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Dema, A., Perets, E., Schulz, M.S., Deak, V.A., Klussmann, E.

Pharmacological targeting of
AKAP-directed compartmentalized cAMP signalling

Alessandro Dema[1,+], Ekaterina Perets[1,+], Maike Svenja Schulz[1,+], Veronika Anita Deàk[1] and Enno Klussmann[1,2,*]

[+], contributed equally

Affiliations

[1]Max Delbrück Center for Molecular Medicine Berlin in the Helmholtz Association (MDC), Robert-Rössle-Straße 10, 13125 Berlin, Germany,

[2]DZHK, German Centre for Cardiovascular Research, Oudenarder Straße 16,13347 Berlin, Germany

Short title: Targeting AKAPs

Keywords: A-kinase anchoring protein; protein kinase A; protein-protein interaction; cardio-vascular; cancer

[*]**Address for correspondence**

Enno Klussmann

Max Delbrück Center for Molecular Medicine Berlin

in the Helmholtz Association (MDC)

Robert-Rössle-Str. 10, 13125 Berlin

Germany

Tel.   +49-30-9406 2596

FAX   +49-30-9406 2593

E-mail: enno.klussmann@mdc-berlin.de

Acknowledgements

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 mito-chondria in leukaemia cells where it regulates apoptosis [18]. Another example is Optic Atro-phy 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 suggest-ed 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 subu-nits 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 intro-duced 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 hip-pocampus 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 nanomo-lar 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 nano-bodies 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 intra-body 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 pro-tein-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 frac-tion of the Ca^{2+} exits the cells, the larger part is pumped back into the SR. More than 10 dif-ferent 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 con-tractility), 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 nega-tive 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 over-load 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. Pre-clinical 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 up-regulated 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].

