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Pharmacological interference with protein-protein interactions of A-kinase anchoring proteins as a strategy for the treatment of disease

Veronika A. Deák[1], Enno Klussmann[1,2*]

Affiliation

[1]Max Delbrueck Center for Molecular Medicine Berlin (MDC), Germany

[2]DZHK, German Centre for Cardiovascular Research, Berlin, Germany

*Address for correspondence

Enno Klussmann

Max Delbrueck Center for Molecular Medicine (MDC) Berlin-Buch

Robert-Roessle-Str. 10, 13125 Berlin

Germany

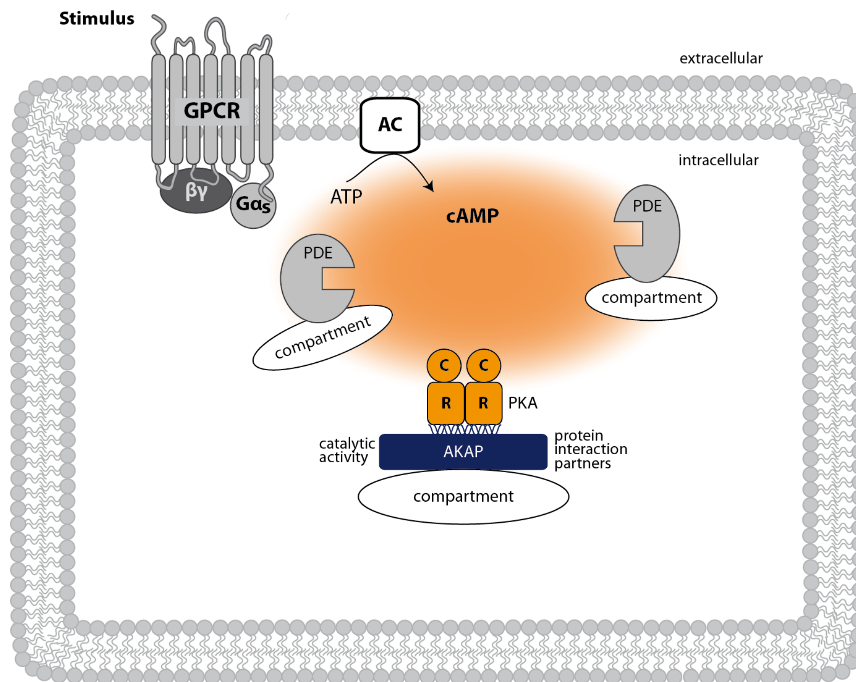
Tel. +49-30-9406 2596

FAX +49-30-9406 2593

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

Running title: AKAPs as potential drug targets

Graphical abstract



Keywords

AKAP; protein-protein interaction; compartmentalized cAMP signaling; peptide; peptidomimetics; PKA; small molecules

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Abstract

A-kinase anchoring proteins (AKAPs) control the localization of cAMP-dependent protein kinase A (PKA) by tethering PKA to distinct cellular compartments. Through additional direct protein-protein interactions with PKA substrates and other signaling molecules they form multi-protein complexes. Thereby, AKAPs regulate the access of PKA to its substrates in a temporal and spatial manner as well as the local crosstalk of cAMP/PKA with other signaling pathways.

Due to the increasing information on their molecular functioning and three-dimensional structures, and their emerging roles in the development of diseases, AKAPs move into the focus as potential drug targets. In particular, targeting AKAP-dependent protein-protein interactions for interference with local signal processing inside cells potentially allows for the development of therapeutics with high selectivity and fewer side effects.

Introduction

A-kinase anchoring proteins (AKAPs) are a family of more than 50 scaffolding proteins (including splice variants) whose common denominator is the ability to directly bind cAMP-dependent protein kinase (protein kinase A, PKA), a serine/threonine kinase. In its inactive state PKA holoenzyme consists of two catalytic (C) and two regulatory (R) subunits. Stimulation of G protein-coupled receptors (GPCRs) on the surface of cells and subsequent activation of the stimulatory G protein G_s leads to activation of adenylyl cyclase (AC) to synthesize the second messenger cAMP. Upon binding of two molecules of cAMP to each R subunit of PKA conformational changes occur and PKA is activated: the C subunits are released and in this active form phosphorylate nearby targets (Fig. 1). Four different R subunit (RIα, RIβ, RIIα and RIIβ) and three different C subunit isoforms (Cα, Cβ, Cγ) are expressed in mammalian cells, and various splice variants have been reported. The C and R subunits can assemble in several combinations and give rise to a variety of PKA holoenzymes [1-5]. AKAPs bind R subunits of PKA. Based on their R subunit selectivity, AKAPs are categorized as RI-, RII- or dual-specific (D-)AKAPs if they bind both RI and RII [6-8].

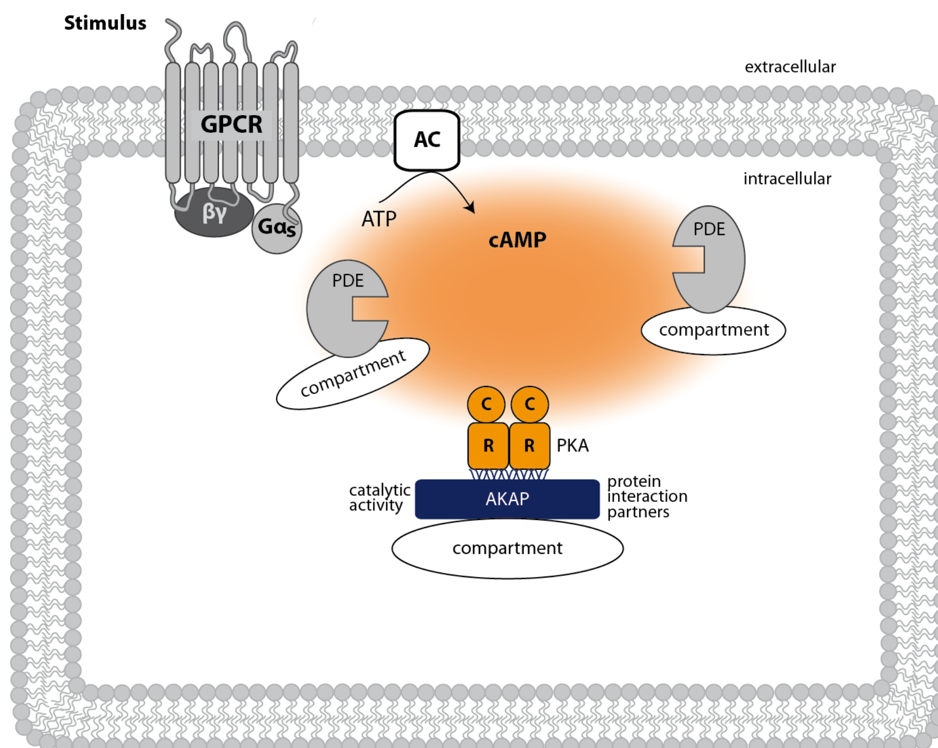

Figure 1: **Compartmentalized cAMP signaling.** Levels of the second messenger cAMP increase in response to a plethora of stimuli that activate G protein-coupled receptors (GPCR), which in turn stimulate the heterotrimeric G protein G_s (α, β and γ subunits) and synthesis of cAMP by adenylyl cyclase (AC). cAMP is degraded by phosphodiesterases (PDEs). The strategic positioning of PDEs establishes gradients of cAMP inside cells, which are sensed by cAMP effectors such as protein kinase A (PKA). The effectors are tethered to defined cellular sites and transform local cAMP elevations into specific cellular responses to each of the external stimuli. This compartmentalization of PDEs and cAMP effectors is achieved by scaffolding proteins such as A-kinase anchoring proteins (AKAPs). AKAPs tether multi-protein complexes to defined cellular locations and spatially and temporally coordinate cellular signaling processes. Aberrations in compartmentalized cAMP signaling are associated with a range of diseases, including cancer, cardiovascular, neurological and inflammatory diseases. Targeting components of this system pharmacologically may pave the way to new concepts for the treatment of diseases with an unmet medical need. R and C, regulatory and catalytic subunits of PKA, respectively.

A plethora of extracellular cues leads to activation of the G_s/AC/PKA system of which all components are ubiquitously expressed. Through unique anchoring domains AKAPs tether PKA to defined cellular compartments (e.g. to the cytoskeleton, plasma membrane, the Golgi, vesicles, nucleus or mitochondria), and thereby regulate PKA signaling spatially and temporally (Fig. 2) [7, 9, 10]. The interaction of AKAPs with PKA increases the specificity of PKA signaling and facilitates specific cellular

responses to each of the extracellular cues. AKAPs are engaged in further direct protein-protein interactions. Interactions with PKA substrates enable PKA to phosphorylate its local protein substrates and thereby to modulate their activities. In addition, AKAPs bind GPCRs [11], ACs [12, 13], exchange proteins directly activated by cAMP (Epac) [14], phosphodiesterases (PDEs) [15, 16], protein kinases (e.g. protein kinases C, D and N) [17], protein phosphatases such as PP1 [18] and PP2B (calcineurin) [19, 20] and ion channels and pumps (e.g. L-type Ca^{2+} channels, Na^{+}-Ca^{2+} exchangers [21]). A few AKAPs additionally possess catalytic activity, e.g. the Rho guanine nucleotide exchange factor (RhoGEF) activity of AKAP-Lbc (Lymphoid blast crisis) that activates the small GTPase RhoA [22] and the GTPase activity of Rab32 hydrolyzing GTP [23]; as activities of some of the interacting proteins are modulated by second messengers other than cAMP, e.g. Ca^{2+}, AKAPs are not simple scaffolds but rather coordinate the crosstalk of cellular signaling processes.

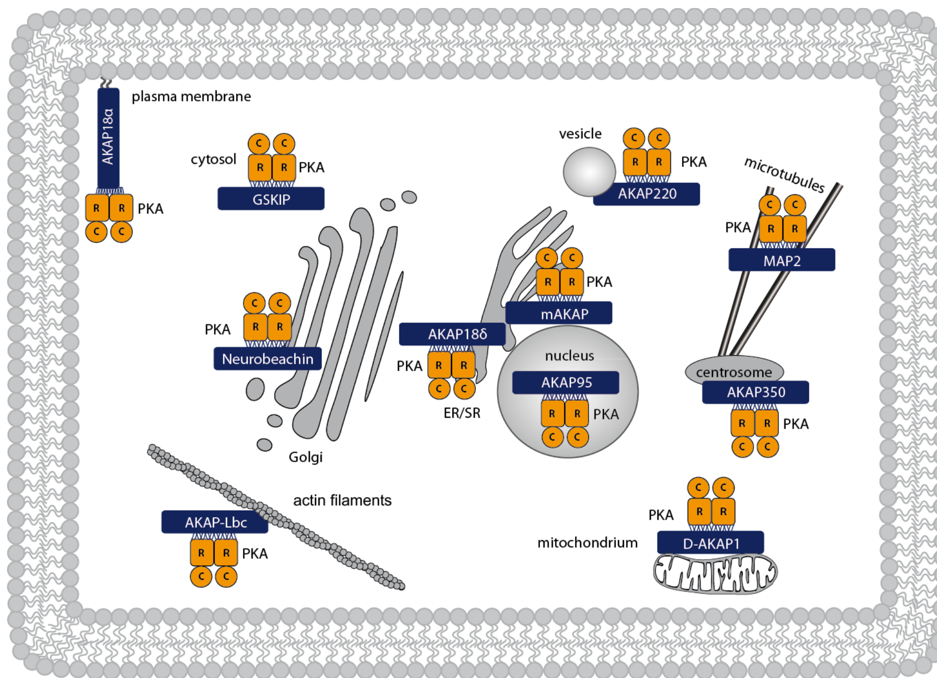

Figure 2: **Every organelle and subcellular compartment such as F-actin within the cytoplasm possesses one or more AKAPs. Several organelles and the F-actin cytoskeleton are shown with an exemplary set of AKAPs.**

In vitro, cell-based and *in vivo* studies including knockdown approaches and gene targeting are shedding light on physiological functions and the pathophysiology of AKAPs. From the variety of studies it is clear that many cellular processes such as the regulation of cell cycle and cell migration [24], cardiac contractility [21, 25-28], sperm motility [29-31], insulin secretion [32] and T cell immune responses [33] are dependent on AKAPs. The pharmacological interference with defined AKAP-dependent protein-protein interactions has elucidated roles of specific interactions. Most prominent, interactions between AKAPs and PKA have been disrupted with various pharmacological agents to reveal, for example, that these interactions are crucial for arginine-vasopressin (AVP)-mediated water reabsorption in renal principal cells [34] and cardiac myocyte contractility [27, 35-37].

