. While the STAD peptides bind R subunits of PKA with nanomolar affinity, the affinity of terpyridine mimetics is around 30 µM. Their chemical structure, however, is amenable to optimisation by medicinal chemistry. Thus the K_D values may be improved [42].

The most promising alternative to peptides are small molecules. They can combine the specificity of peptides and the stability of peptidomimetics, show enhanced plasma membrane permeation and a good bioavailability. The development of small molecules directed against specific AKAP-dependent protein-protein interactions is desirable as small molecules permit a spatially and temporally defined interference with an AKAP function, and in addition, this is becoming increasingly important as AKAP-dependent protein-protein interactions emerge as novel drug targets in a variety of diseases with an unmet medical need such as heart failure and cancer as discussed below [14, 44, 63]. On the down side, their small size makes the inhibition of the binding between two typically large, flat and mainly nonpolar surfaces like the ones that are involved in AKAP-PKA interactions difficult. However, allosteric sites that regulate an interaction between two proteins may be targeted. The identification of FMP-API-1 demonstrated that inhibition of AKAP-PKA interactions *via* a small molecule is feasible. The small molecule binds to an allosteric site C-terminally from the D/D domain of RII subunits. It inhibits AKAP-PKA interactions non-selectively, and also activates PKA [41, 46].

Pharmacological agents for inhibition of interactions between PKA and non-canonical AKAPs are not available. Since these interactions apparently involve larger surfaces or discontinuous binding motifs such as the 100 amino acid residue-spanning domain of pericentrin or the bipartite motif of neurochrondrin (see 1.3), respectively, this will require novel approaches. An alluring strategy to interfere with non-canonical AKAPs might be the development of intra/nanobodies (see 2.2).

In some cases peptides have been synthesized to inhibit interactions between AKAPs and proteins other than PKA. For example, disrupting the interaction between AKAP18δ (AKAP7δ) and phospholamban (PLB) employing a peptide derived from the PLB interacting region reduces Ca^{2+} reuptake into the sarcoplasmic reticulum (SR) of cardiac myocytes [64]. Another example relates to AKAP18α (AKAP7α), which was the source of a peptide interfering with the binding of AKAP18α to the Ca^{2+} channel $Ca_V2.1$, so preventing the β-adrenoreceptor-induced increases of Ca^{2+} entry into cardiac myocytes [65].

2.2 A novel way to inhibit AKAP-dependent protein-protein interactions: Nanobodies

An attractive, even though unexplored alternative to peptides, peptidomimetics and small molecules for targeting AKAP-dependent protein-protein interactions may be antibody-related molecules.

Camelid single-domain antibodies and their derivatives, recombinant nanobodies are used as research tools, diagnostics and therapeutics, e.g. in the field of EGFR and GPCR research [66, 67]. Nanobodies represent a unique class of heavy chain antibodies first expressed in and obtained from the serum of camelids [68]. Nanobodies are the smallest antibody-based functional biologics consisting of a 12-15 kDa variable domain of a heavy chain of an antibody with the full capability of binding an antigen; the affinity for antigen binding is within the nano- to picomolar range [69]. Nanobodies are cost-effective, highly target-specific, stable, temperature and low pH resistant, and show unique flexible paratope structure and small size, which enables them to bind molecular pockets and cavities, e.g. enzyme active sites [66]. Even though humanized nanobodies have a tendency to aggregate, they are not immunogenic and, therefore, are of special interest for the engineering of antibody-based therapeutics [70-72]. Nanobodies can cross the blood-brain barrier. Recently, nanobodies were used to derive bispecific molecules into which a therapeutic “arm” (a blood-brain barrier-transcytosing moiety) has been incorporated [73]. Those new biologics display enhanced

brain delivery and can be regarded as a new antibody-based delivery platform for targeted pharmaceutical interventions in the central nervous system.

Since nanobodies are too bulky to cross plasma membranes, most of the attention focuses on their use as binders of extracellular antigens. A recent study, however, shows how nanobodies raised against a protein-protein interaction can be used intracellularly as intrabodies upon lentiviral transduction. CapG, an actin-capping protein able to increase cell motility and chemotaxis, and actin interact directly; their interaction was effectively interrupted by intrabody administration, leading to decreased metastasis in a breast cancer model [74]. These results point out the potential of nanobodies for use as disruptors of AKAP-dependent protein-protein interactions.

3 AKAPs in the regulation of cardiovascular functions – implication for cardiac hypertrophy, heart failure and hypertension

Cardiac contractility is regulated by a tightly controlled cycle of Ca^{2+} entry and removal from the cytosol of cardiac myocytes. For contraction (systole), Ca^{2+} enters the cytosol of the cells from outside across the plasma membrane (sarcolemma) and from the main intracellular storage site, the SR, and activates contractile proteins. For relaxation (diastole) a minor fraction of the Ca^{2+} exits the cells, the larger part is pumped back into the SR. More than 10 different AKAPs are found in the heart, several of them play a role in the control of this Ca^{2+} cycling (Fig. 2).

3.1 AKAPs in the control of β-adrenoceptor-induced increases in cardiac contractility

Cardiac contractility is enhanced by β-adrenergic stimulation of cardiac myocytes through the sympathetic nervous system; β-agonists have so called positive inotropic (increase in contractility), positive lusitropic (increase in relaxation) and positive chronotropic (increase in frequency) effects. β-agonists stimulate the G_S/adenylyl cyclase system to produce cAMP and activate PKA. AKAPs, such as AKAP79 (AKAP5) and AKAP450 (AKAP9) target PKA to adenylyl cyclases, including adenylyl cyclase VI, the major isoform in the heart, and facilitate their phosphorylation and consequent inhibition by PKA [41, 75, 76]. This constitutes a negative feedback loop limiting cAMP synthesis and thus β-adrenergic signalling. The necessity of tight control of cAMP levels and thereby PKA activity is emphasized by observations made in

a transgenic mouse model overexpressing catalytic subunits of PKA. The overexpression results in an impaired cardiac function resembling that associated with dilated cardiomyopathy [77]. Also, reduced levels of PKA RI and RII subunits together with unchanged levels of cAMP and PKA C subunits are found in failing hearts [78].

Activation of the G_S/adenylyl cyclase system through β-agonists and subsequent PKA phosphorylation leads to an increase in the opening probability of the L-type Ca^{2+} channel, $Ca_v1.2$ to facilitate enhanced Ca^{2+} entry across the sarcolemma. Various studies have so far not yet unequivocally defined the relevant phosphorylation site/s of $Ca_v1.2$ [79]. Initial mutation studies revealed serine 1928 in the distal C terminus of the Ca^{2+} conducting pore, α1c to be crucial for channel regulation [80], whereas other studies argued against the relevance of this residue [81, 82] and suggested phosphorylation sites closer to the proximal C terminus [83, 84]. An explanation for the discrepancies may be that *in vitro* heterologous expression systems fail to reconstitute the complex *in vivo* interplay of the L-type Ca^{2+} channel regulatory network (extensively reviewed in [79] and [85]). A decreased L-type Ca^{2+} channel expression and a correspondently reduced sarcolemmal Ca^{2+} current is associated with cardiac failure, as shown in hearts from spontaneously hypertensive rats [86]. Although β-adrenoceptor-stimulated increases in Ca^{2+} currents through $Ca_v1.2$ seemed to depend on AKAP18α anchoring of PKA (AKAP15) [65, 87], isolated cardiac myocytes from AKAP18 knockout mice showed no difference to wild type cells with regard to isoproterenol (β-agonist)-induced $Ca_v1.2$ phosphorylation, L-type Ca^{2+} current and PKA phosphorylation of PLB at serine 16. AKAP79 has also been shown to facilitate the PKA-catalysed phosphorylation of the channel [88]. AKAP79 compensation in the AKAP18 knockout was ruled out by using a double knockout AKAP18/AKAP79 [89]. Recently, AKAP79 and AKAP18 were found to compete for $Ca_v1.2$ binding [90]. Taken together, the involvement of yet another AKAP facilitating PKA phosphorylation and thus increasing $Ca_v1.2$ channel open probability is likely. One candidate is cypher, which also facilitates PKA phosphorylation of the channel [91]. All three AKAPs, AKAP18α, AKAP79 and cypher, mediate PKA phosphorylation of $Ca_v1.2$ at serine 1928. Possibly, another AKAP controls PKA in a way that facilitates the phosphorylation of another serine or threonine residue involved in the β-adrenoceptor-induced increase in channel opening.

Ca^{2+} entering cardiac myocytes through L-type Ca^{2+} channels activates Ryanodine R2 receptors (RyR). They are located in the SR membrane and allow Ca^{2+}-dependent Ca^{2+} release from the SR into the cytosol. Phosphorylation of RyR by PKA increases the Ca^{2+} flux. RyR regulation was described to be coordinated by a macromolecular complex containing mAKAPβ, PKA, the stabilising protein FKBP12, which ensures coupled gating of neighbour-

ing channels, and the protein phosphatases PP1 and PP2, which dephosphorylate the PKA-phosphorylated channel [92, 93]. In hypertrophic and failing hearts, hyperphosphorylation of RyR was observed [94]. The hyperphosphorylation reduces FKBP12 binding, leading to an impaired channel function [95]. However, these findings are highly controversial, as reviewed in [96]. The mAKAPβ signalosome is now known to be even more complex and to be preferentially located at the perinuclear membrane. The mAKAPβ complex comprises PKA, RyR2, PDE4D3, PP2A and calcineurin (PP2B), adenylyl cyclase 5, ERK5, the exchange protein directly activated by cAMP 1 (Epac1), phospholipase Cε, nuclear histone deacetylase 4 (HDAC4) and the protein kinase PKCε and PKD1 [97-101]. Such a large macromolecular complex regulates and integrates several pathways, including cAMP, Ca^{2+}, MAP kinase signalling and hypoxia-induced signalling. In the context of stress-induced cardiac hypertrophy, mAKAP modulates gene expression regulated by the transcription factors NFATc, MEF2, HIF-1α and type II histone deacetylase [102].

In order to reduce cytosolic Ca^{2+} levels, terminate contraction and ensure cardiac muscle relaxation, SERCA2 (cardiac/slow-twitch isoform 2a) ATP-dependently pumps Ca^{2+} from the cytosol into the SR [103, 104]. It is located in the membrane of the SR and is inhibited by the interaction with PLB. In rat cardiac myocytes, AKAP18δ mediates the PKA phosphorylation of PLB following β-adrenoceptor stimulation, and so directs the PLB dissociation from SERCA2 [64, 105]. This dissociation activates the SERCA2 Ca^{2+} pump [106]. The Na^{+}-Ca^{2+} exchanger (NCX) facilitates Ca^{2+} exit from the cytosol across the plasmalemma and is reported to bind to mAKAPβ [102, 107]. The physiological significance of this interaction is still unknown.

Coordinated repolarization of cardiac myocytes depends on the tethering of PKA by the AKAP450 splice variant, Yotiao to the voltage-gated potassium channel alpha subunit KCNQ1. PKA phosphorylation of KCNQ1 at serine 27 increases the slow outward potassium current I_{KS} that opposes the inward Ca^{2+} current [108]. Mutations within binding regions that disrupt the complex formation are associated with long-QT syndrome, an inherited form of cardiac arrhythmia [109].

The sarcomeric proteins troponin I (TnI) and cardiac myosin-binding protein C (MyBP-C) are phosphorylated by PKA, which is anchored to cardiac myofilaments by myomegalin and troponin T (TnT) [110-112]. Phosphorylation of TnI reduces its interaction with troponin C (TnC) and the Ca^{2+} sensitivity of contraction, and by that augments the cardiac muscle relaxation rate [113, 114]. A deletion of lysine 210 of cardiac TnT is found in patients affected by famil-

iar dilated cardiomyopathy, and it is suggested to cause an increase in the PKC-dependent phosphorylation of TnT and reduced levels of phosphorylated TnI and MyBP-C [115].

3.3 AKAPs and cardiac hypertrophy and heart failure

The heart responds to stress by an adaptive process resulting in cardiac hypertrophy; is the stress persistent, hypertrophy can progress towards heart failure. Major risk factors for the development of myocardial hypertrophy and thus heart failure are pressure and volume overload resulting for example from hypertension and myocardial infarction. Cardiovascular diseases are the top cause of death worldwide and according to the WHO the number of deaths will increase to more than 23 million per year within the next 15 years. Hypertension alone causes annually the premature death of 7.6 million people and leads to 92 million disability-adjusted life years world-wide [116].

Hypertrophy involves reexpression of foetal genes (e.g. ANP, BNP, α-Actinin) that are switched off in the normal post-natal heart [117]. Besides its role in contraction, intracellular Ca^{2+} exerts transcriptional control *via* calcineurin and activation of the transcription factor NFAT, which favours the expression of hypertrophic genes such as BNP [118, 119]. mAKAPβ at the perinuclear membrane recruits both calcineurin Aβ and NFATc3, promoting the calcineurin-dependent dephosphorylation of NFAT and NFAT's subsequent translocation into the nucleus, where it induces transcription of hypertrophic genes [105, 120]. Other hypertrophic pathways that involve mAKAP scaffolding are the cytokine-induced ERK5 pathway [121, 122] and the PKD/HDAC4/MEF2 pathway [123, 124] that is also controlled by AKAP-Lbc (see below). In neurons, a PKA binding site deletion of AKAP79 diminishes NFAT nuclear signalling [125]. Thus AKAP79 may also have a role in cardiac hypertrophy.

The mainly cytosolic AKAP-Lbc that anchors PKA, PKC and PKD [124, 126, 127] also acts as a guanine exchange factor (GEF) for RhoA [128]. While AKAP-Lbc null mice show severe developmental defects and die at embryonic day 10.5 [129], deletion of the Rho-GEF and PKD-binding domains does not affect development in mice [130]. In the basal state PKA phosphorylation of AKAP-Lbc at serine 1565 recruits the regulatory protein 14-3-3, which inhibits its Rho-GEF activity [131]. α1-adrenoceptor stimulation can induce hypertrophy of cardiac myocytes in a way that depends on the AKAP-Lbc-induced activation of RhoA [132].

AKAP-Lbc expression is increased upon cardiac hypertrophy, as modelled by stimulation with the α1-adrenergic agonist phenylephrine [133]. This hypertrophic stimulus leads to acti-

vation of AKAP-Lbc-bound PKD1 [124], subsequent phosphorylation of nuclear histone deacetylase 5 (HDAC5), which then is the trigger for its export from the nucleus [134, 135]. In the basal state, HDAC5 represses the transcription factor myocyte enhancer factor (MEF) 2 [136]. Nuclear export of HDAC5 enables MEF2-activated transcription of hypertrophic genes [133].