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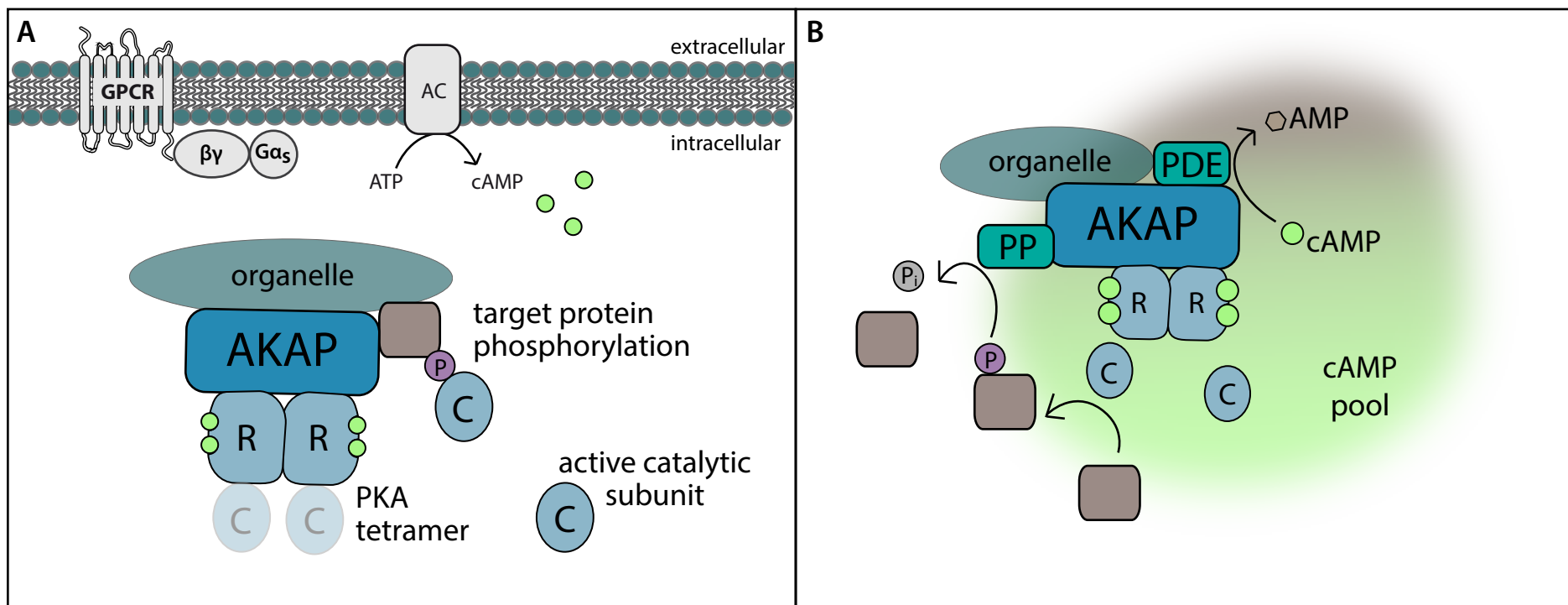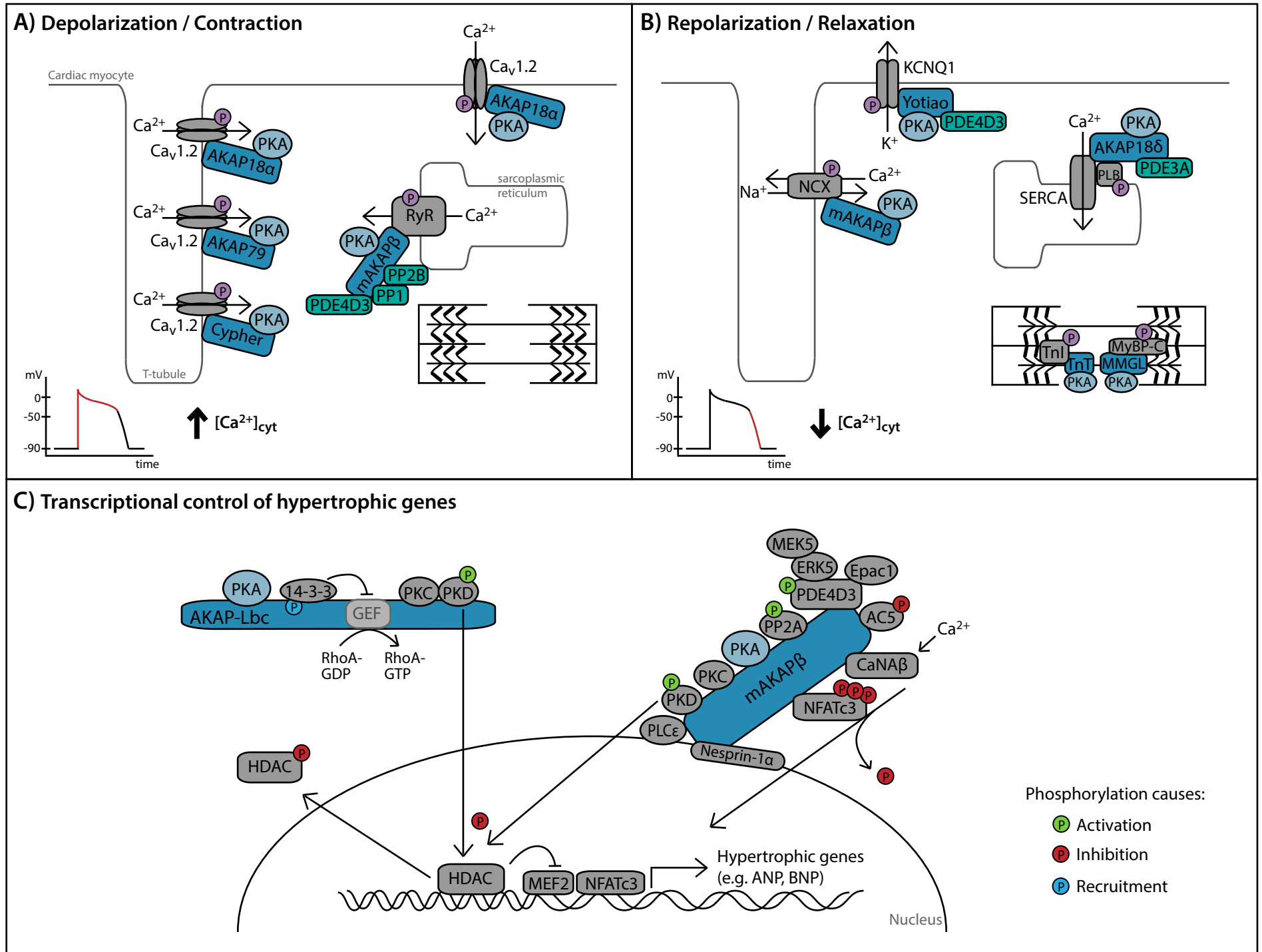
Figure 1

Figure 2

Figure 3

A   Interference sites

anchoring domain

AKAP

target
protein BD

AKB

D/D domain

R   R

C   C

B   AKAP-PKA disruptors

peptides

e.g. Ht31,STAD peptides

peptidomimetics

e.g. terpyridines

small molecules

H$_2$N   NH$_2$

HO   OH

e.g. FMP-API-1

others

nanobodies