The increasing insight into their physiological functioning and structures, and the emerging roles of AKAPs in the development of diseases, move AKAPs into the focus as potential drug targets. In particular, AKAP-dependent protein-protein interactions appear promising for pharmacological interference with local signal processing inside cells and the development of therapeutics with potentially high selectivity and efficacy. However, AKAPs are not yet established drug targets.

Here, we review new insights into AKAP-mediated signaling events, the relevance of AKAPs in health and various diseases (Tab. 1), structural aspects of AKAPs and their interactions, the current developments of pharmacological agents targeting AKAPs and their interactions (Tab. 2), and allude to potential therapeutic options involving AKAPs.

Physiological relevance of AKAPs and their implication in disease - options for pharmacological interference with AKAP-dependent protein-protein interactions

AKAPs in the heart

β-adrenoreceptor stimulation on the surface of cardiac myocytes mediates activation of PKA. PKA phosphorylates several substrates, amongst others L-type Ca^{2+} channels, type 2 ryanodine receptors (RyR2), phospholamban (PLN), phospholemman, Troponin I (TnI) and myosin-binding protein (MyBP). The net effect of the PKA phosphorylations is an increase in contractility (positive inotropic, positive chronotropic and positive lusitropic effects). The phosphorylations are facilitated by interactions of PKA with AKAPs and the tethering of the AKAP-PKA complexes to PKA substrates. One example is the complex comprising AKAP18δ, PKA, PLN and sarcoplasmic reticulum (SR) Ca^{2+} ATPase 2 (SERCA2). AKAP18δ directly interacts with PKA and PLN and facilitates β-adrenoreceptor-induced and PKA-catalyzed phosphorylation of PLN. Phosphorylated PLN is released from its direct interaction with SERCA2, activating SERCA2. The result is an enhanced Ca^{2+} re-uptake into the SR and relaxation of the myocyte [38].

There are more than 10 AKAPs expressed in cardiac myocytes, e.g. Yotiao controlling $KCNQ_1$ and mAKAP controlling RyR2 [21]; AKAP79, AKAP18α (Fig. 3) and Cypher all interact with L-type Ca^{2+} channels [19, 39-41], although the functional implications of these interactions are not entirely clear [42]. For the majority of cardiac AKAPs the physiological function remains to be determined.

The heart responds to stress by an adaptive process resulting in cardiac hypertrophy, a condition characterized by increases in cardiac myocyte size and total cardiac mass; 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.

The protein expression level of several AKAPs is altered in hypertrophic cardiac myocytes. A prototypical example of an AKAP involved in cardiac hypertrophy is AKAP-Lbc (Fig. 3). Its expression is upregulated in mouse hearts upon cardiac stress, induced by phenylephrine and angiotensin II infusion or thoracic aortic constriction (TAC) [26]. Heart biopsies from patients suffering from hypertrophic cardiomyopathy revealed increased AKAP-Lbc transcript levels [26]. AKAP-Lbc's multiple interactions with PKA, protein kinases C, D, Nα and p38 MAPK as well as its ability to act as a RhoGEF to activate RhoA are all involved in the development of hypertrophy [17, 43-47]. In line, knockdown of AKAP-Lbc in cultured cardiac myocytes using shRNA reduces α_1-adrenoceptor-mediated RhoA activation and hypertrophic responses [48]. In cardiac fibroblasts, AKAP-Lbc is involved in the reprogramming towards cardiomyoblasts, cells that cause fibrosis, a maladaptation occurring during the remodeling of the heart and that is associated with the development towards hypertrophy and heart failure [49]. Surprisingly, AKAP-Lbc apparently has not only pro but also anti-hypertrophic functions. In a mouse model where AKAP-Lbc is deficient for PKD binding, the animals become sensitized to stress induced by angiotensin II and phenylephrine treatment as well as to TAC. The mice display attenuated compensatory cardiac hypertrophy, increased collagen deposition and

apoptosis, compared to their wild type littermates indicating the relevance of the AKAP-Lbc-PKD1 interaction to enhance cardiac performance in response to stress [47]. A second AKAP whose role in cardiac hypertrophy has been intensively studied is mAKAP. For details the reader is referred to a recent review [50].

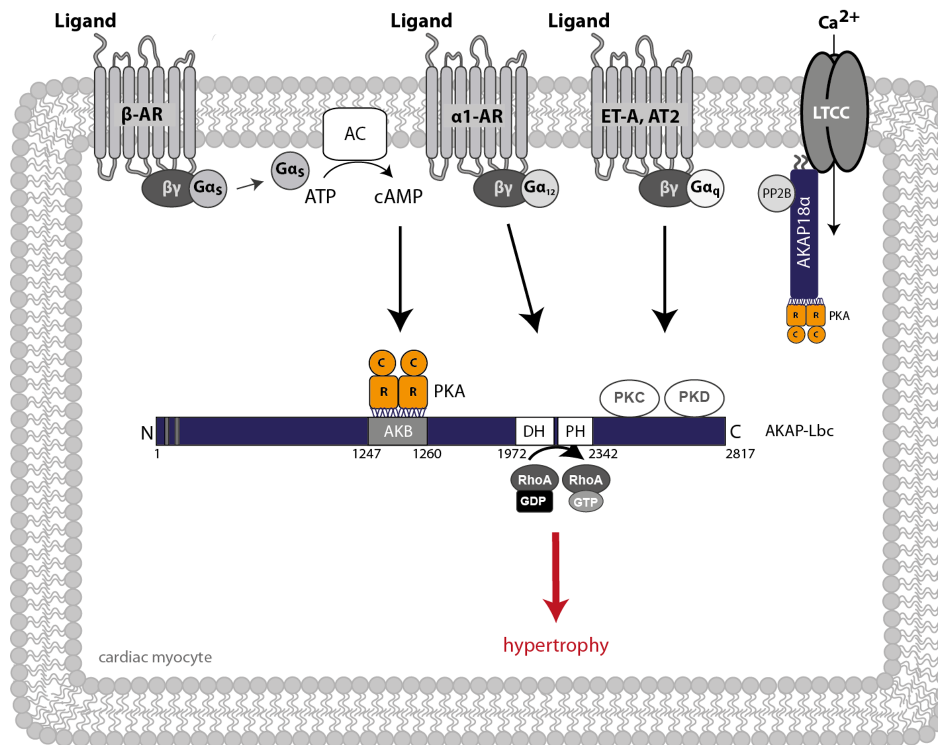

Figure 3: **A role of AKAP-Lbc in the development of cardiac myocyte hypertrophy.** Stimulation of α1- or β-adrenoreceptors leads to activation of G protein-mediated signaling pathways in cardiac myocytes involving AKAP-Lbc. PKA anchors R subunits of PKA *via* the AKB while the DH/PH domain of AKAP-Lbc binds and activates the small GTPase RhoA, followed e.g. by Rho-mediated cytoskeletal remodeling. C-terminally of the DH/PH domain PKC and PKD are bound. Upregulation of AKAP-Lbc is involved in the hypertrophic response of the heart. Several other AKAP play roles in the physiology and pathophysiology in cardiac myocytes (for details see text); one example is AKAP18α. It interacts with the plasma membrane as well as L-type Ca^{2+} channels (LTCC) and participates in the control of Ca^{2+} influx. ET-A: endothelin receptor type A; AT2: angiotensin receptor type 2..

In failing hearts the phosphorylation of regulatory RII subunits of PKA is reduced. Phosphorylated RII has a similar affinity for the binding of AKAP18α, AKAP-Lbc and mAKAP, dephosphorylated RII binds e.g. AKAP-Lbc 660 fold weaker than AKAP18α. Indeed, in failing hearts the interactions of PKA with the AKAPs SPHKAP and AKAP2 (each 6 fold), AKAP18 (2.4 fold) and MAP2 (12 fold) increase, while its interactions with AKAP1 (by 50 %) and Yotiao (by 15 %) decrease [51]. Thus the localization of PKA in failing hearts could be altered [52].

This broad involvement of AKAPs and their interactions in the development of cardiac hypertrophy and heart failure opens avenues for novel therapeutic strategies. For example, inhibition of AKAP-PKA interactions with peptides resulted in an increased rate and amplitude of cell shortening and relaxation compared to control cardiac myocytes [37]. Small molecules, which inhibit AKAP-PKA interactions but at the same time activate PKA also increase contractility and may thus be beneficial for the treatment of hypertrophy and heart failure [36]. However, in another study peptides for the disruption of AKAP-PKA interactions had negative effects on chronotropy (frequency), inotropy (contraction), and lusitropy (relaxation) on cultured cardiac myocytes and isolated hearts [35], and, in line, prevented β-adrenoceptor-induced increases in L-type Ca^{2+} channel currents in isolated cardiac myocytes [53]. Targeting the interaction of AKAP18 and PLN may lower the energy expenditure by SERCA2, which is favorable in the failing heart when energy for contraction is limited

[54]. Interference with the RhoGEF activity of AKAP-Lbc may interfere with α_1-adrenoceptor-induced development of cardiac hypertrophy.

AKAPs in kidney-related diseases

A renal process that relies on compartmentalized cAMP signaling is AVP-mediated water reabsorption [34, 55-57]. In renal collecting duct principal cells, AVP binds vasopressin V_2 receptors (V2R) on the basolateral surface, leading to an activation of adenylyl cyclase and cAMP elevation which is followed by PKA activation and a PKA-dependent phosphorylation of the water channel aquaporin-2 (AQP2) at Serine 256. Under resting conditions AQP2 resides on intracellular vesicles. The phosphorylation by PKA is the key trigger for its redistribution into the plasma membrane. The membrane insertion increases the osmotic water permeability of the collecting duct and water is reabsorbed from primary urine (Fig. 4) [56, 58-60]. Defects in the mechanism leading to the redistribution of AQP2 cause nephrogenic diabetes insipidus (NDI), a disease characterized by polyuria and polydipsia [56, 61-64]. Elevated levels of AVP such as in late stages of heart failure (NYHA II-IV), in the syndrome of inappropriate antidiuretic hormone secretion (SIADH) or in liver cirrhosis enhance the membrane association of AQP2 and lead to excessive water retention [61, 65-67].

Two AKAPs have been identified which are involved in the redistribution of AQP2: AKAP18δ and AKAP220 (Fig. 4) [55, 68-70]. Both AKAPs tether PKA to AQP2-bearing vesicles and apparently facilitate PKA phosphorylation of the channel. AKAP18δ additionally directly binds PDE4D3. Under resting conditions, PDE4D3 maintains a low level of cAMP and thus low PKA activity in the vicinity of AQP2. This prevents an inappropriate AQP2 phosphorylation and redistribution to the plasma membrane and excessive water reabsorption under resting conditions. Upon AVP stimulation the level of cAMP raises beyond a threshold that allows for PKA activation because local cAMP hydrolysis by PDE4D3 is saturated. PKA then phosphorylates AQP2 inducing its translocation into the plasma membrane and water reabsorption [71].