Another hypertrophic pathway mediated by AKAP-Lbc engages the scaffolding of PKA and its substrate Src homology 2 domain containing phosphatase (Shp2, also known as Ptnp11) [137, 138]. Shp2 deletion is associated with malfunctions in cardiac myocytes and mutations cause Noonan/LEOPARD syndrome, which is frequently associated with hypertrophic cardiomyopathy [139, 140]. In the hypertrophic heart, AKAP-Lbc expression is upregulated, so that more Shp2 is phosphorylated by PKA and its phosphatase activity declines. Similarly, a Shp2 missense mutation that represses its phosphatase activity is found in most Noonan/LEOPARD syndrome patients [141]. The mutated Shp2 fails to dephosphorylate GAB1, which favours growth factor induced GAB1/phosphatidylinositol 3-kinase (PI3K) interaction and results in increased PI3K activity, Akt and GSK3β phosphorylation. The deregulated PI3K/Akt/GSK3β signalling promotes growth factor-stimulated cell size increase and hypertrophic gene expression in cardiac myocytes [142]. In a Noonan/LEOPARD syndrome mouse model, the Akt/mTOR signalling pathway is hyperactivated, resulting in hypertrophic cardiomyopathy ameliorable by rapamycin treatment [143]. A therapeutic compound directed against the catalytic pocket of Shp2 shows effective prevention of Shp2-induced hypertrophy in cultured cardiac myocytes [144].

AKAP-Lbc facilitates PKA phosphorylation of the small heat shock protein Hsp20, supporting its cardio-protective function [145] (reviewed in [146]). The cardioprotective role of Hsp20 is suggested by the association of its downregulation with sepsis-mediated cardiovascular injury. Conversely, transgenic overexpression of Hsp20 in mice ameliorated the endotoxin-induced cardiac dysfunction and apoptosis [147], and Hsp20 overexpression improved recovery from ischemia/reperfusion (I/R) injury with reduction of apoptotic cell death [148]. Expression of a constitutively phosphorylated Hsp20 (HSP20-S16D; phosphomimic) in adult cardiac myocytes showed an anti-apoptotic effect, which may be caused by a direct interaction with Bax, sequestering it to prevent pro-apoptotic signalling [148, 149]. Hsp20 co-immunoprecipitates with AKAP-Lbc and AKAP-Lbc facilitates the cardio-protective phosphorylation of serine 16 by PKA [145]. Association of the PDE4D with Hsp20 impedes its phosphorylation. Disrupting this interaction with a peptide re-establishes the protective role of Hsp20 upon hypertrophic stimulation of neonatal cardiac myocytes with isoproterenol. The interfering peptides increase PKA-driven Hsp20 phosphorylation and decrease the hyper-

trophic response. Moreover, *in vivo* the peptides protect against cardiac remodelling after aortic banding, an animal model for heart failure [150, 151].

Besides its role in cardiac myocytes, AKAP-Lbc is central for the stress-induced differentiation of cardiac fibroblasts to myofibroblasts. This step initiates the pathological remodelling of the heart towards cardiac fibrosis, stiffening of the heart and impaired function [152].

It is unknown whether the increase in the expression of AKAPs like mAKAP and AKAP-Lbc in hypertrophic cardiac myocytes plays a role in the transition towards heart failure [153]. However, AKAP-PKA interactions are changed in failing hearts. While interactions of the AKAPs SPHKAP, AKAP-KL (AKAP2), AKAP18 and MAP2 with PKA were increased up to 12-fold (MAP2), D-AKAP1 (AKAP1) and Yotiao showed reduced PKA binding [154]. Thus specific modulation of these AKAP-PKA interactions may reverse pathological changes and return cAMP signalling to its physiological state. The PKA substrates PLB and TnI display reduced phosphorylation in failing hearts [78, 155], which may result from reduced R subunit autophosphorylation and thus lower affinity for binding to AKAPs [156, 157].

3.4 AKAPs and PDE interactions

Besides the negative feedback loop involving AKAP450, AKAP79 and adenylyl cyclases which limits cAMP synthesis, cAMP signalling is controlled through hydrolysis of cAMP by PDEs [158-160] (Fig. 1B). In cardiac myocytes, several AKAPs bind PDEs to locally shape cAMP dynamics. One example is mAKAP, which in addition to the above-mentioned signalling proteins directly binds the cAMP-specific PDE4D3 (Fig. 2C). The loss of PDE4D3 from the mAKAP-based signalling hub at RyR in cardiac myocytes of PDE4D knock out mice accelerates the development of heart failure after myocardial infarction [161]. The cAMP/PKA microdomain is controlled by mAKAP-directed feedback loops involving PDE4D3, PKA, PP2, Epac1 and ERK5 [122]. PDE4D3 catalytic activity is enhanced by PKA phosphorylation at serine 54 [162], whereas it is decreased by ERK phosphorylation at serine 579 [163, 164]. ERK5, in turn, is suppressed by Epac1, which needs higher cAMP levels for activation than PKA [122]. PKA-activated PP2A initially decreases PDE4D3 activity by dephosphorylation of serine 54. Upon the consequent increase of the local cAMP level, Epac1 becomes activated and suppresses ERK5, which prevents the inhibitory phosphorylation of PDE4D3, thus acting as a feedback inhibition of cAMP signalling. The mAKAP-centred spatiotemporal control of cAMP levels orchestrates the stress-activated induction of hypertrophic gene expression, which is mediated by the transcription factors MEF2 and NFAT, and HDAC nuclear export (see above) [102, 122].

Another example is yotiao, which targets PDE4D3 to the potassium channel KCNQ1. PDE4D3 defines the local cAMP pool, regulates I_{KS} in the basal state and presumably reduces cAMP levels after β-adrenergic stimulation to downregulate the channel current that is increased by PKA phosphorylation [165].

The enzymes of the two PDE3 subfamilies, PDE3A and PDE3B, hydrolyse cAMP and cGMP competitively. PDE3A exists in three isoforms, PDE3A1 (136 kDa), PDE3A2 (118 kDa) and PDE3A3 (94 kDa), which are located in different cellular compartments in human myocardium: PDE3A1 is a membrane protein, while PDE3A2 and PDE3A3 are both cytosolic and membrane-associated. They all contain the same catalytic region and are similar concerning catalytic activity and inhibitor sensitivity [166, 167]. In human myocardium, PDE3A together with AKAP18 associates with SERCA2 and PLB and thereby control the local level of cAMP and thus PKA activity at the SR that controls Ca^{2+} reuptake into the SR during diastole [167, 168]. PKA phosphorylation of PDE3A1 at S292/S293 increases its association with the signalosome and may lead to local inhibition of PKA with reduced PLB phosphorylation and consequent reassociation of PLB with and inhibition of SERCA2 [167]. Impaired SERCA2 activity is associated with heart failure [169-171], and decreased SERCA2a expression correlates with increased expression of the hypertrophy marker atrial natriuretic factor (ANF) [172]. Moreover, shifting the SERCA2a/PLB ratio by either PLB down-regulation or increased SERCA2a expression enhances contractility of failing cardiac myocytes [173].

Mutations in the gene encoding PDE3A cause hypertension with Brachydactyly E [174]. The mutations are adjacent to each other and responsible for amino acid substitutions in a conserved region that is present in PDE3A1 (T445N, T445A, T445S, A447T, A447V, G449V) and PDE3A2 and to which a function has not been ascribed, but which is in close proximity to the catalytic domain. The mutations increased PKA-mediated PDE3A phosphorylation of serine 428 and serine 438 and resulted in gain-of-function, with increased cAMP hydrolytic activity. The hypertension is due to an increase in peripheral resistance, apparently induced by proliferation of vascular smooth muscle cells (VSMC). Surprisingly, the patients with hypertension with Brachydactyly E do not develop cardiac hypertrophy or heart failure [175]. The underlying mechanism and whether it involves AKAP18 is unclear.

A recently developed cAMP biosensors expressed in mice elucidated a hitherto unrecognized level of cooperativity between PDE3 and PDE4 in the control of cAMP levels in close proximity to the SERCA2-PLB microdomain in cardiac myocytes. The sensor is based on the cAMP-binding domain of Epac and targeted to the microdomain through fusion with PLB. The experiments revealed that PDE3 and PDE4 are both involved in the regulation of local

cAMP in the SERCA2 microdomain and contractility of healthy hearts. However, in heart failure, the influence of the local PDE4 is decreased, whereas the influence of PDE3 was not affected [176]. Both PDEs interact with AKAP18 [167, 168, 177] and PDE4D3 with mAKAP (see above); it remains to be determined whether the observed effects involve tethering of the PDEs to the SERCA2 microdomain by one or both of these or yet another AKAP.

3.5 Pharmacological interference with AKAP functions in the cardiovascular system

Due to the specificity and diversity of protein-protein interactions the most specific strategy to modulate an AKAP function would be interference with its protein-protein interactions. Indeed, in order to elucidate many of the above-described roles of the interactions of AKAPs with PKA and other binding partners in cardiac contractility, inhibitors of such interactions had been applied (see 2.1). Such inhibitors are not only useful as tools for scrutinizing functions of AKAP-dependent protein-protein interactions, but are also valuable for defining the potential of an interaction as a drug target. For example, inhibition of AKAP-PKA interactions with the peptides Ht31 and AKAP18δ-L314E revealed that intact AKAP-PKA interactions are a prerequisite for β-adrenoceptor-induced Ca^{2+} currents through $Ca_v1.2$ channels, as the peptides prevent β-adrenoceptor-induced increases in Ca^{2+} influx into cultured cardiac myocytes [52, 65]. In addition, adenoviral expression of the AKAP-PKA disruptor peptide Ht31 reduces the phosphorylation of the PKA substrates TnI, MyBP-C and PLB in cardiac myocytes [178]. Similar effects were observed when a D-AKAP2 (AKAP10)-derived peptide was employed that was coupled to a sequence from the HIV-1 TAT protein to render it membrane-permeant. The peptide impaired the isoproterenol-induced phosphorylation of PLB and TnI. Moreover, contraction and relaxation of the peptide-treated cardiac myocytes was diminished, and negative chronotropic, inotropic and lusitropic effects were observed for isolated, perfused hearts [179]. In line, disruption of the interaction of AKAP18δ and PLB inhibited the PKA-catalysed phosphorylation of PLB and decreased the rate of Ca^{2+} reuptake into the SR [64].

These effects of displacement of PKA from its cognate cellular location are comparable to those of β-blockers [52]. Although β-blockers are generally well tolerated and are a first-line treatment of e.g. chronic heart failure and hypertension, they can induce unwanted side effects such as bradycardia, AV block, cold extremities, worsening of claudication, hypotension and a life-threatening increase in airway resistance in patients with asthma and bronchospastic chronic obstructive pulmonary disease (COPD), depression and a loss of libido. All of these are temporary and reversible but limit the compliance of affected persons. Disruption of AKAP-PKA interactions in such cases may constitute an alternative.

Inhibition of PKA-AKAP interactions was also achieved with a small molecule, FMP-API-1. In contrast to Ht31, FMP-API-1 increased contractility of cultured cardiac myocytes and isolated perfused rat hearts, presumably, because this molecule also activates PKA and enhances PKA-dependent phosphorylation of PLB and TnI. FMP-API-1 does not globally induce phosphorylation of PKA substrates or affect phosphatase or PDE4 activities [41, 46].

Although global interference with AKAP-PKA interactions appears beneficial and as an option for the treatment of cardiovascular diseases, targeting AKAP-PKA interactions non-selectively may cause side effects, as these interactions are relevant in many cellular processes in different cell types (e.g. insulin release from pancreatic β-cells or H^+ secretion from gastric parietal cells). Thus agents selectively inhibiting defined AKAP-PKA interactions are needed. Also, only disruption of a selected AKAP-dependent protein-protein interaction will show an involvement of individual interactions in a defined biological process.

In terms of pharmacological modulation of signalling in cardiac myocytes for the treatment of cardiovascular disease, targeting SERCA2 appeared as the most promising approach. Preclinical studies had revealed a reduction of SERCA2 in heart diseases. The consequent reduction in Ca^{2+} reuptake into the SR during diastole prevents normal Ca^{2+} cycling. This affects contractility and triggers a hypertrophic response [104, 180]. A clinical trial, CUPID (Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease) indicated that upregulation of SERCA2 upon adenoviral transfer of SERCA2 is beneficial in advanced heart failure without apparent side effects [181] However, a follow up clinical trial phase 2b (CUPID-2b) did not meet the endpoints, e.g. did not show a significantly reduced frequency and/or delay of heart failure-related hospitalizations in patients with advanced heart failure.

Regulation of the cAMP pool in close vicinity of SERCA2 involves PDE3A [168]. Global inhibition of PDE3A with drugs such as cilostamide globally increases cAMP levels in cardiac myocytes and enhances contractility of failing hearts in the short-term but long-term treatment leads to an increase in sudden cardiac death [182]. A novel approach towards targeting the SERCA2 protein complex may arise from a recent study of clinical effects in patients with mutations in the gene encoding PDE3A that cause hypertension with BDE. As described above, the mutations lead to hyperphosphorylation of PDE3A and protect against heart failure [175]. Compared to the wild type enzyme, the mutant enzymes are targeted to different cellular compartments in HeLa cells [175]. Since PKA phosphorylation of serine 292/293 causes interaction of PDE3A with the SERCA2 complex, the hyperphosphorylated mutants may engage in new protein-protein interactions that are cardioprotective. Thus displacing

PDE3A from the SERCA2 complex by novel pharmacological agents may have a similar effect. Such agents may improve contractility parameters of failing hearts without the adverse effects of enzyme blockade. Displacement of PKA from AKAP18 may have a similar effect; it would withdraw the cAMP effector PKA from the SERCA2 microdomain reducing PDE3A phosphorylation and association with the microdomain.

4 AKAPs in cancer

4.1 AKAPs' involvement in cancer

Despite decades of intensive clinical and molecular research that has advanced the understanding of mechanisms underlying cancer development and progression, cancer remains one of the leading causes of death in the developed world [183]. Several AKAPs are differentially expressed in cancer cells and tissues [184, 185] where they play important roles by regulating proliferation, migration, invasion and survival [186-189].

One example is AKAP-Lbc, which regulates cancer cell proliferation by enhancing ERK signalling. In this pathway, AKAP-Lbc sequesters RAF in proximity to MEK1 enabling the phosphorylation of Kinase Suppressor of Raf (KRS) by PKA, which leads to MEK activation and increases proliferation [2, 190]. In addition, AKAP-Lbc's GEF domain catalyses the GDP-GTP exchange reaction required for tumour growth as well as RhoA-activated stress fiber formation necessary for tumour invasion and metastasis [191]. Overexpression in uterine leiomyoma or mutations resulting in truncated forms of AKAP-Lbc (the so called onco-Lbc) is associated with cancer progression [192-195]. AKAP-Lbc facilitates PKA phosphorylation and inhibition of Shp2 in the heart, which may lead to cardiac hypertrophy [137] (see 3.3). Shp2 is known to initiate pro-survival signalling cascades that include PI3K, MAPK/ERK and STAT3 [196]. Thus AKAP-Lbc may fulfil a similar role in cancer cells as in cardiac myocytes leading to Shp2-initiated increased apoptosis resistance thus promoting tumourigenesis.

An enhanced migratory and invasion ability of cells are hallmarks of tumourigenesis. Several AKAPs are involved in the regulation of metastasis and cytoskeletal remodelling which is associated with cell migration. AKAP450 is highly expressed in colorectal carcinoma cells and its knockdown resulted in attenuation of proliferation and metastasis in a Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1)-dependent mechanism [197]. Moreover, single nucleotide polymorphisms (SNPs) in the encoding AKAP9 gene result in variants associated with breast cancer [187, 198]. AKAP450 promotes initiation of DNA synthesis,

thus favouring cell cycle progression [199]. This AKAP was also suggested to maintain PKA anchored at the centrosomes during interphase and release it at mitosis entry, so permitting the mitosis-specific phosphorylation of PKA substrates [200]. Therefore, interfering with the AKAP450-PKA interaction may potentially prevent mitosis entry and inhibit cell cycle progression in cancer cells.

Gravin (AKAP12) anchors both PKA and PKC, thus regulating multiple tumourigenic processes, including cell cycle, senescence and angiogenesis [201-203]. Its expression is down-regulated in various solid tumours and possibly in myeloid leukaemia [204-206]. A gravin knockout mouse model shows prostatic hyperplasia with focal dysplasia [201]. In addition, gravin negatively regulates metastasis and tumour growth by recruiting Src kinase from focal adhesion kinase (FAK) and by PKC-mediated activation of ERK signalling in the prostate cancer cell lines MAT-LyLu and CWR22Rv1 [207]. In contrast, in a melanoma model gravin can act as a promoter of tumour growth. A specific variant, AKAP12v2, induces a series of PKA-mediated phosphorylation events in response to hypoxia in metastatic melanoma. Upon knockdown of gravin, these phosphorylation events were abrogated and hypoxia-induced migration/invasion was inhibited. Silencing of gravin with shRNA *in vivo* in a mouse model resulted in reduced melanoma growth and distant metastasis [208].

Signalling initiated by binding of the ligand Netrin-1 to its receptor DCC (deleted in colorectal carcinoma) influences angiogenesis, epithelial cell migration and tumour pathogenesis; in particular, the stimulation of DCC is responsible for apoptosis resistance *via* TP53-dependent apoptosis suppression [209, 210], and for the inhibition of the pro-apoptotic activity of DCC in the absence of Netrin-1 [211]. Netrin-1 is able to increase cAMP levels in HEK293 cells upon overexpression [212] and to activate PKA in NG108-15 cells, a neuroblastoma x glioma hybrid neuronal cell line [213]. In NG108-15 cells, the AKAP function of Ezrin-Radixin-Moesin (ERM) family members is required for DCC-mediated activation of PKA and subsequent PKA-mediated phosphorylation of Mena/VASP, proteins involved in the regulation of the cytoskeleton. Using the AKAP-PKA interaction disruptor peptide, Ht31 resulted in a significant reduction in PKA-mediated Mena/VASP phosphorylation associated with augmentation of filopodia formation in response to DCC activation [213]. Filopodia are actin-containing spikes formed at the leading edge of the cell that are required for cell migration during metastasis [214]. However, the implication of PKA and cAMP in the Netrin-1 signalling is still controversial; a study performed in embryonic rat spinal commissural neurons has shown that Netrin-1 does not alter the level of cAMP or activity of PKA across a wide range of Netrin-1 concentrations, indicating that Netrin-1 does not regulate PKA in these cells. Instead, modulating PKA activity regulated the sensitivity of chemo-attraction to Netrin-1: PKA inhibition reduced and

PKA activation increased the distance over which axons turned towards a source of Netrin-1 [215]. The exact mechanism by which the cAMP/PKA axis regulates Netrin-1/DCC signalling in cancer cells is unclear and requires further research.

Other AKAPs that may play a role in metastasis include AKAP79, which directly interacts with the IQ domain of GTPase-activating protein 1 (IQGAP1) [216]. IQGAPs are scaffolding proteins regulating the actin cytoskeleton and microtubule dynamics and frequently are deregulated in tumourigenesis in order to promote cancer progression and metastasis. siRNA-mediated knockdown of IQGAP1 dramatically inhibited thyroid cancer cell invasion and colony formation in *in vitro* studies [217]. IQGAP2, in contrast, appears to act as a tumour suppressor and its expression was significantly downregulated in gastric carcinoma [218]. IQGAP3 is exclusively expressed in proliferating cells of the small intestine. This expression may be associated with IQGAP3-induced ERK activation and may have a role in the regulation of cellular proliferation. IQGAP1 interacts with Cdc42, Rac1, E-cadherin, β-catenin, calmodulin and components of the mitogen-activated protein kinase (MAPK) pathway, all prominent regulators of cytoskeletal dynamics and cellular motility. Therefore, AKAP79 may be involved in one or more of these regulatory pathways and influence metastasis [217-219].

AKAP220 was also shown to interact with IQGAP1 and anchor it to leading edges of migratory MCF-7 breast cancer cells. The silencing of AKAP220 resulted in an apparent decrease in the mobility of HT1080 human fibrosarcoma cells. These observations led to the hypothesis that AKAP220 and IQGAP1 act synergistically to ensure the combined participation of signalling enzymes such as GSK3β, PP1 and PKA together with microtubule effectors that are required for the polarization of microtubule dynamics at the leading edge of the cells. The proposed mechanism states that AKAP220-anchored PKA inhibits GSK3β activity and dephosphorylates serines 533 and 537 of CLIP-associating protein 2. This dephosphorylated form of CLASP2 can then interact with microtubule-associated proteins and the AKAP220-anchored IQGAP1. The ultimate outcome is enhanced cell motility [220]. These finding suggest that disruption of the AKAP220/IQGAP1 as well as AKAP220/PKA interactions may hold promise in attenuating metastasis in cancer cells.

In addition, AKAP220 forms a complex with PKA and IQGAP2 in HEK293 cells. The formation of the complex allows PKA to phosphorylate threonine 716 of IQGAP2 resulting in recruitment of the active form of the GTPase Rac that regulates the actin cytoskeleton and enhances membrane ruffling. Overexpression of an IQGAP2 phosphomimetic mutant (IQGAP2-T716D) enhances the formation of actin-rich membrane ruffles while in contrast, expression IQGAP2-T716A, a mutant that cannot be phosphorylated or gene silencing of

AKAP220 suppresses formation of membrane ruffles [221, 222]. The formation of actin-rich membrane ruffles is associated with metastatis of cancer cells and is considered to be an indicator of cancer cell motility and metastatic potential [223]. These findings suggest that disrupting the formation of AKAP220/IQGAP2/PKA complex may prevent IQGAP2 phosphorylation by PKA and potentially reduce the metastatic potential of cancer cells.

AKAP4 is a testis-specific protein involved in the control of sperm motility [224, 225] and aberrantly expressed in various malignant tissues [226]. High expression of AKAP4 mRNA and protein compared to healthy tissues was detected in prostate, lung, breast, ovarian, and cervical cancer. The AKAP4 expression correlated with cancer stage and severity in non-small cell lung cancer and its expression was reduced in patients undergoing remission and upregulated in patients undergoing recurrence. This correlation can be exploited for diagnostics and cancer staging as well as for treatment [227]. AKAP4 belongs to a family of Cancer testis antigens (CTAs) [228] that are widely expressed in tumour tissues and circulating tumour cells. Due to the fact that CTAs are not found in somatic tissues except testes and often trigger humoral immune responses in cancer patients they have been suggested as potential targets for antigen-specific immunotherapy [229]. AKAP4 initiates humoral immune responses, demonstrated by the presence of IgG antibodies against AKAP4. This immune response was observed in non-small cell lung, prostate and ovarian cancer, and multiple myeloma [227, 228, 230-232].

4.2 Pharmacological opportunities for AKAPs in the cancer field

AKAPs are not yet recognized targets for cancer therapy, because their roles are not yet clear. However, several AKAPs and in particular some of their protein-protein interactions that apparently play a role in cancer may be suitable targets under specific conditions. In addition to the options indicated above, there may be further. A decreased AKAP-Lbc-PKA interaction could, for example, decrease the inhibitory phosphorylation of Shp2 by PKA. Since Shp2 negatively regulates apoptosis resistance *via* JAK/STAT3 signalling this reduction may result in cancer cell death. However, since Shp2 also positively regulates proliferation and migration *via* MAPK/ERK signalling, reducing its PKA-mediated inhibition may boost proliferation and/or metastasis in non-small-cell lung cancer cells [196]. Thus further insight into the exact mode of Shp2 regulation by the AKAP-Lbc-PKA interaction is required prior to pursuing therapeutic disruption of the AKAP-PKA interaction.

The interaction of AKAP450 with PKA drives cell cycle progression in acute lymphocytic leukaemia cells [200] and could be exploited to slow down proliferation. The influence of AKAP220 on the actin cytoskeleton suggests it as a target for metastasis-directed therapy,

since the forces generated by actin filaments permit cell movement and migration [17, 233, 234]. The disruption of the AKAP220-PKA interaction could result in a decreased recruitment of Rac, thus reducing actin cytoskeleton remodelling and hampering metastatic cell motility. The disruption of the AKAP220-GSK3β interaction has therapeutic potential since GSK3β has multiple roles in the regulation of tumourigenic signalling pathways, including apoptosis, metastasis, cell cycle progression, and metabolic reprogramming [235].

PKA activity and anchoring by AKAPs is required for metastasis of the ovarian carcinoma cell, SKOV-3. However, the specific AKAP responsible has yet to be determined [236]. Global disruption of AKAP-PKA interactions may thus interfere with ovarian cancer metastasis. Such global uncoupling of PKA from AKAPs is not generally an option. Recently, contradictory roles of PKA/AKAP signalling were demonstrated in colon carcinoma cells; AKAP149 (D-AKAP1, AKAP1) was shown to be required for TGFβ-mediated PKA activation leading to apoptosis in response to cellular stress, such as oxidative stress, radiation, nutrient deprivation as well as cellular damage [237]. In contrast, a recently characterized AKAP, Praja2 [238] was shown to mediate PKA activation in a cAMP-dependent manner to inhibit apoptosis in the same cell line [188]. Such opposing roles of AKAP/PKA signalling, especially in the same cells, must be taken into account when searching for drug targets to ensure specificity of the treatment. The necessity to ensure specificity of treatment is particularly relevant for gravin. Due to gravin's opposing roles in melanoma and prostate cancer cells, further insight into the molecular pathways involved is required before any conclusion can be drawn on the safety of pharmacologically targeting of the gravin-PKA interaction in cancer therapy. This is also true for AKAP4. Even if previously published research suggested AKAP4 to be involved in cervical cancer cell line growth [239], lack of insight into the molecular pathways involved fails to clarify how AKAP4 contributes to cancer progression. Thus, for now, due to its aberrant expression in cancer tissue, its value is limited to use as a cancer biomarker. However, cancer immunotherapy is a novel rapidly growing field and AKAP4 is currently being analysed as a potential target for immunotherapy of cervical cancer [228, 239]. The ability to elicit immune response makes AKAP4 a potential candidate for development of cancer-specific vaccines and other targeted immunotherapies.

Similarly, further research is required to understand if the recent observation of PKA-mediated Netrin-1/DCC signalling regulation could be affected by disrupting the interaction of PKA with AKAPs of the ERM family not only in NG108-15 cells [213] but also in other cancer cell lines. Disruption of such an interaction may be therapeutically beneficial for treating patients with cancer metastasizing to the brain, such as lung, kidney, breast, and colon cancer [240] and perhaps other cancer types.

The given examples indicate that AKAPs play a role in the various stages of cancer development and progression but also show the yet incomplete understanding of their involvement. In some instances AKAP-dependent protein-protein interactions have even opposing roles in the same cancer cell type. Despite this, the avilable data suggest that a thorough evaluation of their suitability as drug targets may lead to new approaches towards the treatment of cancer.

5 Concluding remarks

Despite all the results described above and many more that could not be mentioned, the understanding of the molecular mechanisms by which AKAPs control cAMP-dependent and cAMP-independent signalling compartments is still incomplete. This lack of understanding is by no means limited to the two fields discussed in this article, cardiovascular and cancer. Thus gaining mechanistic insight into the functioning of AKAPs and compartmentalised signalling is not only essential for the understanding of elementary biological processes. The involvement of compartmentalised signalling and, in particular, of AKAPs organising compartmentalisation of signal transduction in the development and progression of diseases provides opportunities for the design of innovative therapeutic concepts for diseases where no causal treatment is available such as cancer and cardiovascular diseases.

A prerequisite for the elucidation of functions of AKAPs and of their individual protein-protein interactions both in cAMP-dependent and cAMP-independent signalling is the availability of molecular tools. Pharmacological interference with individual AKAP-dependent protein-protein interactions with small molecules is a promising approach. However, so far only one non-selective small molecule inhibitor of AKAP-PKA interactions is available. It binds to R subunits of PKA [41, 46]. PKA-binding (AKB) domains of the AKAP family are structurally conserved. Therefore, specific disruption of the interaction of a defined AKAP with PKA or at least lowering the binding affinity and thus partially inhibit the interaction may require molecules targeting hot spots within AKB domains or bind to allosteric sites within the AKAP. Hot spots are amino acids essential for the interaction with the binding partner [241]. Structural information on the interactions is hardly available, ruling out the *in silico* design of AKAP-specific ligands. Therefore, specific small molecule disruptors can currently only be identified by screening using full length AKAPs and R subunits of PKA. However, the generation of full-length AKAPs for *in vitro* screening is not trivial. Most of them are large proteins that are unstructured in the absence of binding partners and thus are difficult to generate as recombinant proteins. An alternative would be cell-based screening where, for example, fluorescently

tagged interacting partners can be expressed in cells and the disruption of an interaction can be monitored microscopically [242]. Such obstacles extend to other AKAP-dependent protein-protein interactions.