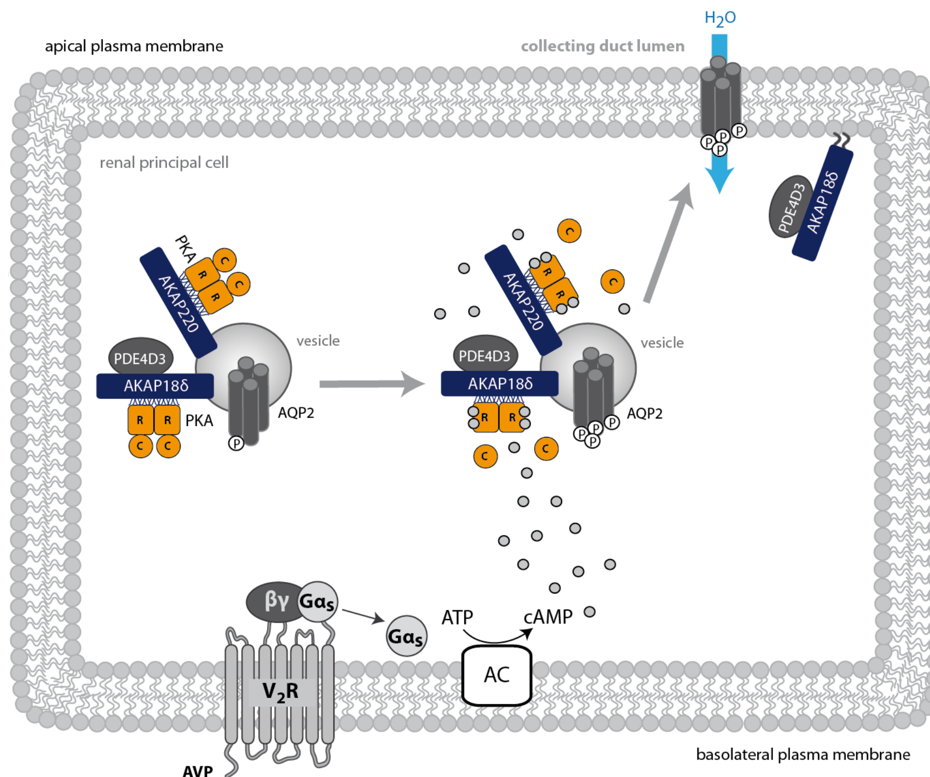

Figure 4: **PKA-triggered phosphorylation elicits the redistribution of AQP2 into the plasma membrane of renal principal cells.** AVP induces *via* V2R and the G protein $G\alpha_s$ the activation of adenylyl cyclase and thus an increase in cAMP and PKA activity. The following PKA-dependent phosphorylation of AQP2 on intracellular vesicles is facilitated by AKAPs (both AKAP220 and AKAP18δ). Upon phosphorylation AQP2 translocates from the vesicles into the plasma membrane, resulting in an increased osmotic permeability of the collecting duct and in the reabsorption of water from primary urine. Defects of this are linked to human diseases, including Diabetes insipidus, heart failure and hyponatraemia. The system is a potential target for the treatment of Polycystic kidney disease (PKD; see text for details).

Targeting AKAP-PKA interactions in renal principal cells could be a therapeutic option for the treatment of diseases associated with excessive AVP-mediated water reabsorption such as the ones mentioned above. Indeed, inhibition of these interactions with peptides prevents the AVP-induced redistribution of AQP2 in cultured renal collecting duct principal cells [34, 55, 57].

Autosomal-dominant polycystic kidney disease (PKD) is caused by mutations in the genes PKD1 or PKD2, encoding polycystin-1 and polycystin-2, respectively. The mutations result in kidney cysts and ultimately renal failure. The levels of cAMP in PKD are elevated in renal cells but also in other cell types including cholangiocytes and vascular smooth muscle cells [72, 73]. Ongoing clinical trials with V2R antagonists and agonists (somatostatin analogs) of GPCRs that couple to inhibitory G proteins (G_i) and lower cAMP have shown encouraging results. However, other therapeutic options are conceivable. An increased cAMP level results in activation of PKA. One consequence is PKA-catalyzed hyperphosphorylation of polycystin-2 in PKD1. This may contribute to cyst initiation in PKD1 patients [74]. Thus displacement of PKA from its site of action may be a therapeutic option. AKAPs targeting PKA to such sites are unknown. So far only AKAP150 has been found relevant in the development of PKD. Dysregulation of a complex consisting of AKAP150, PKA, AC5/6, PDE4C and polycystin-2 is involved in the pathology of PKD by controlling Ca^{2+} entry into cells. Impaired Ca^{2+} entry and the consequent low intracellular Ca^{2+} deinhibits Ca^{2+}-sensitive AC5/6 and thus elevates cAMP [75].

AKAPs in glucose homeostasis and obesity

Glucose homeostasis is predominantly controlled by insulin, which is released from pancreatic β-cells. In diabetes mellitus type 2 (T2D), insulin secretion is initially reduced and eventually lost; decreased islet β-cell numbers, reduced β-cell responsiveness to glucose and insulin resistance are characteristics of T2D. The release of insulin is triggered by a rise in intracellular Ca^{2+}. Ca^{2+} enters the β-cells through L-type Ca^{2+} channels and initiates the fusion of insulin granules with the plasma membrane (Fig. 5). Insulin secretion is modulated by cAMP [76-79]. Studies of various diabetes animal models as well as studies of cultured β-cells have revealed decreased cAMP levels, reduced AC and increased PDE expression [reviewed in [80]]. In addition, AKAP-PKA interactions are necessary for insulin secretion and glucose homeostasis, as their disruption with the peptide Ht31 (see below) diminishes incretin-stimulated insulin secretion in model cells and isolated pancreatic islets [32].

AKAP150 (human ortholog AKAP79), AKAP18α and AKAP18ɣ are expressed in the pancreas and have various functions in insulin release (Fig. 5) [81-83]. While AKAP18α enhances glucose and glucagon-like peptide-1 (GLP1)-stimulated insulin release, AKAP18ɣ inhibits this as shown by knockdown and overexpression studies in rat insulin secreting β-cells (RINm5F cells). In line, glucose stimulates AKAP18α and inhibits AKAP18ɣ mRNA expression [81, 83]. AKAP150 coordinates the PKA-dependent phosphorylation of targets involved in secretion of insulin by interacting with calcineurin and PKA. AKAP150 knockout mice display impaired insulin secretion and increased cAMP production. Studies of AKAP150 knock-in mice either deficient in PKA or calcineurin binding suggest that calcineurin anchoring is critical for the AKAP150-controlled insulin release [84]. In addition, glucose-stimulated cAMP oscillations, which are indispensable for insulin secretion, were abolished in AKAP150 knockout mice. Interestingly, Hinke *et al.* reported increased insulin sensitivity in skeletal muscle in the AKAP150 knockout mice, which was absent in the β-islet-specific AKAP150 knockout mouse, indicating that skeletal muscle selectively adapts to the absence of AKAP150 to compensate for the decrease in insulin. Thus, the interaction between AKAP150 and calcineurin would be a potential target for increasing insulin sensitivity in patients with diabetes and metabolic syndromes [85]. Conventional antidiabetic drugs do not directly target cAMP signaling. For one of the biguanides, metformin, it was however recently discovered that it causes an accumulation of AMP and other nucleotides that inhibit AC. Inhibition of adenylyl cyclases reduces the cAMP level and PKA activity and thus phosphorylation of PKA substrates. This, in turn, blocks glucagon-dependent glucose release from hepatocytes [86]. This observation and the established role of AKAPs and AKAP-dependent protein-protein interactions in the control of glucose and insulin homeostasis suggest that compartmentalized cAMP signaling is a potential target for pharmacological intervention to treat diabetes.

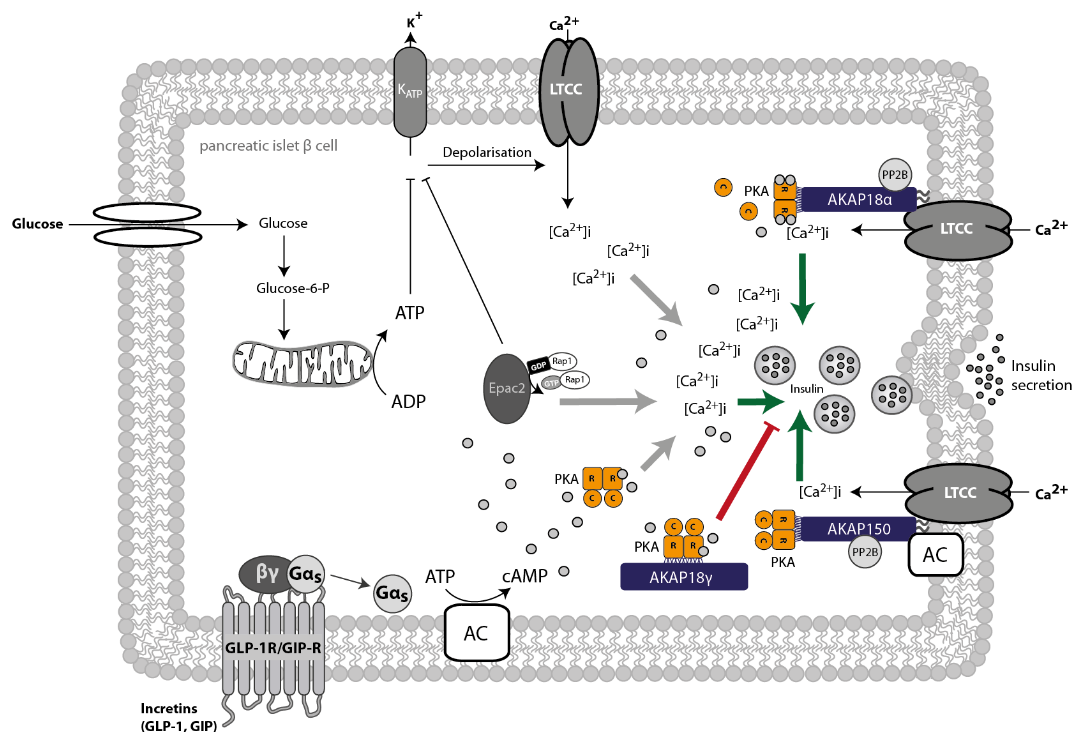

Figure 5: **AKAPs involved in glucose homeostasis and Ca^{2+}-driven insulin release from pancreatic β-cells**. AKAPs coordinate PKA-dependent target phosphorylation involved in insulin secretion. AKAP18α enhances GLP1-stimulated insulin release by enhancing intracellular Ca^{2+} levels while AKAP18γ has the opposing effect (mechanism unknown). Both AKAP18α and AKAP150 interact with PKA and PP2B as well as with L-type Ca^{2+} channels (LTCC). Ca^{2+} enters the cell through LTCC and is the main trigger for insulin release. Glucose is transported into the cell and used for mitochondrial ATP generation. The ATP to ADP ratio controls ATP-sensitive potassium channels (K_{ATP}) located in the β-cell plasma membrane: under resting conditions the channels are continuously open and thus potassium ions exit the cell. In the presence of elevated glucose ATP levels increase and the consequently increased ATP to ADP ratio induces closure of the K_{ATP} channel, causing a depolarization of the membrane, which promotes insulin release. GLP-1R: glucagon-like peptide-1 receptor; GIP/GIP-R: gastric inhibitory polypeptide/receptor.