Thus novel techniques for AKAP analysis and new pharmacological agents for the modulation of their functions are required to gain mechanistic insight into compartmentalised cAMP and cAMP-independent signal transduction processes.

Tab1

Canonical AKAPs show a conserved AKB domain

AKAP name	Conserved domain	Spec.	Ref.
AKAP1 (D-AKAP1)	DRNEEIKRAAFQIISQVISEATEQVLATTVGKVAGRV	D	[243]
AKAP2 (AKAP-KL)	SVDDPLEYQAGLLVQNAIQQAIAEQVDKAVSKTSRDG	RII	[244]
AKAP3	SSVDEVSFYANRLTNLVIAMARKEINEKIDGSENKCV	D	[245]
AKAP4 (FSC1A)	DLSFYVNRLSSLVIQMAHKEIKEKLEGKSKCLHHSIC	D	[246]
AKAP4 (FSC1B)	SISKGLMVYANQVASDMMVSLMKTLKVHSSGKPIPAS	RI	[246]
AKAP5 (AKAP79)	QYETLLIETASSLVKNAIQLSIEQLVNEMASDDNKI	D	[247, 248]
AKAP6 (mAKAP)	HQKDAEDCSVHNFVKEIIDMASTALKSKSQPENEVAA	RII	[249]
AKAP7 (18)α,β,γ (Hs)	PDDAELVRLSKRLVENAVLKAVQQYLEETQNKNKPGE	RII	[87]
AKAP7 (18)δ (rat)	PEDAELVRLSKRLVENAVLKAVQQYLEETQNKKQPGE	RII	[250]
AKAP8 (AKAP95)	TPEEVAADVLAEVITAAVRAVDGEGAPAPESSGEPAE	RII	[247]
AKAP9 (AKAP450)	KIVNLQKIVEEKVAAALVSQIQLEAVQEYAKFCQDNQ	RII	[251]
AKAP10 (D-AKAP2)	AQEELAWKIAKMIVSDIMQQAQYDQPLEKSTKL-	D	[252]
AKAP11 (AKAP220)	VNLDKKAVLAEKIVAEAIEKAERELSSTSLAADSGIG	D	[253]
AKAP12 (gravin)	ELETKSSKLVQNIIQTAVDQFVRTEETATEMLTSELQ	RII	[254]
AKAP13 (AKAP-Lbc)	KGADLIEEAASRIVDAVIEQVKAAGALLTEGEACHMS	RII	[255]
AKAP14	NYEDELTQVALALVEDVINYAVKIVEEERNPLKNIKW	RII	[256]
GSKIP	TDMKDMRLEAEAVVNDVLFAVNNMFVSKSLRCADDVA	RII	[257]
Ezrin	DQIKSQEQLAAELAEYTAKIALLEEARRRKEDEVEE	D	[54, 258]
smAKAP	VILEYAHRLSQDILCDALQQWACNNIKYHDIPYIESE	RI	[259]
SKIP	CITDFAEELADTVVSMATEEIAAICLDNSSGKQPWFC	RI	[260, 261]
PAP7	EERLRLEQQKQQIMAALNSQTAVQFQQYAAQRYPGNY	RI	[262]
$AKAP_{CE}$	GRDSIEESANESALYQFADRFSELVISEALNHRKMHY	RI	[263]
MAP2D (rat)	ELTSADRETAEEVSARIVQVVTAEAVAVLKGEGEKEA	D	[264]

Tab1: Alignment showing the A-kinase-binding domains (AKBs) of several AKAPs. Indicated are the names of the AKAPs, the PKA binding sequences (highlighted in grey are amino acids in conserved positions) and the AKAP specificity for binding to RI, RII or to both (dual (D) specific).

Figure legends

Figure 1. Model of compartmentalized cAMP signalling. A. A-kinase anchoring proteins (AKAPs) spatially and temporally coordinate protein kinase A (PKA)-catalysed phosphorylation of substrate proteins. Ligand binding to G protein-coupled receptors (GPCRs) induces adenylyl cyclase (AC) activity to convert ATP to the second messenger cyclic AMP (cAMP). PKA holoenzyme consists of a dimer of regulatory subunits (RIα, RIβ, RIIα or RIIβ) and two catalytic (C) subunits. Upon binding of cAMP to the R subunits, the catalytic subunits are released to phosphorylate nearby substrates. B. Termination of cAMP signalling at an AKAP-organized signalling hub. Besides an α-helical PKA-R subunit binding motif (A kinase-binding (AKB) domain), AKAPs possess specific anchoring and additional protein-protein interaction domains that define the AKAP signalosome. Phosphodiesterases (PDEs) degrade cAMP to 5'AMP, establishing gradients of the second messenger. PKA will only be activated where cAMP reaches threshold levels. Phosphatases dephosphorylate target proteins, counteracting PKA activity. Compartmentalized PKA in concert with PDEs and phosphatases facilitates coordinated phosphorylation of target proteins only at defined signalling hubs within the cell.

Figure 2. AKAPs involved in depolarization (A), repolarization (B) and transcriptional control of hypertrophy (C) of cardiac myocytes. A. For contraction of cardiac myocytes Ca^{2+} enters the cell through voltage-gated Ca^{2+} channels ($Ca_v1.2$), triggering the efflux of Ca^{2+} from the intracellular store, the sarcoplasmic reticulum into the cytosol. The increased cytosolic Ca^{2+} promotes contraction of the sarcomere. Beta-adrenergic receptor stimulation causes a cAMP-induced activation of the protein kinase A (PKA) catalytic subunits. Phosphorylation of $Ca_v1.2$ and ryanodine receptors (RyR) by AKAP-anchored PKA increases their open-probability, which enhances the force of contraction (positive inotropic effect). B. For relaxation, a small fraction of Ca^{2+} exits the cell across the sarcolemma through the Na^+/Ca^{2+} exchanger (NCX), whereas the major part is actively pumped back to the sarcoplasmic reticulum by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA). Phospholamban (PLB) inhibits SERCA. SERCA is deinhibited by release of PLB upon PLB's phosphorylation by PKA. KCNQ1 conducts a slow outward potassium current that supports repolarization of the cardiac myocyte. C. Hypertrophy is a stress-induced non-mitotic growth of the heart to compensate increased hemodynamic load. AKAPs controlling hypertrophic gene transcription include AKAP-Lbc and mAKAPβ. AKAP-Lbc has intrinsic Rho-GEF activity and mediates the inhibitory phosphorylation of histone acetylases by PKD. Derepression of the transcription factor MEF2 and chromatin decondensation promote transcription of hypertrophic genes. Located

to the perinuclear membrane mAKAPβ scaffolds a multiprotein complex that integrates cAMP/ Ca^{2+}/ MAP kinase and hypoxia signalling pathways. Activated by Ca^{2+}, calcineurin Aβ dephosphorylates NFATc3, which enables it to enter the nucleus and activate transcription of its target genes.

Figure 3. Pharmacological interference with AKAPs has the potential to target distinct cellular pathways involved in pathogenesis. A. Besides the A kinase-binding (AKB) domains of AKAPs that mediate the interaction with the dimerization/docking (D/D) domain of R subunits of PKA, AKAP's anchoring domains or the domains interacting with further proteins can be targeted to interfere with AKAP functions. B. Currently available methods include interference with AKAP-PKA interactions using peptides preferentially binding RI [54, 56] and/or RII subunits [39, 40, 43, 55], peptidomimetics [42, 59, 61] or small molecules [41]. Of use in the future may also be interference with specific AKAP-dependent protein-protein interactions using novel nanobody-based technologies [68, 74].

References

1. Skroblin, P., et al., *Mechanisms of protein kinase a anchoring.* Int Rev Cell Mol Biol, 2010. **283**: p. 235-330.
2. Langeberg, L.K. and J.D. Scott, *Signalling scaffolds and local organization of cellular behaviour.* Nat Rev Mol Cell Biol, 2015. **16**(4): p. 232-44.
3. Taylor, S.S., et al., *PKA: lessons learned after twenty years.* Biochim Biophys Acta, 2013. **1834**(7): p. 1271-8.
4. Taylor, S.S., et al., *Assembly of allosteric macromolecular switches: lessons from PKA.* Nat Rev Mol Cell Biol, 2012. **13**(10): p. 646-58.
5. Bruystens, J.G., et al., *PKA RIalpha homodimer structure reveals an intermolecular interface with implications for cooperative cAMP binding and Carney complex disease.* Structure, 2014. **22**(1): p. 59-69.
6. Wu, J., et al., *PKA type IIalpha holoenzyme reveals a combinatorial strategy for isoform diversity.* Science, 2007. **318**(5848): p. 274-9.
7. Kim, C., et al., *PKA-I holoenzyme structure reveals a mechanism for cAMP-dependent activation.* Cell, 2007. **130**(6): p. 1032-43.
8. Christensen, B.M., et al., *Localization and regulation of PKA-phosphorylated AQP2 in response to V(2)-receptor agonist/antagonist treatment.* Am J Physiol Renal Physiol, 2000. **278**(1): p. F29-42.
9. Ellerbroek, S.M., K. Wennerberg, and K. Burridge, *Serine phosphorylation negatively regulates RhoA in vivo.* J Biol Chem, 2003. **278**(21): p. 19023-31.
10. Ahmad, F., et al., *Cyclic nucleotide phosphodiesterases: important signaling modulators and therapeutic targets.* Oral Dis, 2015. **21**(1): p. e25-50.
11. Baillie, G.S., J.D. Scott, and M.D. Houslay, *Compartmentalisation of phosphodiesterases and protein kinase A: opposites attract.* FEBS Lett, 2005. **579**(15): p. 3264-70.
12. Nygren, P.J. and J.D. Scott, *Therapeutic strategies for anchored kinases and phosphatases: exploiting short linear motifs and intrinsic disorder.* Front Pharmacol, 2015. **6**: p. 158.
13. Wong, W. and J.D. Scott, *AKAP signalling complexes: focal points in space and time.* Nat Rev Mol Cell Biol, 2004. **5**(12): p. 959-70.
14. Troger, J., et al., *A-kinase anchoring proteins as potential drug targets.* Br J Pharmacol, 2012. **166**(2): p. 420-33.
15. Szaszak, M., et al., *Compartmentalized cAMP signalling in regulated exocytic processes in non-neuronal cells.* Cell Signal, 2008. **20**(4): p. 590-601.
16. Soderling, S.H., et al., *A WAVE-1 and WRP signaling complex regulates spine density, synaptic plasticity, and memory.* J Neurosci, 2007. **27**(2): p. 355-65.
17. Frugtniet, B., W.G. Jiang, and T.A. Martin, *Role of the WASP and WAVE family proteins in breast cancer invasion and metastasis.* Breast Cancer (Dove Med Press), 2015. **7**: p. 99-109.
18. Kang, R., et al., *WAVE1 regulates Bcl-2 localization and phosphorylation in leukemia cells.* Leukemia, 2010. **24**(1): p. 177-86.
19. Frezza, C., et al., *OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion.* Cell, 2006. **126**(1): p. 177-89.
20. Song, Z., et al., *Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion.* Mol Biol Cell, 2009. **20**(15): p. 3525-32.
21. Pidoux, G., et al., *Optic atrophy 1 is an A-kinase anchoring protein on lipid droplets that mediates adrenergic control of lipolysis.* EMBO J, 2011. **30**(21): p. 4371-86.
22. Belenguer, P. and L. Pellegrini, *The dynamin GTPase OPA1: more than mitochondria?* Biochim Biophys Acta, 2013. **1833**(1): p. 176-83.
23. Newlon, M.G., et al., *The molecular basis for protein kinase A anchoring revealed by solution NMR.* Nat Struct Biol, 1999. **6**(3): p. 222-7.

24. Newlon, M.G., et al., *A novel mechanism of PKA anchoring revealed by solution structures of anchoring complexes.* EMBO J, 2001. **20**(7): p. 1651-62.
25. Kinderman, F.S., et al., *A dynamic mechanism for AKAP binding to RII isoforms of cAMP-dependent protein kinase.* Mol Cell, 2006. **24**(3): p. 397-408.
26. Gold, M.G., et al., *Molecular basis of AKAP specificity for PKA regulatory subunits.* Mol Cell, 2006. **24**(3): p. 383-95.
27. Doxsey, S.J., et al., *Pericentrin, a highly conserved centrosome protein involved in microtubule organization.* Cell, 1994. **76**(4): p. 639-50.
28. Diviani, D., et al., *Pericentrin anchors protein kinase A at the centrosome through a newly identified RII-binding domain.* Curr Biol, 2000. **10**(7): p. 417-20.
29. Delaval, B. and S.J. Doxsey, *Pericentrin in cellular function and disease.* J Cell Biol, 2010. **188**(2): p. 181-90.
30. Chaturvedi, D., et al., *Subcellular localization and biological actions of activated RSK1 are determined by its interactions with subunits of cyclic AMP-dependent protein kinase.* Mol Cell Biol, 2006. **26**(12): p. 4586-600.
31. Kostenko, S., et al., *Cross-talk between protein kinase A and the MAPK-activated protein kinases RSK1 and MK5.* J Recept Signal Transduct Res, 2011. **31**(1): p. 1-9.
32. Kurosu, T., et al., *Alpha/beta-tubulin are A kinase anchor proteins for type I PKA in neurons.* Brain Res, 2009. **1251**: p. 53-64.
33. Rivard, R.L., et al., *AKAP-independent localization of type-II protein kinase A to dynamic actin microspikes.* Cell Motil Cytoskeleton, 2009. **66**(9): p. 693-709.
34. Hausken, Z.E., et al., *Type II regulatory subunit (RII) of the cAMP-dependent protein kinase interaction with A-kinase anchor proteins requires isoleucines 3 and 5.* J Biol Chem, 1994. **269**(39): p. 24245-51.
35. Hausken, Z.E., et al., *Mutational analysis of the A-kinase anchoring protein (AKAP)-binding site on RII. Classification Of side chain determinants for anchoring and isoform selective association with AKAPs.* J Biol Chem, 1996. **271**(46): p. 29016-22.
36. Hermann, J.S., et al., *Neurochondrin is an atypical RIIalpha-specific A-kinase anchoring protein.* Biochim Biophys Acta, 2015.
37. Wang, H., et al., *Norbin: A promising central nervous system regulator.* Commun Integr Biol, 2010. **3**(6): p. 487-90.
38. Uematsu, K., et al., *Protein kinase A directly phosphorylates metabotropic glutamate receptor 5 to modulate its function.* J Neurochem, 2015. **132**(6): p. 677-86.
39. Alto, N.M., et al., *Bioinformatic design of A-kinase anchoring protein-in silico: a potent and selective peptide antagonist of type II protein kinase A anchoring.* Proc Natl Acad Sci U S A, 2003. **100**(8): p. 4445-50.
40. Burns-Hamuro, L.L., et al., *Designing isoform-specific peptide disruptors of protein kinase A localization.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 4072-7.
41. Christian, F., et al., *Small molecule AKAP-protein kinase A (PKA) interaction disruptors that activate PKA interfere with compartmentalized cAMP signaling in cardiac myocytes.* The Journal of biological chemistry, 2011. **286**(11): p. 9079-96.
42. Schafer, G., et al., *Highly functionalized terpyridines as competitive inhibitors of AKAP-PKA interactions.* Angew Chem Int Ed Engl, 2013. **52**(46): p. 12187-91.
43. Wang, Y., et al., *Isoform-selective disruption of AKAP-localized PKA using hydrocarbon stapled peptides.* ACS Chem Biol, 2014. **9**(3): p. 635-42.
44. Deak, V.A. and E. Klussmann, *Pharmacological interference with protein-protein interactions of A-kinase anchoring proteins as a strategy for the treatment of disease.* Curr Drug Targets, 2015.
45. Hundsrucker, C. and E. Klussmann, *Direct AKAP-mediated protein-protein interactions as potential drug targets.* Handb Exp Pharmacol, 2008(186): p. 483-503.
46. Schachterle, C., et al., *Screening for Small Molecule Disruptors of AKAP-PKA Interactions.* Methods Mol Biol, 2015. **1294**: p. 151-66.
47. Carr, D.W., et al., *Interaction of the regulatory subunit (RII) of cAMP-dependent protein kinase with RII-anchoring proteins occurs through an amphipathic helix binding motif.* J Biol Chem, 1991. **266**(22): p. 14188-92.