WASP-family verprolin homologous protein 1 (WAVE-1) regulates actin cytoskeleton dynamics, apoptosis and glycolysis. It functions as an AKAP and a WAVE-1-coordinated protein complex consisting of Bcl-2 antagonist of cell death (BAD), glucokinase (GK, Hexokinase D), PKA and protein phosphatase 1 (PP1) is involved in glucose-driven mitochondrial respiration in the liver: PKA is targeted *via* WAVE-1 to mitochondria, phosphorylates and thereby inactivates BAD, which leads to dissociation of the protein complex; PP1 instead activates BAD *via* dephosphorylation [87, 88]. As GK only binds to active BAD, the phosphorylation status of BAD determines the binding of the glycolysis enzyme GK to the above-mentioned complex. Mice either BAD-deficient or expressing a BAD variant which cannot be phosphorylated show abnormal glucose homeostasis and glucose tolerance in correlation with diminished GK activity. GK couples blood glucose level changes to insulin release for maintaining glucose homeostasis and the pathophysiology of T2D is linked to both gain-of-function and loss-of-function mutations in the GK-encoding gene [89, 90]. In addition, GK inactivity or over-expression disturbs glucose uptake and has been shown to be defective in T2D patients [90, 91]. In addition, GK activation has been recognized as a treatment strategy for T2D and targeted pharmacologically by GK activating chemical compounds such as piragliatin, leading to decreased hepatic glucose output in T2D patients [92]. As BAD is phosphorylated in an AKAP-dependent manner by PKA, targeting the PKA-WAVE-1 interaction within the protein complex potentially induces more BAD activity and thus ultimately increases GK activity. Influencing GK activity *via* targeting the WAVE-1/BAD/GK/PKA/PP1 protein assembly might be beneficial in reestablishing glucose uptake in T2D patients.

Diabetes and obesity are associated with deregulated fat metabolism; for example, triglycerides accumulate in the liver of late stage Diabetes patients causing fatty liver. Pidoux *et al.* identified optic atrophy 1 (OPA1) as a dual-specific AKAP forming a complex with PKA and perilipin on lipid droplets [93]. Lipid droplets are characteristic cytosolic compartments of adipocytes and serve as triglyceride and cholesterol storages [94]. Peptides interfering with AKAP-PKA interactions inhibit OPA1-mediated anchoring of PKA to the lipid droplet surface and PKA-dependent phosphorylation of perilipin and, thereby, adrenergic stimulation of lipolysis [93, 95]. Thus targeting OPA1 may lower the triglyceride load in Diabetes patients. AKAP149 (AKAP1, D-AKAP1) was found to be the most abundant AKAP in adipocytes [96]. A fourfold reduction of AKAP149 gene expression was detected in adipose tissue of obese patients [95-97]. AKAP1 localizes predominantly type II PKA and other interaction partners to the outer mitochondrial membrane (reviewed in [98]). AKAP1 associates with mRNAs, e.g. encoding mitochondrial ATP synthase F0-f subunit, manganese superoxide dismutase and steoridogenic acute regulator, localizing them close to the outer mitochondrial membrane and suggesting an implication in steroidogenesis [99, 100]. Of note, AKAP1 binds lipoprotein lipase (LPL) mRNA and thereby inhibits its translation, suggesting that reduced AKAP1 levels in obesity might cause an increase in LPL and thus increased LPL-dependent adipose tissue lipogenesis and fatty acid uptake [95]. Thus interference with AKAP1-mRNA interactions may be a therapeutic strategy for the treatment of obesity.

AKAPs in sickle cell disease

Sickle cell disease (sickle cell anemia) is a hereditary disorder that affects hemoglobin and is accompanied by severe pain attacks. A sickle-cell crisis can be initiated by adhesion of red blood cells to the vascular wall leading ultimately to vasoocclusion. Red blood cell deformability is crucial for maintaining normal blood flow and changes in deformability occur e.g. upon mechanical stress. cAMP and Ca^{2+}-dependent signaling pathways play a role in the regulation of red blood cell membrane properties related to aggregability and filterability [101].

Sickle cell red blood cells do not only contain more than fourfold higher cAMP levels than unaffected red blood cells [102], but also acquire a PKA-dependent adhesion mechanism by which they bind to the vascular wall. Cytoadherence of red blood cells to the endothelial basal lamina *via* the basal cell adhesion molecule/Lutheran (BCAM/Lu) receptor is important in vasoocclusive episodes of sickle cell disease [103, 104]. It is well established that cAMP is crucial for BCAM/Lu receptor activation [102]. However, the involvement of AKAPs as well as their assistant function in BCAM/Lu receptor phosphorylation has only recently been uncovered (Fig. 6). AKAPs are present in sickle red blood cell plasma membranes [105]. By using the stearate-coupled AKAP-PKA disruptor peptide Ht31, Maciaszek *et al.* demonstrated the requirement of intact AKAP-PKA interactions in normal as well as sickle cell disease red blood cell adhesion [105]. This finding may lead to an alternative strategy for the reduction of vasoocclusion in sickle cell disease, namely disruption of AKAP-PKA interactions or displacement of the relevant AKAP/s (not identified yet) from its cognate location. An alternative to the current treatment with Hydroxyurea is highly desirable as this can cause severe side effects. Hydroxyurea is used to prevent painful episodes and to reduce the need for blood transfusions in sickle cell disease but is also used for the treatment of skin and other cancers. Hydroxyurea reduces BCAM/Lu receptor expression and interferes with the interaction between the BCAM/Lu receptor and laminin, a constituent of the endothelial basal lamina [106].

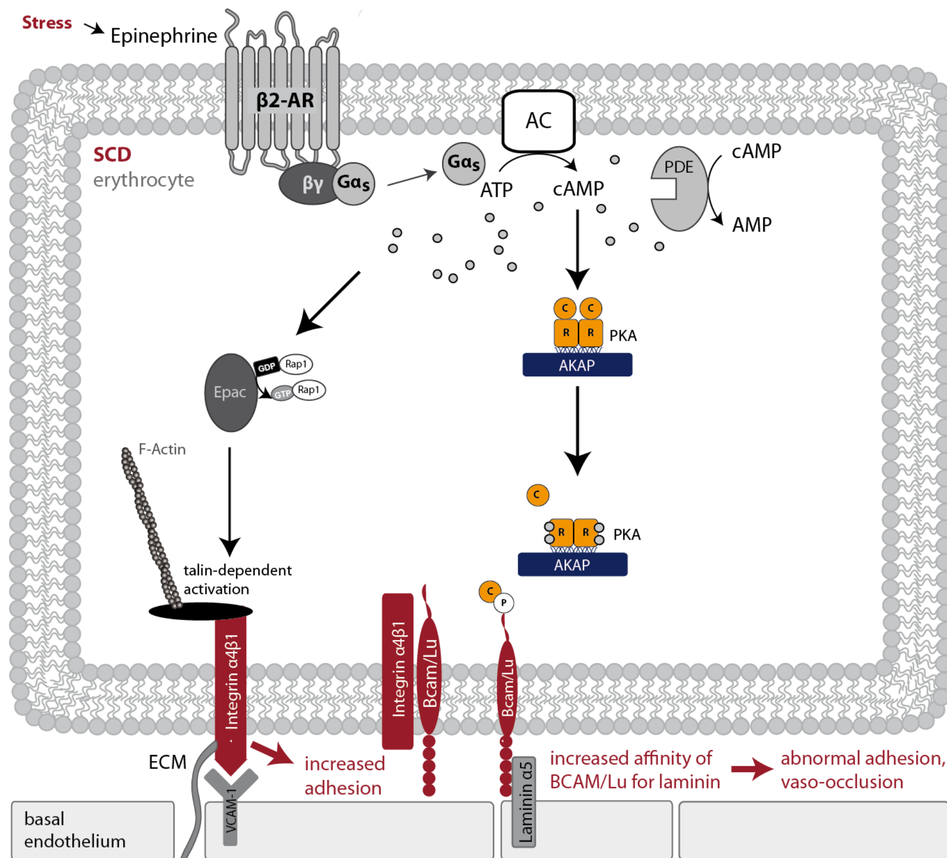

Figure 6: **The function of AKAPs in abnormal adhesion of and vaso-occlusion by erythrocytes as characteristic features of sickle cell disease (SCD).** Epinephrine induces GPCR-mediated activation of AC, increased intracellular cAMP levels and thus activation of PKA as well as Epac. Epacs promote the talin-dependent activation of integrin α4β1 receptors which serve as a bridge between the actin cytoskeleton and the extracellular matrix (ECM) around and below the basal endothelium, e.g. by recognizing VCAM-1 on the surface of the basal endothelium. AKAP-facilitated PKA phosphorlyation assists the adhesion of extracellular Bcam/Lu to Lamininα5. SCD erythrocytes show increased cAMP levels, upregulated expression of integrins as well as Bcam/Lu receptors. These observations are in line with an increased affinity of the cellular membrane receptors for laminin and ECM proteins in SCD erythrocytes ultimately enhancing the adhesion of red blood cells to the vascular wall and vaso-occlusion.

AKAPs in cancer

Cancer cells acquire the ability to increase proliferation, which is associated with upregulated Ras-Raf-MEK-ERK signaling, e.g. caused by mutations in the gene encoding BRAF [107]. Cancer cell proliferation can be controlled by the interplay between the Ras-Raf-MEK-ERK pathway and the cAMP/PKA system and involves scaffolding proteins for the local and temporal control of the crosstalk including AKAPs. AKAP-Lbc is not only involved in cardiac myocyte hypertrophy but also has a role in cancer. It mediates cAMP/PKA phosphorylation of kinase suppressor of Raf (KSR) and thus the subsequent increase of MEK and ERK activity through a signalosome composed of AKAP-Lbc, PKA, Raf and KSR1 [108]. The consequence is a proliferative effect (Fig. 7). Targeting the AKAP-Lbc signalosome may therefore be antiproliferative.

At least three splice variants of AKAP-Lbc have been identified. Only full-length AKAP-Lbc (2813 amino acids) has a PKA anchoring domain [22, 109, 110]. A single nucleotide polymorphism (*Lys*526*Gln*) was associated with increased familial breast cancer risk in genome-wide association studies. The polymorphism is not located in any DNA region that encodes a defined domain of AKAP-Lbc; it may alter the secondary structure of the protein [111]. Overexpression of AKAP-Lbc is linked to uterine leiomyoma [112]. All AKAP-Lbc isoforms possess a RhoGEF domain [22, 113]. The truncated form of AKAP-Lbc known as Onco-Lbc consists of the RhoGEF domain and 70 N-terminal amino acids (amino acids 1922-2346 of AKAP-Lbc). Onco-Lbc was identified in material from myeloid leukemia

patients [114] and induces Rho-dependently cell cycle progression [115], the formation of actin stress fibers as well as the assembly of focal adhesions in fibroblasts [113, 116]. The third form is termed Proto-Lbc and consists of the Rho GEF domain plus an extended C-terminal region that is absent in Onco-Lbc [117] (amino acids 1922-2813 of AKAP-Lbc). Proto-Lbc is expressed in testis and estrogen-sensitive tissues. While the RhoGEF domain of full-length AKAP-Lbc underlies tight regulation by PKA and homodimerization [118], the two truncated versions possess constitutively active RhoGEF domains that increase Rho activity [113]. Activated Rho is involved as a molecular switch in numerous cellular processes, e.g. cytoskeletal dynamics, cell polarity and motility [119]. Activation of RhoA by RhoGEFs requires direct protein-protein interactions. Thus targeting the interaction of the RhoGEF domain of the AKAP-Lbc variants with RhoA in cancer cells may have an antiproliferative effect.

Of note, cytoskeletal regulation as well as cell migration are PKA-dependent processes and particularly interesting with regard to physiological changes leading to tumor cell invasion and metastatic cell spreading. One aspect to consider is the dynamic regulation of PKA by integrin-mediated cell adhesion to extracellular matrix (ECM). Furthermore, PKA activity is required for key activation processes in cell migration and cytoskeletal organization, e.g. Rac and Cdc42 activation and the assembly of actin filament assembly, reviewed in [120]. cAMP/PKA signaling is spatially regulated by AKAP-anchoring of PKA with various proteins of the actin cytoskeleton, an example of which is the PKA substrate Vasodilator-stimulated phosphoprotein (VASP), being a critical player in actin remodeling and actin-based cell motility [121].