48. Walker-Gray, R. and M.G. Gold, *Structure-based bacteriophage screening for AKAP-selective PKA regulatory subunit variants.* Methods Mol Biol, 2015. **1294**: p. 167-80.
49. Kennedy, E.J. and J.D. Scott, *Selective disruption of the AKAP signaling complexes.* Methods Mol Biol, 2015. **1294**: p. 137-50.
50. Klussmann, E., et al., *Protein kinase A anchoring proteins are required for vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells.* J Biol Chem, 1999. **274**(8): p. 4934-8.
51. Hundsrucker, C., W. Rosenthal, and E. Klussmann, *Peptides for disruption of PKA anchoring.* Biochem Soc Trans, 2006. **34**(Pt 4): p. 472-3.
52. Hundsrucker, C., et al., *High-affinity AKAP7delta-protein kinase A interaction yields novel protein kinase A-anchoring disruptor peptides.* Biochem J, 2006. **396**(2): p. 297-306.
53. Ruehr, M.L., M.A. Russell, and M. Bond, *A-kinase anchoring protein targeting of protein kinase A in the heart.* J Mol Cell Cardiol, 2004. **37**(3): p. 653-65.
54. Carlson, C.R., et al., *Delineation of type I protein kinase A-selective signaling events using an RI anchoring disruptor.* J Biol Chem, 2006. **281**(30): p. 21535-45.
55. Gold, M.G., et al., *Engineering A-kinase anchoring protein (AKAP)-selective regulatory subunits of protein kinase A (PKA) through structure-based phage selection.* J Biol Chem, 2013. **288**(24): p. 17111-21.
56. Wang, Y., et al., *PKA-type I selective constrained peptide disruptors of AKAP complexes.* ACS Chem Biol, 2015. **10**(6): p. 1502-10.
57. Craik, D.J., et al., *The future of peptide-based drugs.* Chem Biol Drug Des, 2013. **81**(1): p. 136-47.
58. Otvos, L., Jr. and J.D. Wade, *Current challenges in peptide-based drug discovery.* Front Chem, 2014. **2**: p. 62.
59. Torheim, E.A., et al., *Design of proteolytically stable RI-anchoring disruptor peptidomimetics for in vivo studies of anchored type I protein kinase A-mediated signalling.* Biochem J, 2009. **424**(1): p. 69-78.
60. Tasken, K. and A.J. Stokka, *The molecular machinery for cAMP-dependent immunomodulation in T-cells.* Biochem Soc Trans, 2006. **34**(Pt 4): p. 476-9.
61. Singh, M., et al., *The RIAD peptidomimetic inhibits HIV-1 replication in humanized NSG mice.* Eur J Clin Invest, 2013.
62. Flaherty, B.R., et al., *The Stapled AKAP Disruptor Peptide STAD-2 Displays Antimalarial Activity through a PKA-Independent Mechanism.* PLoS One, 2015. **10**(5): p. e0129239.
63. Esseltine, J.L. and J.D. Scott, *AKAP signaling complexes: pointing towards the next generation of therapeutic targets?* Trends Pharmacol Sci, 2013. **34**(12): p. 648-55.
64. Lygren, B., et al., *AKAP complex regulates Ca2+ re-uptake into heart sarcoplasmic reticulum.* EMBO Rep, 2007. **8**(11): p. 1061-7.
65. Hulme, J.T., et al., *Beta-adrenergic regulation requires direct anchoring of PKA to cardiac CaV1.2 channels via a leucine zipper interaction with A kinase-anchoring protein 15.* Proc Natl Acad Sci U S A, 2003. **100**(22): p. 13093-8.
66. Mujic-Delic, A., et al., *GPCR-targeting nanobodies: attractive research tools, diagnostics, and therapeutics.* Trends Pharmacol Sci, 2014. **35**(5): p. 247-55.
67. Zarschler, K., et al., *Diagnostic nanoparticle targeting of the EGF-receptor in complex biological conditions using single-domain antibodies.* Nanoscale, 2014. **6**(11): p. 6046-56.
68. Hamers-Casterman, C., et al., *Naturally occurring antibodies devoid of light chains.* Nature, 1993. **363**(6428): p. 446-8.
69. Harmsen, M.M. and H.J. De Haard, *Properties, production, and applications of camelid single-domain antibody fragments.* Appl Microbiol Biotechnol, 2007. **77**(1): p. 13-22.
70. Holt, L.J., et al., *Domain antibodies: proteins for therapy.* Trends Biotechnol, 2003. **21**(11): p. 484-90.

71. Siontorou, C.G., *Nanobodies as novel agents for disease diagnosis and therapy.* Int J Nanomedicine, 2013. **8**: p. 4215-27.
72. Unciti-Broceta, J.D., et al., *Novel therapy based on camelid nanobodies.* Ther Deliv, 2013. **4**(10): p. 1321-36.
73. Farrington, G.K., et al., *A novel platform for engineering blood-brain barrier-crossing bispecific biologics.* FASEB J, 2014.
74. Van Impe, K., et al., *A nanobody targeting the F-actin capping protein CapG restrains breast cancer metastasis.* Breast Cancer Res, 2013. **15**(6): p. R116.
75. Cooper, D.M. and V.G. Tabbasum, *Adenylate cyclase-centred microdomains.* Biochem J, 2014. **462**(2): p. 199-213.
76. Scott, J.D., C.W. Dessauer, and K. Tasken, *Creating order from chaos: cellular regulation by kinase anchoring.* Annu Rev Pharmacol Toxicol, 2013. **53**: p. 187-210.
77. Antos, C.L., et al., *Dilated cardiomyopathy and sudden death resulting from constitutive activation of protein kinase a.* Circ Res, 2001. **89**(11): p. 997-1004.
78. Zakhary, D.R., et al., *Protein kinase A (PKA)-dependent troponin-I phosphorylation and PKA regulatory subunits are decreased in human dilated cardiomyopathy.* Circulation, 1999. **99**(4): p. 505-10.
79. Weiss, S., et al., *Regulation of cardiac L-type Ca(2)(+) channel CaV1.2 via the beta-adrenergic-cAMP-protein kinase A pathway: old dogmas, advances, and new uncertainties.* Circ Res, 2013. **113**(5): p. 617-31.
80. Gao, T., et al., *cAMP-dependent regulation of cardiac L-type Ca2+ channels requires membrane targeting of PKA and phosphorylation of channel subunits.* Neuron, 1997. **19**(1): p. 185-96.
81. Ganesan, A.N., et al., *Beta-adrenergic stimulation of L-type Ca2+ channels in cardiac myocytes requires the distal carboxyl terminus of alpha1C but not serine 1928.* Circ Res, 2006. **98**(2): p. e11-8.
82. Minobe, E., et al., *A new phosphorylation site in cardiac L-type Ca2+ channels (Cav1.2) responsible for its cAMP-mediated modulation.* Am J Physiol Cell Physiol, 2014. **307**(11): p. C999-C1009.
83. Fu, Y., et al., *Phosphorylation sites required for regulation of cardiac calcium channels in the fight-or-flight response.* Proc Natl Acad Sci U S A, 2013. **110**(48): p. 19621-6.
84. Fuller, M.D., et al., *Molecular mechanism of calcium channel regulation in the fight-or-flight response.* Sci Signal, 2010. **3**(141): p. ra70.
85. Hofmann, F., et al., *L-type CaV1.2 calcium channels: from in vitro findings to in vivo function.* Physiol Rev, 2014. **94**(1): p. 303-26.
86. Pluteanu, F., et al., *Early subcellular Ca2+ remodelling and increased propensity for Ca2+ alternans in left atrial myocytes from hypertensive rats.* Cardiovasc Res, 2015. **106**(1): p. 87-97.
87. Fraser, I.D., et al., *A novel lipid-anchored A-kinase Anchoring Protein facilitates cAMP-responsive membrane events.* EMBO J, 1998. **17**(8): p. 2261-72.
88. Nichols, C.B., et al., *Sympathetic stimulation of adult cardiomyocytes requires association of AKAP5 with a subpopulation of L-type calcium channels.* Circ Res, 2010. **107**(6): p. 747-56.
89. Jones, B.W., et al., *Cardiomyocytes from AKAP7 knockout mice respond normally to adrenergic stimulation.* Proc Natl Acad Sci U S A, 2012. **109**(42): p. 17099-104.
90. Fuller, M.D., et al., *Differential regulation of CaV1.2 channels by cAMP-dependent protein kinase bound to A-kinase anchoring proteins 15 and 79/150.* J Gen Physiol, 2014. **143**(3): p. 315-24.
91. Lin, C., et al., *Cypher/ZASP is a novel A-kinase anchoring protein.* J Biol Chem, 2013. **288**(41): p. 29403-13.
92. Yang, J., et al., *A-kinase anchoring protein 100 (AKAP100) is localized in multiple subcellular compartments in the adult rat heart.* J Cell Biol, 1998. **142**(2): p. 511-22.

93. Marx, S.O., K. Ondrias, and A.R. Marks, *Coupled gating between individual skeletal muscle Ca2+ release channels (ryanodine receptors).* Science, 1998. **281**(5378): p. 818-21.
94. Reiken, S., et al., *Protein kinase A phosphorylation of the cardiac calcium release channel (ryanodine receptor) in normal and failing hearts. Role of phosphatases and response to isoproterenol.* J Biol Chem, 2003. **278**(1): p. 444-53.
95. Marx, S.O., et al., *PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts.* Cell, 2000. **101**(4): p. 365-76.
96. Houser, S.R., *Role of RyR2 phosphorylation in heart failure and arrhythmias: protein kinase A-mediated hyperphosphorylation of the ryanodine receptor at serine 2808 does not alter cardiac contractility or cause heart failure and arrhythmias.* Circ Res, 2014. **114**(8): p. 1320-7; discussion 1327.
97. Kapiloff, M.S., et al., *An adenylyl cyclase-mAKAPbeta signaling complex regulates cAMP levels in cardiac myocytes.* J Biol Chem, 2009. **284**(35): p. 23540-6.
98. Rababa'h, A., et al., *Compartmentalization role of A-kinase anchoring proteins (AKAPs) in mediating protein kinase A (PKA) signaling and cardiomyocyte hypertrophy.* Int J Mol Sci, 2015. **16**(1): p. 218-29.
99. Kapiloff, M.S., N. Jackson, and N. Airhart, *mAKAP and the ryanodine receptor are part of a multi-component signaling complex on the cardiomyocyte nuclear envelope.* J Cell Sci, 2001. **114**(Pt 17): p. 3167-76.
100. Kapiloff, M.S., et al., *mAKAP: an A-kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes.* J Cell Sci, 1999. **112 (Pt 16)**: p. 2725-36.
101. Pare, G.C., et al., *The mAKAP complex participates in the induction of cardiac myocyte hypertrophy by adrenergic receptor signaling.* J Cell Sci, 2005. **118**(Pt 23): p. 5637-46.
102. Passariello, C.L., et al., *mAKAP-a master scaffold for cardiac remodeling.* J Cardiovasc Pharmacol, 2015. **65**(3): p. 218-25.
103. Andersen, J.P. and B. Vilsen, *Structure-function relationships of cation translocation by Ca(2+)- and Na+, K(+)-ATPases studied by site-directed mutagenesis.* FEBS Lett, 1995. **359**(2-3): p. 101-6.
104. Kranias, E.G. and R.J. Hajjar, *Modulation of cardiac contractility by the phospholamban/SERCA2a regulatome.* Circ Res, 2012. **110**(12): p. 1646-60.
105. Diviani, D., et al., *A-kinase anchoring proteins: scaffolding proteins in the heart.* Am J Physiol Heart Circ Physiol, 2011. **301**(5): p. H1742-53.
106. Asahi, M., et al., *Regulation of sarco(endo)plasmic reticulum Ca2+ adenosine triphosphatase by phospholamban and sarcolipin: implication for cardiac hypertrophy and failure.* Trends Cardiovasc Med, 2003. **13**(4): p. 152-7.
107. Schulze, D.H., et al., *Sodium/calcium exchanger (NCX1) macromolecular complex.* J Biol Chem, 2003. **278**(31): p. 28849-55.
108. Marx, S.O., et al., *Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel.* Science, 2002. **295**(5554): p. 496-9.
109. Chen, L., et al., *Mutation of an A-kinase-anchoring protein causes long-QT syndrome.* Proc Natl Acad Sci U S A, 2007. **104**(52): p. 20990-5.
110. Uys, G.M., et al., *Myomegalin is a novel A-kinase anchoring protein involved in the phosphorylation of cardiac myosin binding protein C.* BMC Cell Biol, 2011. **12**: p. 18.
111. Sumandea, C.A., et al., *Cardiac troponin T, a sarcomeric AKAP, tethers protein kinase A at the myofilaments.* J Biol Chem, 2011. **286**(1): p. 530-41.
112. Garvey, J.L., E.G. Kranias, and R.J. Solaro, *Phosphorylation of C-protein, troponin I and phospholamban in isolated rabbit hearts.* Biochem J, 1988. **249**(3): p. 709-14.
113. Rao, V., et al., *PKA phosphorylation of cardiac troponin I modulates activation and relaxation kinetics of ventricular myofibrils.* Biophys J, 2014. **107**(5): p. 1196-204.
114. Zhang, R., et al., *Cardiac troponin I phosphorylation increases the rate of cardiac muscle relaxation.* Circ Res, 1995. **76**(6): p. 1028-35.