Several AKAPs are associated with cancer, in particular through their control over mitosis and the cell cycle and thus cancer progression [reviewed in [7, 122-124]]. For example, an increased risk of breast cancer is linked with single nucleotide polymorphisms in AKAP9 and D-AKAP2. AKAP9 promotes colorectal cancer cell proliferation, migration and invasion and has been shown to be upregulated in these cells [125]. In addition, the expression of the tumor suppressor Gravin and its rodent ortholog Src-suppressed C kinase substrate (SSeCKS)/AKAP12 [24] [recently reviewed in detail [124]] is suppressed in various solid tumors [126-128], partly through hypermethylation of the gravin promoter [129-131]. Gravin was thought to inhibit Src activity, but has recently been shown to rather sequester Src to reduce mitogenic signaling [132]. The assembly of Gravin, PKC and inactive Src is anchored to caveolin-rich lipid rafts and thereby removes Src from both focal adhesion kinase (FAK)-associated adhesion and PKC-dependent activation of the Ras-Raf-MEK-ERK cascade. In the absence of Gravin, Src kinase is active and induces PKC-mediated activation of ERK signaling along with increased proliferation, unregulated cell growth and secretion of matrix metalloproteinases [133]; focal adhesions are reduced due to active FAK leading to cell migration and invasion (Fig. 7). Loss of Gravin and the degree of its decreased expression correlate with poor prognosis, e.g. in acute myeloid leukemia [134]. Increased hypermethylation of the gravin promoter coincides with increased metastasis and Gravin has been suggested as a cancer progression biomarker. Whether selective elevation of Gravin protein abundance would be an option for cancer treatment is unclear so far, but in the context of the Gravin-PKC-Src assembly a stabilization of the protein complex would be favorable.

The testis-specific AKAP4 is important in sperm motility [135, 136]. AKAP4 is aberrantly expressed in various malignant tissues [137]. Its expression triggers humoral immune responses in ovarian cancer and is associated with malignant properties of cervical, breast and prostate cancer [138]. AKAP4 is

currently analyzed as a potential target for cervical cancer immunotherapy [139, 140]. The strong expression of AKAP4 in multiple myeloma led to its classification as cancer testes antigen [137].

Taken together, cancer-related AKAP interactions are worth validating as possible targets for pharmacological interventions.

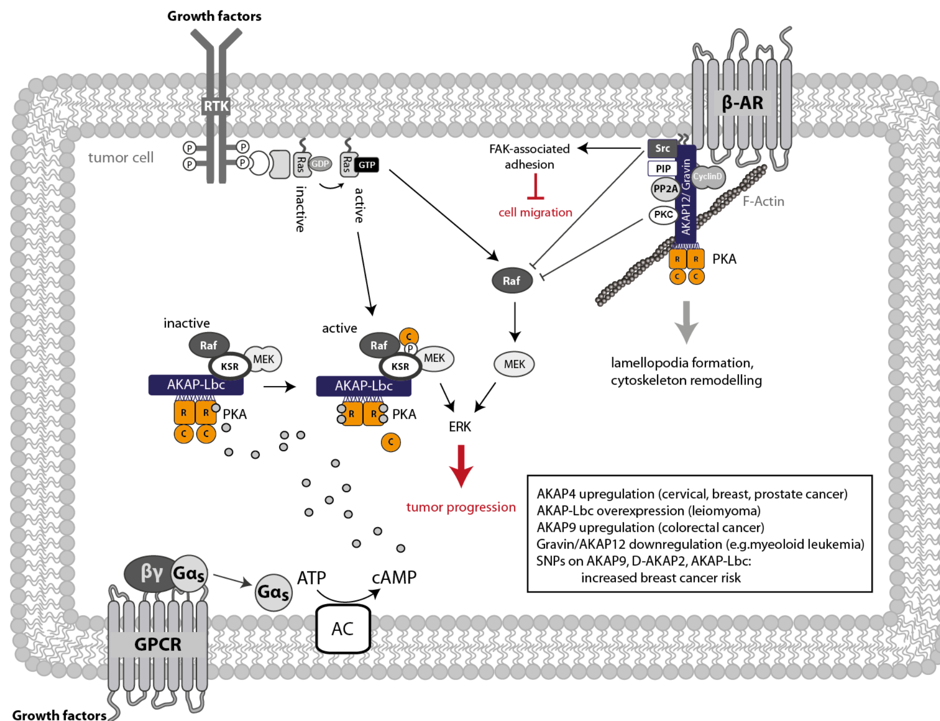

Figure 7: **Function of selected AKAPs in cancer.** Growth factors on the one hand activate the Ras-Raf-MEK-ERK cascade *via* receptor tyrosin kinases (RTK) and the AKAP-Lbc-anchored complex consisting of PKA, RAF, KSR and MEK, and on the other hand induce the production of cAMP and increase PKA activity via GPCR signalling. Upon Raf activation, AKAP-Lbc-anchored PKA phosphorylates KSR and thus induces a conformational change of MEK, resulting in the activation of ERK, which in turn enhances cell cycle progression and cellular growth. In case of AKAP-Lbc upregulation such as in cancer tissue the progression of tumor growth is promoted by the subsequent increase of MEK and ERK activity through the AKAP-Lbc/ PKA/ Raf/ KSR1 signalosome. The assembly of the AKAP Gravin with PKC and Src removes Src from both focal adhesion kinase (FAK)-associated cell migration and PKC-dependent activation of the Ras-Raf-MEK-ERK cascade. Gravin is down-regulated in cancer, hence its absence supports Ras-Raf-MEK-ERK signalling as well as the disassembly of focal adhesions (as FAK is consequently bound by the available Src) resulting in increased cell motility and metastasis. PP2A: protein phosphatase 2A; PIP: phosphatidylinositolphosphate.

AKAPs in the immune system

The current knowledge about the role of AKAPs in the immune system is mainly restricted to T cells and HIV infection. In T cells of HIV patients, the levels of cAMP and PKA type I activity are elevated [33, 141-143], and cAMP inhibits T cell receptor (TCR)-induced T cell proliferation [144]. In line, TCR-induced T cell activation is inhibited e.g. by GPCR-mediated signalling *via* prostaglandin E_2 (PGE_2), which signals amongst others through the G_S/adenylyl cyclase system.

A few AKAPs have been identified in T cells (recently reviewed in [145]). The mitochondrial AKAP1 is indispensable for efficient HIV replication [146, 147] through its interaction with HIV-1 reverse transcriptase, the enzyme converting single-strand viral RNA into double-strand DNA for the integration into the host genome. Thus the interaction of AKAP1 with HIV reverse transcriptase constitutes a potential target for the pharmacological inhibition of early viral replication.

Ezrin, a member of the ERM family of proteins, is a dual-specific AKAP that binds F-actin and links the cytoskeleton and the plasma membrane. A complex comprising Ezrin, PKA type I and C-terminal Src kinase (Csk) is present in lipid rafts of T cell plasma membranes in close proximity to the TCR during T

cell activation [148]. PGE_2 production is enhanced in response to continuous antigen presentation such as in HIV infection and leads to PGE_2-induced stimulation of AC and subsequent PKA activation. Ezrin-anchored PKA guides the phosphorylation of Csk that inhibits T cell proliferation and function (recently reviewed in [149]). Hyperactivation of the PKA-Csk pathway is involved both in HIV infection and in murine AIDS [142, 150]. The disruption of the PGE_2-cAMP-PKA pathway with peptides targeting AKAP-PKA interactions causes an increase in effector T cell function, e.g. reduced sensitivity to PGE_2-dependent T cell inhibition. Mice overexpressing the AKAP-PKA disruptor RIAD had improved resistance to murine leukemia virus-induced immunodeficiency, in line with hyperactive PKA-Csk signaling [151]. Ezrin knockdown in cultured T lymphoblasts enhances HIV-1-induced cell-cell fusion, described as syncytium formation that assists in spreading of the virus [152]. Thus Ezrin limits the spread of HIV-1 by suppressing membrane fusion events.

As AKAP1 is implicated in HIV early replication and Ezrin in HIV transmission through cell fusion disruption of AKAP-dependent protein-protein interactions and/or the displacement of a defined AKAP from its cognate cellular location may be a therapeutic option for the treatment of AIDS. With the inhibition of RI-AKAP interactions in a murine AIDS model first attempts have been successful already [153].

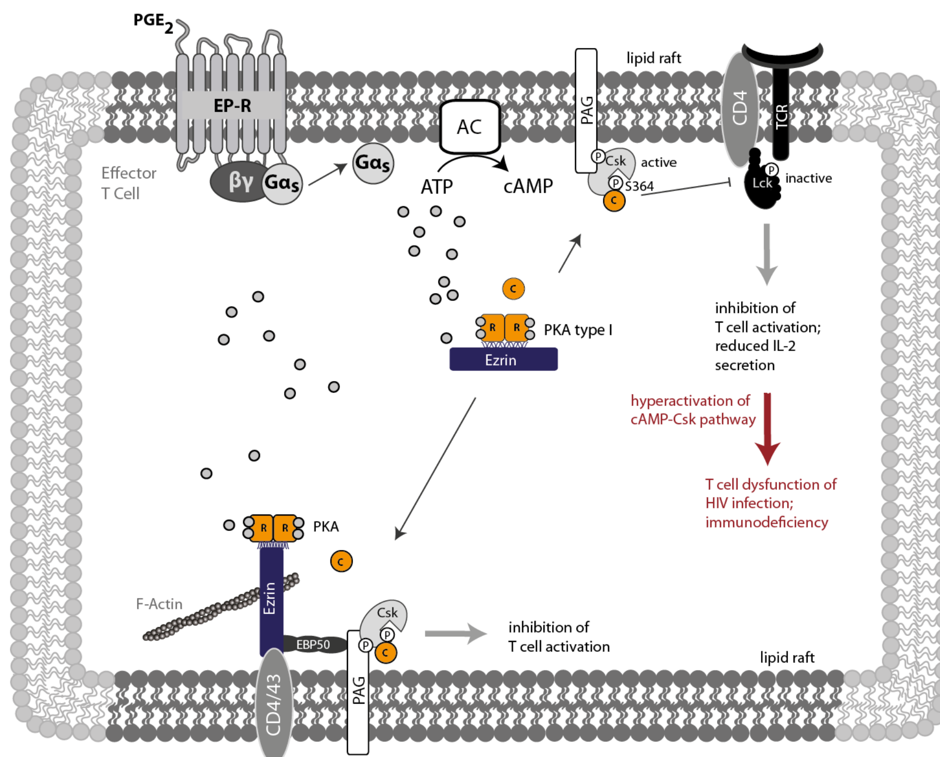

Figure 8: **AKAP-dependent processes involved in T cell activation.** PGE_2-induced GPCR activation signals through the $G\alpha_s$/AC system and leads to subsequent PKA activation. The cAMP-PKA pathway is involved in the regulation and modulation of immune responses. Ezrin-anchoring in lipid rafts (dark grey plasma membrane region) positions PKA close to its substrate Csk (C-terminal Src kinase). Csk phosphorylation by PKA in the vicinity of TCR results in the inhibition of Lck in line with inhibited CD4(+) T cell proliferation and function. CD43 regulates multiple T-cell functions, including T-cell activation, proliferation, apoptosis, and migration by linking Ezrin to the actin cytoskeleton. Hyperactive cAMP/Csk signaling is involved in HIV infection and acquired T cell deficiency. Lck: Src family tyrosine kinases; PAG: phosphoprotein associated with glycosphingolipid-enriched membrane microdomains; EBP50: ERM (Ezrin/Radixin/Moesin)-binding phosphoprotein 50.