115. Sfichi-Duke, L., et al., *Cardiomyopathy-causing deletion K210 in cardiac troponin T alters phosphorylation propensity of sarcomeric proteins.* J Mol Cell Cardiol, 2010. **48**(5): p. 934-42.
116. Lawes, C.M., et al., *Global burden of blood-pressure-related disease, 2001.* Lancet, 2008. **371**(9623): p. 1513-8.
117. Barry, S.P., S.M. Davidson, and P.A. Townsend, *Molecular regulation of cardiac hypertrophy.* Int J Biochem Cell Biol, 2008. **40**(10): p. 2023-39.
118. Chien, K.R., et al., *Transcriptional regulation during cardiac growth and development.* Annu Rev Physiol, 1993. **55**: p. 77-95.
119. Molkentin, J.D., et al., *A calcineurin-dependent transcriptional pathway for cardiac hypertrophy.* Cell, 1998. **93**(2): p. 215-28.
120. Li, J., et al., *The mAKAPbeta scaffold regulates cardiac myocyte hypertrophy via recruitment of activated calcineurin.* J Mol Cell Cardiol, 2010. **48**(2): p. 387-94.
121. Takahashi, N., et al., *Hypertrophic responses to cardiotrophin-1 are not mediated by STAT3, but via a MEK5-ERK5 pathway in cultured cardiomyocytes.* J Mol Cell Cardiol, 2005. **38**(1): p. 185-92.
122. Dodge-Kafka, K.L., et al., *The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways.* Nature, 2005. **437**(7058): p. 574-8.
123. Kritzer, M.D., et al., *The scaffold protein muscle A-kinase anchoring protein beta orchestrates cardiac myocyte hypertrophic signaling required for the development of heart failure.* Circ Heart Fail, 2014. **7**(4): p. 663-72.
124. Carnegie, G.K., et al., *AKAP-Lbc nucleates a protein kinase D activation scaffold.* Mol Cell, 2004. **15**(6): p. 889-99.
125. Murphy, J.G., et al., *AKAP-anchored PKA maintains neuronal L-type calcium channel activity and NFAT transcriptional signaling.* Cell Rep, 2014. **7**(5): p. 1577-88.
126. Diviani, D., L. Baisamy, and A. Appert-Collin, *AKAP-Lbc: a molecular scaffold for the integration of cyclic AMP and Rho transduction pathways.* Eur J Cell Biol, 2006. **85**(7): p. 603-10.
127. Klussmann, E., et al., *Ht31: the first protein kinase A anchoring protein to integrate protein kinase A and Rho signaling.* FEBS Lett, 2001. **507**(3): p. 264-8.
128. Diviani, D., J. Soderling, and J.D. Scott, *AKAP-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation.* J Biol Chem, 2001. **276**(47): p. 44247-57.
129. Mayers, C.M., et al., *The Rho guanine nucleotide exchange factor AKAP13 (BRX) is essential for cardiac development in mice.* J Biol Chem, 2010. **285**(16): p. 12344-54.
130. Spindler, M.J., et al., *AKAP13 Rho-GEF and PKD-binding domain deficient mice develop normally but have an abnormal response to beta-adrenergic-induced cardiac hypertrophy.* PLoS One, 2013. **8**(4): p. e62705.
131. Diviani, D., et al., *Anchoring of both PKA and 14-3-3 inhibits the Rho-GEF activity of the AKAP-Lbc signaling complex.* EMBO J, 2004. **23**(14): p. 2811-20.
132. Appert-Collin, A., et al., *The A-kinase anchoring protein (AKAP)-Lbc-signaling complex mediates alpha1 adrenergic receptor-induced cardiomyocyte hypertrophy.* Proc Natl Acad Sci U S A, 2007. **104**(24): p. 10140-5.
133. Carnegie, G.K., et al., *AKAP-Lbc mobilizes a cardiac hypertrophy signaling pathway.* Mol Cell, 2008. **32**(2): p. 169-79.
134. Grozinger, C.M. and S.L. Schreiber, *Regulation of histone deacetylase 4 and 5 and transcriptional activity by 14-3-3-dependent cellular localization.* Proc Natl Acad Sci U S A, 2000. **97**(14): p. 7835-40.
135. Vega, R.B., et al., *Protein kinases C and D mediate agonist-dependent cardiac hypertrophy through nuclear export of histone deacetylase 5.* Mol Cell Biol, 2004. **24**(19): p. 8374-85.
136. Chang, S., et al., *An expression screen reveals modulators of class II histone deacetylase phosphorylation.* Proc Natl Acad Sci U S A, 2005. **102**(23): p. 8120-5.
137. Burmeister, B.T., et al., *Protein kinase A (PKA) phosphorylation of Shp2 inhibits its phosphatase activity and modulates ligand specificity.* J Biol Chem, 2015.

138. Burmeister, B.T., et al., *Src homology 2 domain-containing phosphatase 2 (Shp2) is a component of the A-kinase-anchoring protein (AKAP)-Lbc complex and is inhibited by protein kinase A (PKA) under pathological hypertrophic conditions in the heart.* J Biol Chem, 2012. **287**(48): p. 40535-46.
139. Tartaglia, M., et al., *Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome.* Nat Genet, 2001. **29**(4): p. 465-8.
140. Kontaridis, M.I., et al., *Deletion of Ptpn11 (Shp2) in cardiomyocytes causes dilated cardiomyopathy via effects on the extracellular signal-regulated kinase/mitogen-activated protein kinase and RhoA signaling pathways.* Circulation, 2008. **117**(11): p. 1423-35.
141. Hanna, N., et al., *Reduced phosphatase activity of SHP-2 in LEOPARD syndrome: consequences for PI3K binding on Gab1.* FEBS Lett, 2006. **580**(10): p. 2477-82.
142. Edouard, T., et al., *Functional effects of PTPN11 (SHP2) mutations causing LEOPARD syndrome on epidermal growth factor-induced phosphoinositide 3-kinase/AKT/glycogen synthase kinase 3beta signaling.* Mol Cell Biol, 2010. **30**(10): p. 2498-507.
143. Schramm, C., et al., *The PTPN11 loss-of-function mutation Q510E-Shp2 causes hypertrophic cardiomyopathy by dysregulating mTOR signaling.* Am J Physiol Heart Circ Physiol, 2012. **302**(1): p. H231-43.
144. Schramm, C., M.A. Edwards, and M. Krenz, *New approaches to prevent LEOPARD syndrome-associated cardiac hypertrophy by specifically targeting Shp2-dependent signaling.* J Biol Chem, 2013. **288**(25): p. 18335-44.
145. Edwards, H.V., J.D. Scott, and G.S. Baillie, *The A-kinase-anchoring protein AKAP-Lbc facilitates cardioprotective PKA phosphorylation of Hsp20 on Ser(16).* Biochem J, 2012. **446**(3): p. 437-43.
146. Fan, G.C. and E.G. Kranias, *Small heat shock protein 20 (HspB6) in cardiac hypertrophy and failure.* J Mol Cell Cardiol, 2011. **51**(4): p. 574-7.
147. Wang, X., et al., *Overexpression of Hsp20 prevents endotoxin-induced myocardial dysfunction and apoptosis via inhibition of NF-kappaB activation.* J Mol Cell Cardiol, 2009. **47**(3): p. 382-90.
148. Fan, G.C., et al., *Novel cardioprotective role of a small heat-shock protein, Hsp20, against ischemia/reperfusion injury.* Circulation, 2005. **111**(14): p. 1792-9.
149. Fan, G.C., et al., *Small heat-shock protein Hsp20 phosphorylation inhibits beta-agonist-induced cardiac apoptosis.* Circ Res, 2004. **94**(11): p. 1474-82.
150. Martin, T.P., et al., *Targeted disruption of the heat shock protein 20-phosphodiesterase 4D (PDE4D) interaction protects against pathological cardiac remodelling in a mouse model of hypertrophy.* FEBS Open Bio, 2014. **4**: p. 923-7.
151. Sin, Y.Y., et al., *Disruption of the cyclic AMP phosphodiesterase-4 (PDE4)-HSP20 complex attenuates the beta-agonist induced hypertrophic response in cardiac myocytes.* J Mol Cell Cardiol, 2011. **50**(5): p. 872-83.
152. Cavin, S., D. Maric, and D. Diviani, *A-kinase anchoring protein-Lbc promotes pro-fibrotic signaling in cardiac fibroblasts.* Biochim Biophys Acta, 2014. **1843**(2): p. 335-45.
153. Kritzer, M.D., et al., *AKAPs: The architectural underpinnings of local cAMP signaling.* Journal of molecular and cellular cardiology, 2012. **52**(2): p. 351-8.
154. Aye, T.T., et al., *Reorganized PKA-AKAP associations in the failing human heart.* J Mol Cell Cardiol, 2012. **52**(2): p. 511-8.
155. Schwinger, R.H., et al., *Reduced Ca(2+)-sensitivity of SERCA 2a in failing human myocardium due to reduced serin-16 phospholamban phosphorylation.* J Mol Cell Cardiol, 1999. **31**(3): p. 479-91.
156. Zakhary, D.R., C.S. Moravec, and M. Bond, *Regulation of PKA binding to AKAPs in the heart: alterations in human heart failure.* Circulation, 2000. **101**(12): p. 1459-64.
157. Manni, S., et al., *Phosphorylation of the cAMP-dependent protein kinase (PKA) regulatory subunit modulates PKA-AKAP interaction, substrate phosphorylation, and calcium signaling in cardiac cells.* J Biol Chem, 2008. **283**(35): p. 24145-54.

158. Mika, D., et al., *Differential regulation of cardiac excitation-contraction coupling by cAMP phosphodiesterase subtypes.* Cardiovasc Res, 2013. **100**(2): p. 336-46.
159. Lomas, O. and M. Zaccolo, *Phosphodiesterases maintain signaling fidelity via compartmentalization of cyclic nucleotides.* Physiology (Bethesda), 2014. **29**(2): p. 141-9.
160. Edwards, H.V., F. Christian, and G.S. Baillie, *cAMP: novel concepts in compartmentalised signalling.* Semin Cell Dev Biol, 2012. **23**(2): p. 181-90.
161. Lehnart, S.E., et al., *Phosphodiesterase 4D deficiency in the ryanodine-receptor complex promotes heart failure and arrhythmias.* Cell, 2005. **123**(1): p. 25-35.
162. Dodge-Kafka, K.L., et al., *cAMP-stimulated protein phosphatase 2A activity associated with muscle A kinase-anchoring protein (mAKAP) signaling complexes inhibits the phosphorylation and activity of the cAMP-specific phosphodiesterase PDE4D3.* J Biol Chem, 2010. **285**(15): p. 11078-86.
163. Sette, C. and M. Conti, *Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation.* J Biol Chem, 1996. **271**(28): p. 16526-34.
164. Hoffmann, R., et al., *The MAP kinase ERK2 inhibits the cyclic AMP-specific phosphodiesterase HSPDE4D3 by phosphorylating it at Ser579.* EMBO J, 1999. **18**(4): p. 893-903.
165. Terrenoire, C., et al., *The cardiac IKs potassium channel macromolecular complex includes the phosphodiesterase PDE4D3.* J Biol Chem, 2009. **284**(14): p. 9140-6.
166. Hambleton, R., et al., *Isoforms of cyclic nucleotide phosphodiesterase PDE3 and their contribution to cAMP hydrolytic activity in subcellular fractions of human myocardium.* The Journal of biological chemistry, 2005. **280**(47): p. 39168-74.
167. Ahmad, F., et al., *Regulation of Sarcoplasmic Reticulum Ca2+ ATPase 2 (SERCA2) Activity by Phosphodiesterase 3A (PDE3A) in Human Myocardium: PHOSPHORYLATION-DEPENDENT INTERACTION OF PDE3A1 WITH SERCA2.* J Biol Chem, 2015. **290**(11): p. 6763-76.
168. Beca, S., et al., *Phosphodiesterase type 3A regulates basal myocardial contractility through interacting with sarcoplasmic reticulum calcium ATPase type 2a signaling complexes in mouse heart.* Circ Res, 2013. **112**(2): p. 289-97.
169. Schultz Jel, J., et al., *Accelerated onset of heart failure in mice during pressure overload with chronically decreased SERCA2 calcium pump activity.* Am J Physiol Heart Circ Physiol, 2004. **286**(3): p. H1146-53.
170. Shareef, M.A., L.A. Anwer, and C. Poizat, *Cardiac SERCA2A/B: therapeutic targets for heart failure.* Eur J Pharmacol, 2014. **724**: p. 1-8.
171. Janczewski, A.M. and E.G. Lakatta, *Modulation of sarcoplasmic reticulum Ca(2+) cycling in systolic and diastolic heart failure associated with aging.* Heart Fail Rev, 2010. **15**(5): p. 431-45.
172. Arai, M., et al., *Alterations in sarcoplasmic reticulum gene expression in human heart failure. A possible mechanism for alterations in systolic and diastolic properties of the failing myocardium.* Circ Res, 1993. **72**(2): p. 463-9.
173. del Monte, F., et al., *Targeting phospholamban by gene transfer in human heart failure.* Circulation, 2002. **105**(8): p. 904-7.
174. Maass, P.G., et al., *PDE3A mutations cause autosomal dominant hypertension with brachydactyly.* Nat Genet, 2015.
175. Toka, O., et al., *Clinical Effects of Phosphodiesterase 3A Mutations in Inherited Hypertension With Brachydactyly.* Hypertension, 2015.
176. Sprenger, J.U., et al., *In vivo model with targeted cAMP biosensor reveals changes in receptor-microdomain communication in cardiac disease.* Nat Commun, 2015. **6**: p. 6965.
177. Stefan, E., et al., *Compartmentalization of cAMP-dependent signaling by phosphodiesterase-4D is involved in the regulation of vasopressin-mediated water reabsorption in renal principal cells.* J Am Soc Nephrol, 2007. **18**(1): p. 199-212.

178. Fink, M.A., et al., *AKAP-mediated targeting of protein kinase a regulates contractility in cardiac myocytes.* Circ Res, 2001. **88**(3): p. 291-7.
179. Patel, H.H., et al., *Disruption of protein kinase A localization using a trans-activator of transcription (TAT)-conjugated A-kinase-anchoring peptide reduces cardiac function.* J Biol Chem, 2010. **285**(36): p. 27632-40.
180. Braunwald, E., *The war against heart failure: the Lancet lecture.* Lancet, 2015. **385**(9970): p. 812-24.
181. Jessup, M., et al., *Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID): a phase 2 trial of intracoronary gene therapy of sarcoplasmic reticulum Ca2+-ATPase in patients with advanced heart failure.* Circulation, 2011. **124**(3): p. 304-13.
182. Amsallem, E., et al., *Phosphodiesterase III inhibitors for heart failure.* Cochrane Database Syst Rev, 2005(1): p. CD002230.
183. Society, A.C., *Global cancer facts and figures.* 2015.
184. Uhlen, M., et al., *A human protein atlas for normal and cancer tissues based on antibody proteomics.* Mol Cell Proteomics, 2005. **4**(12): p. 1920-32.
185. Uhlen, M., et al., *Proteomics. Tissue-based map of the human proteome.* Science, 2015. **347**(6220): p. 1260419.
186. Diviani, D. and J.D. Scott, *AKAP signaling complexes at the cytoskeleton.* J Cell Sci, 2001. **114**(Pt 8): p. 1431-7.
187. Frank, B., et al., *Association of a common AKAP9 variant with breast cancer risk: a collaborative analysis.* J Natl Cancer Inst, 2008. **100**(6): p. 437-42.
188. Hedrick, E.D., et al., *Differential PKA activation and AKAP association determines cell fate in cancer cells.* J Mol Signal, 2013. **8**(1): p. 10.
189. Song, M.H., et al., *Identification of the cancer/testis antigens AKAP3 and CTp11 by SEREX in hepatocellular carcinoma.* Oncol Rep, 2012. **28**(5): p. 1792-8.
190. Smith, F.D., et al., *AKAP-Lbc enhances cyclic AMP control of the ERK1/2 cascade.* Nat Cell Biol, 2010. **12**(12): p. 1242-9.
191. Wirtenberger, M., et al., *Association of genetic variants in the Rho guanine nucleotide exchange factor AKAP13 with familial breast cancer.* Carcinogenesis, 2006. **27**(3): p. 593-8.
192. Abdul Azeez, K.R., et al., *The Crystal Structure of the RhoA : AKAP-Lbc DH-PH Domain Complex.* Biochem J, 2014.
193. Rogers, R., et al., *Mechanical homeostasis is altered in uterine leiomyoma.* Am J Obstet Gynecol, 2008. **198**(4): p. 474 e1-11.
194. Lenoir, M., et al., *Structural Insights into the Activation of the RhoA GTPase by the Lbc Oncoprotein.* J Biol Chem, 2014.
195. Sterpetti, P., et al., *Activation of the Lbc Rho exchange factor proto-oncogene by truncation of an extended C terminus that regulates transformation and targeting.* Mol Cell Biol, 1999. **19**(2): p. 1334-45.
196. Karachaliou, N., et al., *Predicting resistance by selection of signaling pathways.* Transl Lung Cancer Res, 2014. **3**(2): p. 107-15.
197. Yang, M.H., et al., *MALAT1 promotes colorectal cancer cell proliferation/migration/invasion via PRKA kinase anchor protein 9.* Biochim Biophys Acta, 2015. **1852**(1): p. 166-74.
198. Milne, R.L., et al., *Common non-synonymous SNPs associated with breast cancer susceptibility: findings from the Breast Cancer Association Consortium.* Hum Mol Genet, 2014. **23**(22): p. 6096-111.
199. Mattaloni, S.M., et al., *Centrosomal AKAP350 modulates the G/S transition.* Cell Logist, 2013. **3**(1): p. e26331.
200. Carlson, C.R., et al., *CDK1-mediated phosphorylation of the RIIalpha regulatory subunit of PKA works as a molecular switch that promotes dissociation of RIIalpha from centrosomes at mitosis.* J Cell Sci, 2001. **114**(Pt 18): p. 3243-54.

201. Akakura, S., et al., *Rb-dependent cellular senescence, multinucleation and susceptibility to oncogenic transformation through PKC scaffolding by SSeCKS/AKAP12.* Cell Cycle, 2010. **9**(23): p. 4656-65.
202. Gelman, I.H., *Emerging Roles for SSeCKS/Gravin/AKAP12 in the Control of Cell Proliferation, Cancer Malignancy, and Barriergenesis.* Genes Cancer, 2010. **1**(11): p. 1147-56.
203. Akakura, S. and I.H. Gelman, *Pivotal Role of AKAP12 in the Regulation of Cellular Adhesion Dynamics: Control of Cytoskeletal Architecture, Cell Migration, and Mitogenic Signaling.* J Signal Transduct, 2012. **2012**: p. 529179.
204. Hayashi, M., et al., *Identification of the A kinase anchor protein 12 (AKAP12) gene as a candidate tumor suppressor of hepatocellular carcinoma.* J Surg Oncol, 2012. **105**(4): p. 381-6.
205. Kuijjer, M.L., et al., *Identification of osteosarcoma driver genes by integrative analysis of copy number and gene expression data.* Genes Chromosomes Cancer, 2012. **51**(7): p. 696-706.
206. Kim, D.H., et al., *A genome-wide association study identifies novel loci associated with susceptibility to chronic myeloid leukemia.* Blood, 2011. **117**(25): p. 6906-11.
207. Su, B., et al., *Adhesion-mediated cytoskeletal remodeling is controlled by the direct scaffolding of Src from FAK complexes to lipid rafts by SSeCKS/AKAP12.* Oncogene, 2013. **32**(16): p. 2016-26.
208. Finger, E.C., et al., *Hypoxic induction of AKAP12 variant 2 shifts PKA-mediated protein phosphorylation to enhance migration and metastasis of melanoma cells.* Proc Natl Acad Sci U S A, 2015. **112**(14): p. 4441-6.
209. Mehlen, P. and C. Furne, *Netrin-1: when a neuronal guidance cue turns out to be a regulator of tumorigenesis.* Cell Mol Life Sci, 2005. **62**(22): p. 2599-616.
210. Arakawa, H., *Netrin-1 and its receptors in tumorigenesis.* Nat Rev Cancer, 2004. **4**(12): p. 978-87.
211. Ko, S.Y., C.R. Dass, and K. Nurgali, *Netrin-1 in the developing enteric nervous system and colorectal cancer.* Trends Mol Med, 2012. **18**(9): p. 544-54.
212. Corset, V., et al., *Netrin-1-mediated axon outgrowth and cAMP production requires interaction with adenosine A2b receptor.* Nature, 2000. **407**(6805): p. 747-50.
213. Deming, P.B., et al., *Anchoring of Protein Kinase A by ERM (Ezrin-Radixin-Moesin) Proteins Is Required for Proper Netrin Signaling through DCC (Deleted in Colorectal Cancer).* J Biol Chem, 2015. **290**(9): p. 5783-96.
214. Machesky, L.M., *Lamellipodia and filopodia in metastasis and invasion.* FEBS Lett, 2008. **582**(14): p. 2102-11.
215. Moore, S.W. and T.E. Kennedy, *Protein kinase A regulates the sensitivity of spinal commissural axon turning to netrin-1 but does not switch between chemoattraction and chemorepulsion.* J Neurosci, 2006. **26**(9): p. 2419-23.
216. Nauert, J.B., J.D. Rigas, and L.B. Lester, *Identification of an IQGAP1/AKAP79 complex in beta-cells.* J Cell Biochem, 2003. **90**(1): p. 97-108.
217. Liu, Z., et al., *IQGAP1 plays an important role in the invasiveness of thyroid cancer.* Clin Cancer Res, 2010. **16**(24): p. 6009-18.
218. White, C.D., M.D. Brown, and D.B. Sacks, *IQGAPs in cancer: a family of scaffold proteins underlying tumorigenesis.* FEBS Lett, 2009. **583**(12): p. 1817-24.
219. Xie, Y., et al., *IQGAP2, A candidate tumour suppressor of prostate tumorigenesis.* Biochim Biophys Acta, 2012. **1822**(6): p. 875-84.
220. Logue, J.S., et al., *Anchored protein kinase A recruitment of active Rac GTPase.* J Biol Chem, 2011. **286**(25): p. 22113-21.
221. Logue, J.S., et al., *AKAP220 protein organizes signaling elements that impact cell migration.* J Biol Chem, 2011. **286**(45): p. 39269-81.
222. Logue, J.S., J.L. Whiting, and J.D. Scott, *Sequestering Rac with PKA confers cAMP control of cytoskeletal remodeling.* Small GTPases, 2011. **2**(3): p. 173-176.
223. Jiang, W.G., *Membrane ruffling of cancer cells: a parameter of tumour cell motility and invasion.* Eur J Surg Oncol, 1995. **21**(3): p. 307-9.

224. Turner, R.M., et al., *Molecular evaluation of two major human sperm fibrous sheath proteins, pro-hAKAP82 and hAKAP82, in stump tail sperm.* Fertil Steril, 2001. **76**(2): p. 267-74.
225. Miki, K., et al., *Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility.* Dev Biol, 2002. **248**(2): p. 331-42.
226. Chiriva-Internati, M., et al., *AKAP-4: a novel cancer testis antigen for multiple myeloma.* Br J Haematol, 2008. **140**(4): p. 465-8.
227. Gumireddy, K., et al., *AKAP4 is a circulating biomarker for non-small cell lung cancer.* Oncotarget, 2015. **6**(19): p. 17637-47.
228. Agarwal, S., et al., *The novel cancer-testis antigen A-kinase anchor protein 4 (AKAP4) is a potential target for immunotherapy of ovarian serous carcinoma.* Oncoimmunology, 2013. **2**(5): p. e24270.
229. Suri, A., et al., *Cancer testis antigens: A new paradigm for cancer therapy.* Oncoimmunology, 2012. **1**(7): p. 1194-1196.
230. Chiriva-Internati, M., et al., *Identification of AKAP-4 as a new cancer/testis antigen for detection and immunotherapy of prostate cancer.* Prostate, 2012. **72**(1): p. 12-23.
231. Gumireddy, K., et al., *AKAP4 is a circulating biomarker for non-small cell lung cancer.* Oncotarget, 2015.
232. Mirandola, L., et al., *Novel antigens in non-small cell lung cancer: SP17, AKAP4, and PTTG1 are potential immunotherapeutic targets.* Oncotarget, 2015. **6**(5): p. 2812-26.
233. Pollard, T.D. and J.A. Cooper, *Actin, a central player in cell shape and movement.* Science, 2009. **326**(5957): p. 1208-12.
234. Case, L.B. and C.M. Waterman, *Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch.* Nat Cell Biol, 2015.
235. McCubrey, J.A., et al., *GSK-3 as potential target for therapeutic intervention in cancer.* Oncotarget, 2014. **5**(10): p. 2881-911.
236. McKenzie, A.J., S.L. Campbell, and A.K. Howe, *Protein kinase A activity and anchoring are required for ovarian cancer cell migration and invasion.* PLoS One, 2011. **6**(10): p. e26552.
237. Chowdhury, S., et al., *Identification of a novel TGFbeta/PKA signaling transduceome in mediating control of cell survival and metastasis in colon cancer.* PLoS One, 2011. **6**(5): p. e19335.
238. Lignitto, L., et al., *Control of PKA stability and signalling by the RING ligase praja2.* Nat Cell Biol, 2011. **13**(4): p. 412-22.
239. Saini, S., et al., *Gene silencing of A-kinase anchor protein 4 inhibits cervical cancer growth in vitro and in vivo.* Cancer Gene Ther, 2013. **20**(7): p. 413-20.
240. Schouten, L.J., et al., *Incidence of brain metastases in a cohort of patients with carcinoma of the breast, colon, kidney, and lung and melanoma.* Cancer, 2002. **94**(10): p. 2698-705.
241. Arkin, Michelle R., Y. Tang, and James A. Wells, *Small-Molecule Inhibitors of Protein-Protein Interactions: Progressing toward the Reality.* Chemistry & Biology, 2014. **21**(9): p. 1102-1114.
242. Laraia, L., et al., *Overcoming Chemical, Biological, and Computational Challenges in the Development of Inhibitors Targeting Protein-Protein Interactions.* Chem Biol, 2015. **22**(6): p. 689-703.
243. Huang, L.J., et al., *Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits.* J Biol Chem, 1997. **272**(12): p. 8057-64.
244. Dong, F., et al., *Molecular characterization of a cDNA that encodes six isoforms of a novel murine A kinase anchor protein.* J Biol Chem, 1998. **273**(11): p. 6533-41.
245. Xu, K. and H. Qi, *Sperm-specific AKAP3 is a dual-specificity anchoring protein that interacts with both protein kinase a regulatory subunits via conserved N-terminal amphipathic peptides.* Mol Reprod Dev, 2014. **81**(7): p. 595-607.