AKAPs have also been identified in dendritic cells, the major antigen presenting cells of the immune system [154]. Dendritic cell maturation and activity is regulated, amongst others, by PKA and cAMP [155]. Human dendritic cells express AKAP79, AKAP1, AKAP95, AKAP-Lbc and Ezrin; their expression upon maturation from a monocyte to a mature dendritic cell is differentially regulated: The

expression of AKAP95 and Ezrin does not change, but that of AKAP79, AKAP1 and AKAP-Lbc increases and this is accompanied by a 1.8-fold increase of RIIα protein expression. Indeed, optimal antigen presentation by dendritic cells and T cell activation requires PKA anchoring [154, 156].

Chronic obstructive pulmonary disease (COPD) is an airway disease involving chronic inflammation, airway obstruction, fibrosis and chronic bronchitis [157]. Secretion of inflammatory mediators such as Interleukin 8 (IL-8) contributes to the pathogenesis [158]. The mRNA expression of IL-8 positively correlates with COPD progression in lung biopsies [159]. Cigarette smoke represents a major risk factor of COPD and induced IL-8 release from inflammatory cells, structural lung cells and airway smooth muscle (ASM) cells [160]. cAMP compartmentalization is involved in important features of COPD such as inflammation and airway remodeling. ASM cells are important players in these processes (recently reviewed in [161]). They switch phenotype between a proliferative and contractile state upon stimulation, e.g. by growth factors. This process involves PKA and Epac [162] that apparently act synergistically [163, 164]. The activation of either Epac or PKA decreased cigarette smoke extract-induced IL-8 mRNA expression and IL-8 release from human ASM cells by a mechanism involving ERK and NFkB signaling [162]. The expression of AKAP5, AKAP12 and Ezrin in human ASM cells [165] suggests that these AKAPs are of importance in ASM cell responses. As there is no curative treatment existing for COPD, the medical need for pharmacological intervention is high. Local PKA signaling might be an appropriate pharmacological target for anti-inflammatory therapy of COPD [162].

AKAPs in neurological processes and disorders

Learning and memory formation are based on long-term potentiation (LTP) and long-term depression (LTD) that define synaptic plasticity, the strength of synaptic transmission [166, 167]. LTD and LPD have been extensively studied in the hippocampus. Many of the studies linked LTD and LTP to receptors which are phosphorylated by PKA [167], in particular AMPA- and NMDA-type glutamate receptors. AKAP-PKA complexes play prominent roles in synaptic plasticity by modulating Ca^{2+} influxes into hippocampal neurons through these receptors [9, 168-171]. Disturbances in the modulation of synaptic strengths are a common phenomenon in pathological conditions and disease. Neuronal AKAP150 is the platform for different multiprotein complexes (recently reviewed in [124]). One complex formed by AKAP150, PP2B/calcineurin, PKC and PKA modulates diverse functions at the post-synaptic membrane [172-175]. AKAP150-dependent and PKA-mediated phosphorylation of the AMPA receptor GluA1 subunit triggers the insertion of the receptor into the post-synaptic membrane during LTD, while dephosphorylation through AKAP150-bound calcineurin has the opposite effect (Figure 9) [175].

AKAP-PKA interactions are also associated with the development of inflammatory pain and increased sensitivity of pain [176]. Sensitivity to pain involves phosphorylation and activation of Transient Receptor Potential Vanilloid 1 (TRPV1) by PKA (Figure 9). AKAP150 is implicated in the phosphorylation of TRPV1 by PKA [177, 178]. In mice lacking the PKA binding site of AKAP150 PGE_2-induced thermal hyperalgesia is inhibited, suggesting that the AKAP150-PKA interaction is essential for TRPV1 function [176]. Calcineurin dephosphorylates TRPV1 independently of its anchoring by AKAP150 [179]. The PKA-dependent reduction of TRPV1 desensitization has been suggested to have a facilitator purpose during inflammation periods marked by continuous channel activation and being involved in chronic pain [180]. In inflammatory processes the disruption of PKA anchoring to TRPV1 may be a strategy for altering the effects of chronic TRPV1 activation.

The AKAP Neurobeachin (NBEA) was identified as a candidate gene for autism spectrum disorders in several unrelated patients with NBEA gene disruptions, including monoallelic gene deletions [181], SNP [182] and copy number variations [183]. NBEA is involved in post-Golgi membrane trafficking and in the process of regulated secretion. Thereby, it contributes to neurotransmission at neuromuscular junctions through controlling neurotransmitter release [184, 185]. Its exact function is still unknown but NBEA is discussed to limit PKA activity spatially in an AKAP-typical manner as both increased and decreased phosphorylation of PKA substrates have been found in platelets of mice heterozygous for NBEA [186].

As discussed above, WAVE-1 is mainly expressed in the brain and an important player in actin organization. The depletion of WAVE-1 in knockout mice causes sensorimotor retardation and reduced learning and memory [187, 188]. In its active dephosphorylated form, WAVE-1 initiates actin polymerization and thus facilitates neurotransmitter-induced neurite outgrowth and neuronal plasticity [189]. Neuronal tyrosine-phosphorylated adaptors for the phosphoinositide 3-kinase (NYAP) family of phosphoproteins have been identified as interaction partners of the WAVE-1 complex in developing neurons [190]. The ternary complex of phosphoinositide 3-kinase (PI3K), NYAPs and the WAVE-1 complex is recognized as another example of spatial and temporal control of signaling components to subcellular compartments in close vicinity of PI3K for regulation of neuronal morphogenesis [191]. Aberrant PI3K signaling is linked to altered developmental processes of the nervous system and to neurological diseases characterized by cognitive and behavioral deficits including intellectual disability, autism, epilepsy and schizophrenia [192].

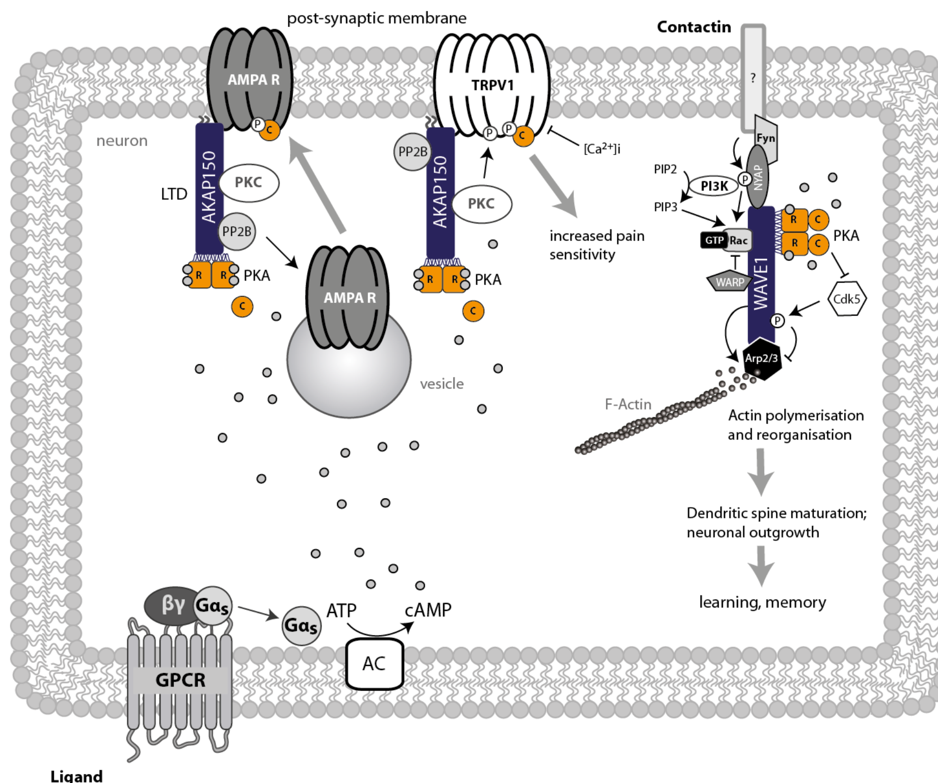

Figure 9: **AKAPs involved in neuronal processes such as increased pain sensitivity (AKAP150) and neuronal development (WAVE-1).** During long-term depression (LTD), the redistribution of AMPA receptors is mediated by an AKAP150-PKA complex: AKAP150-bound PKA induces phosphorylation of A-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA R) and thus the insertion of AMPA R into the post-synaptic membrane, while AKAP150-bound PP2B (calcineurin) triggers AMPA R to reside on intracellular vesicles. PP2B also dephosphorylates TRPV1, while PKA increases TRPV1 phosphorylation, leading to pain sensitivity increases up to chronic pain in case of continuous channel activation. In addition, the complex of WAVE-1, PKA, NYAP and PI3K is involved in neuronal cytoskeleton remodeling. The receptor mediating Contactin-dependently the activation of an Fyn-NYAP-PI3K-Rac pathway is currently unknown. Active NYAP, after phosphorylation by Fyn tyrosine-protein kinase, recruits and activates PI3K as well as the WAVE-1-PKA complex. Cdk5-dependent

phosphorylation of WAVE-1 inhibiting Arp2/3-mediated actin polymerization is inhibited by PKA; hence PKA triggers F-actin filament elongation by Arp2/3.

In addition, altered cAMP signaling is associated with a plethora of neurodegenerative diseases such as Alzheimer´s, Parkinson´s and Huntington´s disease, recently reviewed by Poppinga *et al.* [161]. This points out the obvious physiological relevance of cAMP, PKA and AKAPs in the healthy neuronal system as well as in disease. Thus targeting AKAP-dependent protein-protein interactions in the nervous system with specific pharmacological agents might be an option for the treatment of neuronal disorders.

Table 1: AKAPs and their relevance in pathophysiology

AKAP	HGNC name	Cellular distribution	Gene KO phenotype	Disease relevance
D-AKAP1/ AKAP149	AKAP1	Outer mitochondrial membrane, inner mitochondrial compartment, ER, nuclear envelope	Oocyte meiosis defects, female infertility (mouse KO) [193]	Heart failure (decreased interaction with PKA) [194]; Obesity (reduced gene expression) [96]; HIV replication and infection [146, 147]
AKAP2	AKAP2	Actin cytoskeleton, apical membrane of epithelial cells	N/A	Heart failure (increased interaction with PKA) [194]; Kallmann syndrome, bone anomalies (gene disruption) [195]
AKAP4	AKAP4	Fibrous sheath of sperm tail	Sperm mobility defects, Male infertility (mouse KO) [136]	Cervical, breast, prostate cancer (cancer testes antigen) [137]
AKAP79, AKAP150	AKAP5	Plasma membrane, postsynaptic dendrites	Impaired insulin secretion, increased cAMP production, abolished cAMP oszillations; but increased insulin sensitivity in skeletal muscle (Mouse KO) [84]	Polycystic kidney disease (dysregulation, impaired Ca^{2+} entry, elevated cAMP) [75]; Diabetes, metabolic syndrome [85]
mAKAP	AKAP6	Nuclear envelope (neurons, striated cardiac and skeletal myocytes)	Pathological cardiac remodeling (cardiac myocyte-specific mouse KO) [196]	Cardiac myocyte hypertrophy [197]; mAKAP ablation beneficial in heart disease [196]
AKAP18α	AKAP7	Plasma membrane	Normal response to adrenergic stimulation (KD in cardiac myocytes) [40]	Heart failure (increased interaction with PKA) [194]
AKAP18ɣ, δ	AKAP7	Cytosol, SR, secretory vesicles	Normal response to adrenergic stimulation (KD in cardiac myocytes) [40]	Ca^{2+} reuptake into the sarcoplasmic reticulum of cardiac myocytes during diastole (AKAP18ɣ in human, AKAP18, δ in rat) [38, 198]
AKAP95	AKAP8	Nuclear matrix	viable with no overt phenotype (mouse KO) [199]	N/A
AKAP350/AKAP450	AKAP9	Centrosomes, Golgi (epithelial cells)	N/A	Increased breast cancer risk (SNP) [200]
Yotiao	AKAP9 isoform	Plasma membrane (postsynaptic)	Sertoli cell maturation defects in sperm (mouse KO) [201]	Heart failure (decreased interaction with PKA) [194]; cardiac arrhythmias and long Q-T syndrome (genetic polymorphism Ser1570Lys) [202]; male infertility (allelic mutations) [201]
D-AKAP2	AKAP10	Mitochondria, cytosol, endosomes	Shortened life span due to cardiac arrhythmia (AKAP10ΔRBD gene mutation in mice) [203]	Negative effect on longevity (SNP) [204]; increased basal heart rate, reduced heart rate variability [203]