246. Miki, K. and E.M. Eddy, *Identification of tethering domains for protein kinase A type Ialpha regulatory subunits on sperm fibrous sheath protein FSC1.* J Biol Chem, 1998. **273**(51): p. 34384-90.
247. Herberg, F.W., et al., *Analysis of A-kinase anchoring protein (AKAP) interaction with protein kinase A (PKA) regulatory subunits: PKA isoform specificity in AKAP binding.* J Mol Biol, 2000. **298**(2): p. 329-39.
248. Leiser, M., C.S. Rubin, and J. Erlichman, *Differential binding of the regulatory subunits (RII) of cAMP-dependent protein kinase II from bovine brain and muscle to RII-binding proteins.* J Biol Chem, 1986. **261**(4): p. 1904-8.
249. McCartney, S., et al., *Cloning and characterization of A-kinase anchor protein 100 (AKAP100). A protein that targets A-kinase to the sarcoplasmic reticulum.* J Biol Chem, 1995. **270**(16): p. 9327-33.
250. Henn, V., et al., *Identification of a novel A-kinase anchoring protein 18 isoform and evidence for its role in the vasopressin-induced aquaporin-2 shuttle in renal principal cells.* J Biol Chem, 2004. **279**(25): p. 26654-65.
251. Witczak, O., et al., *Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450.* EMBO J, 1999. **18**(7): p. 1858-68.
252. Huang, L.J., et al., *D-AKAP2, a novel protein kinase A anchoring protein with a putative RGS domain.* Proc Natl Acad Sci U S A, 1997. **94**(21): p. 11184-9.
253. Day, M.E., et al., *Isoform-specific targeting of PKA to multivesicular bodies.* J Cell Biol, 2011. **193**(2): p. 347-63.
254. Nauert, J.B., et al., *Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein.* Curr Biol, 1997. **7**(1): p. 52-62.
255. Miki, K. and E.M. Eddy, *Single amino acids determine specificity of binding of protein kinase A regulatory subunits by protein kinase A anchoring proteins.* J Biol Chem, 1999. **274**(41): p. 29057-62.
256. Kultgen, P.L., et al., *Characterization of an A-kinase anchoring protein in human ciliary axonemes.* Mol Biol Cell, 2002. **13**(12): p. 4156-66.
257. Hundsrucker, C., et al., *Glycogen synthase kinase 3beta interaction protein functions as an A-kinase anchoring protein.* J Biol Chem, 2010. **285**(8): p. 5507-21.
258. Dransfield, D.T., et al., *Ezrin is a cyclic AMP-dependent protein kinase anchoring protein.* EMBO J, 1997. **16**(1): p. 35-43.
259. Burgers, P.P., et al., *A small novel A-kinase anchoring protein (AKAP) that localizes specifically protein kinase A-regulatory subunit I (PKA-RI) to the plasma membrane.* J Biol Chem, 2012. **287**(52): p. 43789-97.
260. Kovanich, D., et al., *Sphingosine kinase interacting protein is an A-kinase anchoring protein specific for type I cAMP-dependent protein kinase.* Chembiochem, 2010. **11**(7): p. 963-71.
261. Means, C.K., et al., *An entirely specific type I A-kinase anchoring protein that can sequester two molecules of protein kinase A at mitochondria.* Proc Natl Acad Sci U S A, 2011. **108**(48): p. E1227-35.
262. Jarnaess, E., et al., *Dual specificity A-kinase anchoring proteins (AKAPs) contain an additional binding region that enhances targeting of protein kinase A type I.* J Biol Chem, 2008. **283**(48): p. 33708-18.
263. Angelo, R. and C.S. Rubin, *Molecular characterization of an anchor protein (AKAPCE) that binds the RI subunit (RCE) of type I protein kinase A from Caenorhabditis elegans.* J Biol Chem, 1998. **273**(23): p. 14633-43.
264. Salvador, L.M., et al., *Neuronal microtubule-associated protein 2D is a dual a-kinase anchoring protein expressed in rat ovarian granulosa cells.* J Biol Chem, 2004. **279**(26): p. 27621-32.

Figure 1

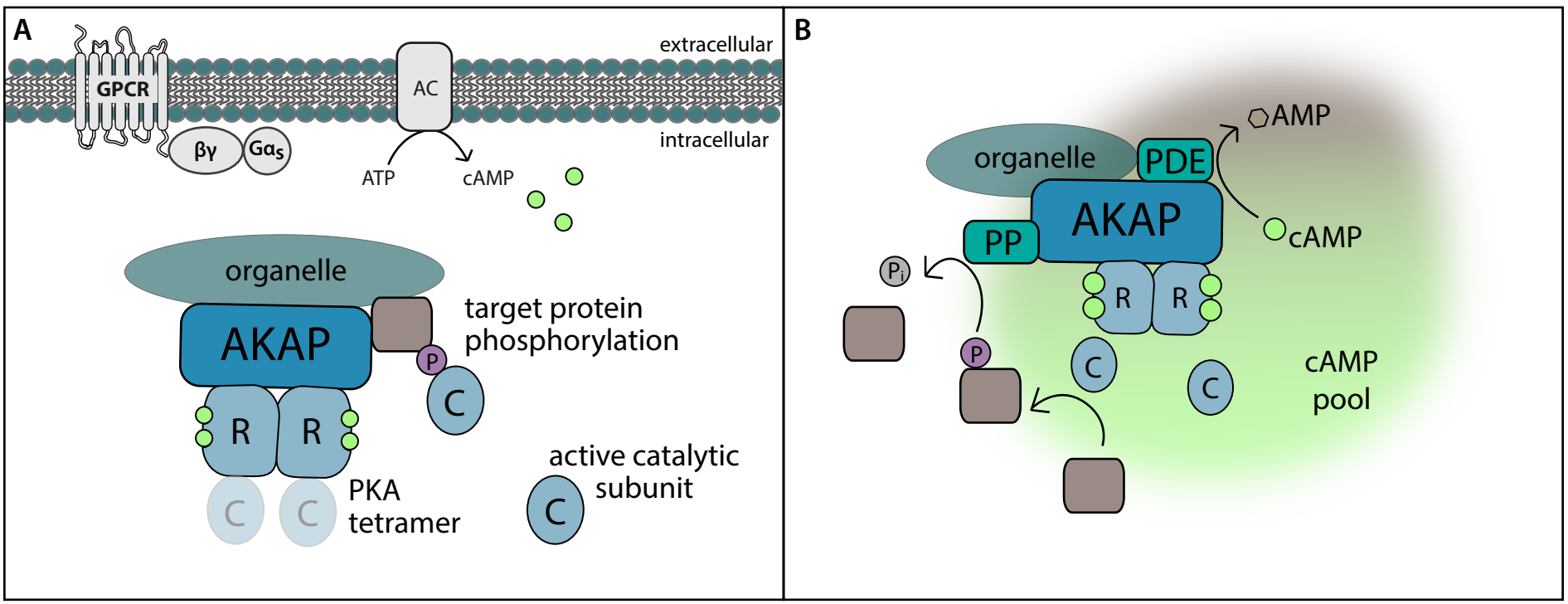
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Figure 2

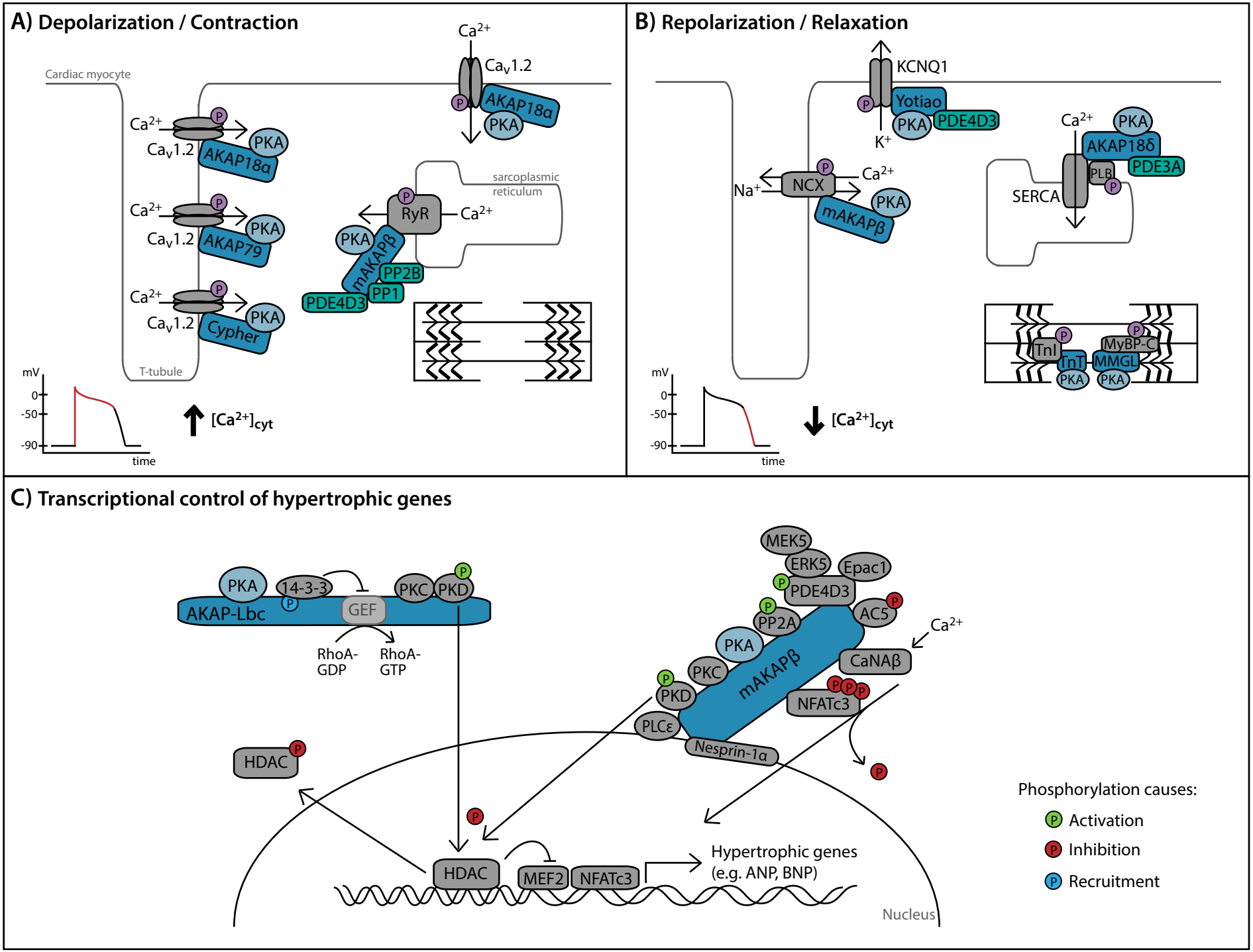
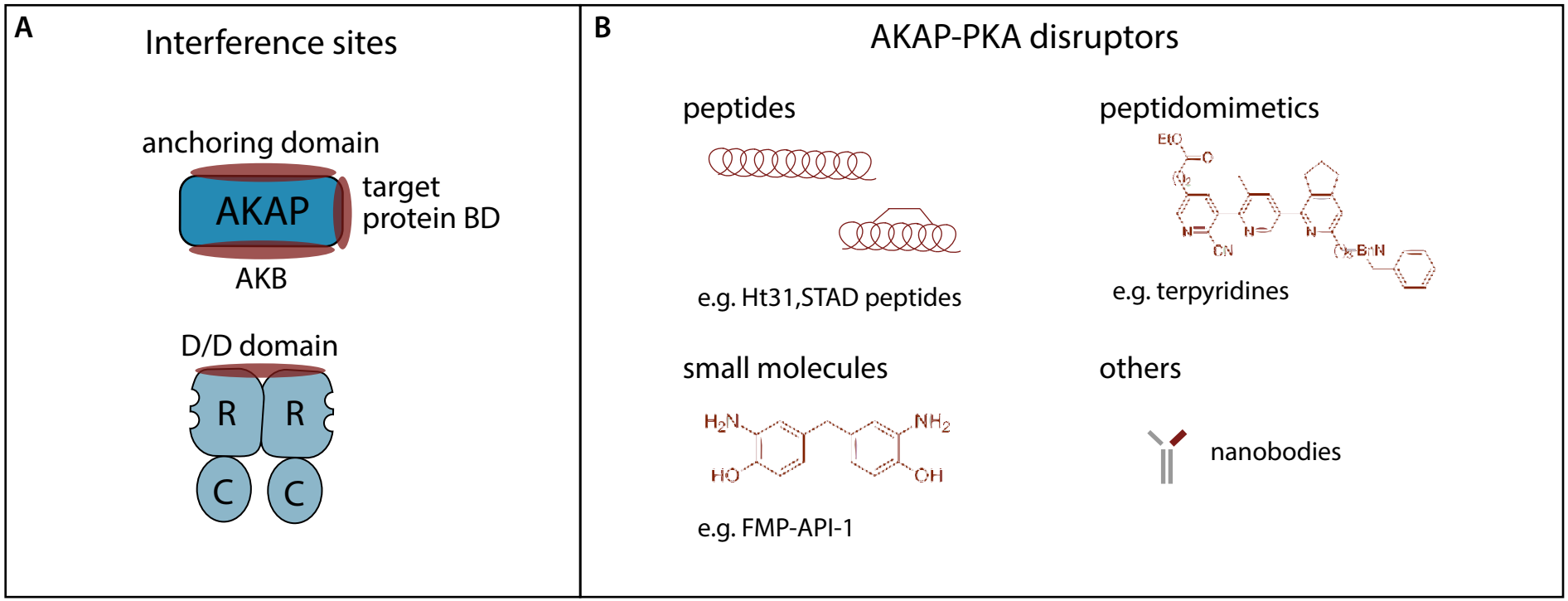

A) Depolarization / Contraction
Cardiac myocyte
T-tubule
sarcoplasmic reticulum
Ca2+
CaV1.2
AKAP18α
AKAP79
Cypher
PKA
RyR
mAKAPβ
PP2B
PP1
PDE4D3
mV
0
-50
-90
time
[Ca2+]cyt
B) Repolarization / Relaxation
KCNQ1
Yotiao
PDE4D3
K+
Na+
NCX
mAKAPβ
SERCA
AKAP18δ
PDE3A
PLB
TnI
TnT
MyBP-C
MMGL
C) Transcriptional control of hypertrophic genes
AKAP-Lbc
14-3-3
GEF
PKC
PKD
RhoA-GDP
RhoA-GTP
HDAC
MEK5
ERK5
Epac1
PDE4D3
PP2A
AC5
CaNAβ
NFATc3
PLCε
Nesprin-1α
MEF2
Hypertrophic genes
(e.g. ANP, BNP)
Nucleus
Phosphorylation causes:
Activation
Inhibition
Recruitment

Figure 3


A
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AKAP
target
protein BD
AKB
D/D domain
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B
AKAP-PKA disruptors
peptides
e.g. Ht31,STAD peptides
peptidomimetics
e.g. terpyridines
small molecules
e.g. FMP-API-1
others
nanobodies