AKAP220	AKAP11	Vesicles, peroxisomes, centrosome	Cell migration decreased (KD in metastatic human cancer cell lines) [205]	Overexpressed in tumors, oral carcinogenesis [206]
AKAP250/Gravin	AKAP12	Cytosol, Actin cytoskeleton	Prostatic hyperplasia [207]	Various solid tumors (Gene expression suppressed, hypermethylation of the promoter, increased metastasis) [126-131] ; acute myeloid leukemia (decreased expression) [134]
AKAP-Lbc	AKAP13	Cytosol, Actin cytoskeleton	Cardiac developmental defects, lethal cardiac arrest (mouse KO) [208]	Familial breast cancer (genetic polymorphism Lys526Gln) [111]; uterine leiomyoma; hypertrophic cardiomyopathy (increased transcript levels, cardiac fibroblast reprogramming towards cardiomyoblasts, α1-adrenoceptor-mediated cardiac hypertrophy) [26, 48, 49]
Ezrin (AKAP78)	EZR	Actin cytoskeleton	Enhanced HIV-1-induced cell-cell fusion assisiting virus spreading (KD in cultured T lymphoblasts) [152]	HIV transmission, immune response (Increased T cell activation) [144]
MAP2	MAP2	Microtubules	reduction in microtubule density in dendrites, reduction of dendritic length [209]	Heart failure (increased interaction with PKA) [194]; preserved speech variant of Rett syndrome (gene deletion) [210]
Neurobeachin	NBEA	Golgi, postsynaptic plasma membrane	PKA target phosphorylation altered (heterozygous KD in mouse platelets) [186]	Autism (gene disruption or partial deletion) [181, 182, 211]
OPA1	OPA1	Inner mitochondrial membrane, mitochondrial intermembrane space	Essential for early embryonic survival, increased mitochoncrial fission and fragmentation [212]	optic nerve degeneration; multisystem neurological disease, e.g. optic atrophy (various gene mutations) [213]
SKIP	SPHKAP	Cytosol	N/A	Heart failure (increased interaction with PKA) [194]
WAVE-1	WASF1	Actin cytoskeleton, mitochondria	Sensorimotor retardation, learning and memory deficits (mouse KO) [188]	N/A

Abbr.: HGNC: human genome nomenclature committee; KO: knockout; KD: knockdown; SNP: single nucleotide polymorphism. N/A, not available.

Recent advances in 3D structural analysis of AKAPs

A detailed understanding of the functioning of AKAPs requires insight into their 3D structures. An understanding of AKAPs and their protein-protein interactions at the atomic level permits rational design of pharmacological agents for selective targeting of individual AKAPs and their protein-protein interactions.

AKAP-PKA interactions

AKAP-PKA interactions are mediated by highly conserved A-kinase binding domains (AKB) of AKAPs and the dimerization and docking (D/D) domains of the N-termini of R subunits of PKA. The D/D domains of R subunits dimerize and form a hydrophobic groove as a docking site for the AKAP. D/D domains are X-type four helix bundle structures [3, 4, 214-218].

AKBs of the AKAP family are structurally conserved amphipathic α-helices of 14-18 amino acids in length [219]. NMR and X-ray crystallographic analyses of AKB-derived peptides of several AKAPs confirmed the α-helical structure and showed that the hydrophobic phase of the helix docks into the hydrophobic groove formed by the D/D domain. The hydrophilic phase of the helix can interact with hydrophilic amino acids at the rim of the D/D domain. Such interactions apparently increase the binding affinity [53, 214, 215, 220, 221].

A consensus sequence of polar and hydrophobic amino acids in conserved positions defines an AKAP signature motif: [AVLISE]-X-X-[AVLIF]-[AVLI]-X-X-[AVLI]-[AVLIF]-X-X-[AVLISE] (X = any amino acid, amino acids in [] represent alternatives at this position) [222, 223]. This AKAP signature motif was used to screen protein databases for new AKAPs. This approach identified GSK3β interaction protein (GSKIP) as an AKAP [223]. Recently, another bioinformatics tool for the mapping of PKA binding domains and the prediction of novel AKAPs, THAHIT (the AKAP/amphipathic helix identification tool), was introduced and a list of new RIα and RIIα binding domains for existing AKAPs was published suggesting that various AKAPs are D-AKAPs [224]. This new tool not only uses sequence information from the PKA binding domains of the known AKAPs but also includes the available structural information. THAHIT identified novel AKAPs, including a so far unknown AKAP of 330 kDa that is expressed in heart.

GSKIP is so far the only AKAP with a nearly fully resolved 3D structure (Protein Data Bank code 1sgo); of its 139 amino acids only the N-terminal 32 amino acids appeared unstructured in NMR analyses. The AKB resides between amino acids 28 and 52. The hydrophobic phase of the helix lies on the surface of the protein; the hydrophilic phase is buried by a β-sheet that follows the AKB in the primary structure (amino acids 49-115). The β-sheet, in turn, is followed by an α-helix that binds GSK3β (amino acids 116-139). GSKIP facilitates the phosphorylation of GSK3β at Serine 9 by PKA and thereby the inactivation of GSK3β [223].

Since 3D structures of other full-length AKAPs are not available, the positions of AKBs within the proteins are unknown. However, this is relevant as AKAP-PKA interactions are apparently regulated by AKAP-inherent mechanisms. The β-sheet covering the AKB of GSKIP may have to be displaced prior to R subunit binding or for modulating the affinity of the interaction [223]. The binding affinity of AKAP18δ for PKA is influenced by its N terminus; a N-terminally truncated version lacking the first 123 amino acids binds RII subunits of PKA with higher affinity than the full-length protein: K_D = 9 nM for AKAP18δ(124-353) and K_D = 31 nM for full-length AKAP18δ [68, 225].

A crystal structure of the central part of the 353 amino acids long AKAP18δ (amino acids 76–292) is

available [226]. Although this structure is lacking the C-terminal AKB (amino acids 301-314) it revealed the positions of positively charged amino acids that form a binding surface for the interaction with negatively charged membrane lipids and thus form the membrane-targeting domain of this AKAP [227]. The positively charged amino acids are distantly located in the primary structure but concentrate in the tertiary structure and form a clear binding surface that can engage in electrostatic interactions with membrane lipids. The defined binding surface does not explain the specific binding of AKAP18δ to AQP2-bearing membranes (see above) [225], as the electrostatic interactions rather point to non-selective binding to any negatively charged membrane and to a yet undefined protein-lipid or protein-protein interaction conferring specificity to the intracellular targeting of this AKAP.

Lessons from AKAP-based signalosome studies

Recent electron microscopy and molecular modeling studies [228] indicated for the first time the 3D arrangement of an AKAP together with PKA in a complex. Scott and colleagues found that AKAP18γ and PKA type II are in a pentameric configuration and suggest that the flexible linker region of the RII subunits that is located C-terminally from the D/D domain confers a radius of 16 nm to the C subunits. Within this distance the C subunits phosphorylate nearby substrates such as PDE4D bound to AKAP18γ. Surprisingly, the authors did not find a complete dissociation of the pentameric complex in response to cAMP elevation in HEK293 cells where the complex components were overexpressed. This is in contrast to earlier findings in renal principal cells where an elevation of cAMP leads to dissociation of the endogenously expressed RII subunits and C subunits from AKAP18δ [68]. Possibly, due to the differences in their N termini AKAP18γ and AKAP18δ behave differently with regard to cAMP responsiveness.

AKAP79 interacts with a variety of protein binding partners, including calcineurin, RII subunits of PKA and the Ca^{2+}-binding protein Calmodulin (CaM). Quantitative biochemical analyses and native mass spectrometry led to a 3D model of an AKAP79-based protein complex comprising these proteins. The model indicates dimerization of AKAP79 and shows binding of two AKBs, four calcineurin heterodimers and two CaMs. AKAP79 concentrates these proteins at the plasma membrane and establishes a local signalosome that can respond to two second messengers, cAMP and Ca^{2+} [229].

In hippocampal neurons, AKAP79 sequesters calcineurin to L-type Ca^{2+} channels and couples Ca^{2+} influx through the channels to the activation of calcineurin and of its substrate, the transcription factor Nuclear factor of activated T-cells (NFAT). X-ray analyses revealed details of the interaction of AKAP79 with calcineurin [230]. A sequence, IAIIIT, of AKAP79 directly binds calcineurin. The AKAP79 site binds to the same site as the PxIxIT motif of NFAT. In line, altering the AKAP79 binding site to decrease the affinity leads to activation of NFAT; *vice versa*, increasing the affinity of the calcineurin-AKAP79 interaction reduces NFAT activation. Although this structural information yields insight into the mechanisms underlying the AKAP79-calcineurin interaction and its functional role with regard to NFAT activation it is unclear whether the 3D structure should be a basis for a drug discovery program. NFAT activation in cardiac myocytes occurs through a signalosome consisting of mAKAPβ, calcineurin, PKA and the RyR2 and controls gene expression but is also involved in the development of cardiac hypertrophy. Thus consequences of pharmacological interference are difficult to predict.

The interaction of AKAP-Lbc and RhoA

AKAP-Lbc belongs to the Dbl family of RhoGEFs, whose RhoGEF activity is conferred by a tandem Dbl homology (DH) and pleckstrin homology (PH) domain (amino acids 1972-2342) [231-233]. DH domains bind GTPases of the Rho family, catalyze the exchange of GDP for GTP and thereby activate the GTPases. AKAP-Lbc selectively activates RhoA but not other members of the Rho family such as Rac and Cdc42 [22]. PH domains can control the localization and/or the activity of DH domains [231]. A crystal structure of the complex between RhoA-GDP and the AKAP-Lbc-DHPH domain showed that the PH domain is connected with the DH domain by a helical linker and that the switch I and II regions of RhoA bind to two distinct regions of the DH domain. The binding of RhoA induces a conformational change in the "GEF switch". The PH domain does not affect the activity of the DH domain [234]. The PH domain does apparently not interact with lipids explaining why Onco-Lbc remains soluble [110].

The affinity for the binding of RhoA to the AKAP-Lbc-DHPH domain is around 20 µM, i.e. a low affinity interaction that can potentially be targeted by inhibitors [234], e.g. for treatment of cardiac hypertrophy and/or cancer (see above).

Dynamic regulation of AKAP signalosomes

AKAP complexes are dynamically regulated at several levels. A redistribution within cells has been observed for some AKAPs, amongst them two actin-associated AKAPs (Gravin, WAVE1). Gravin can undergo trafficking from the cortical plasma membrane to the cytosol in response to stimuli following the elevation of intracellular calcium, involving not only PKA-dependent signaling but also the activation of PKC [235]. WAVE1, in complex with RII subunits of PKA and the actin-interacting Abl tyrosin kinase shuttles cell-cycle-dependently between the nuclear cortex and cytoplasmic foci in oocytes [236]; the translocation is essential for genomic and cytoskeletal dynamics during mammalian fertilization: while WAVE1 is distributed throughout the cytoplasm of metaphase II oocytes (co-localized with RII), WAVE1 relocalization towards the developing pronuclei is observable 8 h after sperm entry (insemination) accompanied by complete RII and Abl reorganization inside the pronuclei. The nuclear WAVE1 distribution stays until the nuclear envelope is broken down again during mitosis. The postsynaptic localization of PKA upon NMDA receptor activation is mediated by the translocation of AKAP150 [237]. Smith *et al.* demonstrated that brief NMDA receptor activation leads to a long-lasting redistribution of AKAP79/150 and PKA-RII, but not PP2B, from postsynaptic membranes to the cytoplasm in hippocampal neurons. AKAP79/150 trafficking requires PP2B activation and is accompanied by dephosphorylation and internalization of AMPA R subunit GluR1. In renal principal cells, the AVP-induced elevation of cAMP leads to the disassembly of the vesicular AKAP18δ-PKA complex: not only dissociate the catalytic subunits from the RII subunits that are bound to the AKAP, the RII subunits also dissociate from AKAP18δ; and AKAP18δ translocates together with AQP2 from intracellular vesicles to the plasma membrane (see above; [68]). The functional significance of the observed dissociation of RII subunits from the AKAP is unknown, possibly the released RII subunits inactivate the free catalytic subunits, constituting a negative feedback loop. Also the interaction of AKAP95 with RII subunits can be regulated. CDK1 mediates the phosphorylation of T54 of RIIα subunits. The phosphorylated RIIα binds to AKAP95. The AKAP95-PKA complex participates in remodeling chromatin during mitosis. Thus AKAPs are not solely stationary anchors in cells but can also serve as dynamic signaling components.

Current strategies to interfere with AKAP-dependent protein-protein-interactions

Pharmacological interference is an approach for elucidating functions of AKAPs and their individual protein-protein interactions. The ongoing efforts focus on targeting AKAP-PKA interactions with peptides, peptidomimetics and small molecules. Other AKAP-dependent interactions are targeted with peptides derived from the binding domain of either the AKAP or its interacting partner.

Disruptor peptides

The first AKAP-PKA disruptor peptide, Ht31, was derived from the AKB of AKAP-Lbc and characterized almost three decades ago [219]. As synthetic peptide or expressed from vectors, Ht31 was rapidly established as a valuable tool to study functional implication of anchored PKA signaling in a variety of cell systems and animal models. Ht31 and other AKAP-derived peptides bind to the D/D domain of PKA and compete with AKAPs for PKA binding. However, they do not distinguish between RI and RII PKA subunits and, therefore, cannot discriminate between RI- and RII-mediated processes. Bioinformatics approaches and peptide-array based optimization processes introduced a new generation of PKA anchoring disruptor peptides [25]. AKAP-*in silico* (AKAP-IS) and AKAP18δ-derived peptides with nanomolar affinities for both R subunits were developed [238]. RI- and RII-preferring and RI- and RII-specific peptides are available now, e.g. the RI anchoring disruptor, RIAD [239] and SuperAKAP-IS for RII [214]; the dual-specific D-AKAP2 was used as template for the generation of RI- and RII-preferring peptides [240]. All peptides are available as membrane-permeant versions for cell-based studies. For membrane permeation tags such stearate, myristoylic acid or TAT sequences have been used [25].

As the mode of interaction of AKAPs with PKA is conserved (see above), all of the disruptor peptides globally uncouple PKA from AKAPs, either type I or type II but in most cases both types of PKA. Recently, Gold *et al.* employed a phage display method to derive peptides from the D/D domain of RII subunits and engineered them in a way that they became binders for a limited number of AKAPs. The peptides R_{Select}AKAP2 and R_{Select}AKAP18 preferentially bound AKAP2 and AKAP18, respectively. These peptides represent new molecular tools that allow studying functions of a limited set of AKAP-PKA interactions [241].

Peptides were also used to target protein-protein interactions of AKAPs other than those with PKA. Some studies have been performed with peptides perturbing disease-related AKAP-dependent protein-protein interactions [e.g. the AKAP18δ-PLN binding [38] and AKAP18α-L-type Ca^{2+} channel interactions [242]].

A drawback of peptides is their generally short half-life, their low membrane-permeation and low oral bioavailability, which limits their use for cell and animal studies. In addition, they are considered difficult for the development towards drugs, although several peptide-based drugs have reached the market [243].

Small molecules

Small molecules are considered as an alternative to peptides. Their stability and ability to cross biological membranes allow their use in cell and animal studies. They have become powerful tools in studying functions of protein-protein interactions. High-throughput screening of small molecule libraries facilitates the discovery of novel compounds targeting the protein or protein-protein interaction of interest. Identified hits can be optimized with regard to specificity and affinity by medicinal chemistry approaches [244, 245].

The first small molecule targeting an AKAP-dependent protein-protein interaction is FMP-API-1. It was identified in a high-throughput screen of 20,000 druggable substances. FMP-API-1 allosterically binds RII subunits and thereby blocks AKAP-PKA interactions [36, 246]. In addition, PKA is activated upon FMP-API-1 binding. Both in rat neonatal cardiac myocytes and *ex vivo* in intact hearts the net effect of FMP-API-1 is an increase in cardiac contractility.

The membrane-associated G protein-coupled estrogen receptor 1 (GPER; initially called GPR30) is structurally unrelated to nuclear ERα or ERβ. In hypertension and ischemia-reperfusion models activation of GPER is protective as it relaxes arteries. Inhibition of AKAP-PKA interactions with FMP-API-1 attenuated the effect of GPER activation on coronary artery relaxation by a mechanism involving activation of myosin light chain phosphatase and inhibition of a RhoA pathway [247].

Peptidomimetics

Peptidomimetics are designed to combine advantages of peptides, i.e. the specificity and target selectivity, with those of small molecules, i.e. increased membrane permeation and stability. The first peptidomimetic for inhibition of an AKAP-dependent protein-protein interaction was based on the peptide RIAD, which selectively inhibits the interaction of RI subunits of PKA with AKAPs. The peptide was modified by inclusion of non-natural amino acids to improve stability [248]. A membrane-permeant version reduced cAMP levels in HIV-infected cultured T cells. In a mouse model, humanized NOD/SCID/IL2γnull (NSG) mice infected with HIV-1 the molecule limited HIV-1 replication and stabilized CD4 cell levels by interference with a cAMP/PKA type I pathway [153].

On the basis of the peptide AKAP18δ-L314E, which binds RII subunits with subnanomolar affinity, polypyridines were developed as helix mimetics [53, 220]. The terpyridin-based compounds mimic the hydrophobic phase of the peptidic α-helix by projecting amino acid-derived side chains from a hydrophobic rod-like axis [220]. The rod-like axis is composed of pyridine, cyclopentyl and benzyl rings and interacts with the bottom of the D/D domain pocket; carboxyl groups engage in interactions with hydrophilic residues at the rim of the D/D domain pocket and increase the binding affinity. K_D values for the interaction with the D/D domain of RIIα range from 30 – 148 µM; The terpyridines inhibit the interaction of RIIα with AKAP18 with IC_{50} values between 38 µM and 138 µM [220]. The newly synthesized terpyridine scaffolds represent the first nonpeptidic, biologically active compounds which interfere with AKAP-PKA interactions *in vitro* as well as *in vivo* in HEK293 cells [220]. Although the K_D and IC_{50} values of the terpyridines are high the new molecules exemplify the design of nonpeptide helix mimetics and pave the way to novel high affinity compounds for disruption of AKAP-PKA interactions.

Tab. 2 summarizes current approaches yielding peptides disruption AKAP-dependent PPIs. Of note, none of the disruptors reached the phase of clinical trial so far.

Table 2: Current inhibitors of AKAP-protein interactions and their physiological consequences

Type	**Name**	**Target**	**2.4.4.1 Effect on cells and isolated organs (Examples)**	**Model system**

Peptide	Ht-31	AKAP-PKA interaction	Stimulation of oocyte maturation; Increased β-AR-stimulated contractility; Reduced AMPA/kainite channel currents; Reduced antigen presentation, inhibition of TNFα and IL-10 production	Mouse oocytes [193]; Rat hearts [249]; Hippocampal neurons [169]; CD4+ T cells [154]
	S-Ht31	AKAP-PKA interaction	Inhibition of vasopressin-stimulated AQP-2 translocation; Inhibition of sperm motility	Renal inner medullary collecting duct (IMCD) cells [34]; Bovine caudal epididymal sperm [31]
	TAT-AKAD	AKAP-PKA interaction	Reduced contractility; Negative effect on chronotropy, ionotropy and lusitropy	Cardiac myocytes [250]
	AKAP15-LZ	AKAP18α-L-type Ca^{2+} channel interaction	Inhibition of voltage-dependent L-type Ca^{2+} channel potentiation; Inhibition of β-adrenoceptor-induced L-type Ca^{2+} currents	Mouse skeletal muscle cells (MM14, DZ1A) [242]; Mouse skeletal mouse [251]
	AKAP18δ-wt	AKAP-PKA interaction	Reduced β-adrenoceptor-induced L-type Ca^{2+} currents	Rat neonatal cardiac myocytes [53]
	Arg9-11-PLN	AKAP18δ-PLN interaction	Reduced andrenoceptor-induced Ca^{2+} reuptake into the SR	Rat neonatal and adult cardiac myocytes [53]
	RIAD-Arg11	AKAP-PKA RI interaction	Uncoupling of cAMP-mediated inhibition of T cell function; Reduced ACTH-stimulated progesterone production	T cells [239]; Mouse Y1 adrenocortical cells [239]
	AKAP-IS	AKAP-PKA interaction	Attenuation of AMPA receptor subunit GluR1 currents; Reduced antigen presentation	HEK293 cells [238]; CD4+ T cells, KG-1 dendritic progenitor cells [154, 156]
	TAT-AKAP-IS	AKAP-PKA interaction	Inhibition of glucagon-induced insulin secretion potentiation	INS-1 pancreatic β-cells [252]
	SuperAKAP-IS	AKAP-PKA RII interaction	Attenuation of AMPA-responsive currents	Hippocampal neurons [214]
	R_{Select}AKAP2 / R_{Select}AKAP18	AKAP2-PKA/ AKAP18-PKA interaction	Colocalization with AKAP18δ and MAP2	*In vitro*; *in vivo* in HEK293 cells [241]
	peptides derived from the	AKAP-PKA RI (PV-38) or AKAP-PKA RII	Colocalization with R subunits	*In vitro*; *in vivo* in 10T(1/2) cells [240]

	AKB of D-AKAP2 mutant versions	interaction		
Peptidomimetics **(inclusion of non-natural amino acids)**	Stabilized RIAD	AKAP-PKA RI interaction	Not determined	*In vitro* [248]
	RIAD-P3	AKAP-PKA RI interaction	Lower intracellular cAMP levels in T cells; Limited HIV-1 replication, stabilized CD4 cell levels	HIV-infected human peripheral blood mononuclear cells (PBMCs) [153]; Humanized NOD/SCID/IL2γnull (NSG) mice infected with HIV [153]
	STADs (Hydrocarbon STapled Anchoring Disruptors)	AKAP-PKA RII interaction	Intracellular access of the peptides, decreased PKA substrate phosphorylation, inhibition of cytosolic PKA activity	*In vitro*; *in vivo* in human cell lines (HeLa, MDA-MB-231, PC-3) [253]
	RI-STADs (RI-STapled Anchoring Disruptors)	AKAP-PKA RI interaction	Cell permeability and RI selectivity proven, AKAP-anchored signaling disrupted	*In vitro*; *in vivo* in human osteosarcoma U2 OS cells [254]
Non-peptide helix mimetics	Terpyridines	AKAP-PKA interaction	Prevents PGE_1-mediated, PKA-dependent negative feedback inhibiting AC	*In vitro*; *in vivo* in HEK293 cells [220]
Small molecules	FMP-API-1	AKAP-PKA R interaction, activation of PKA	Increase in cardiac contractility; Inhibition of G protein coupled estrogen receptor 1 (GPER)-mediated coronary artery vasodilation	Rat hearts; rat neonatal cardiac myocytes [36]; Cultured coronary artery smooth muscle cells (SMCs) [255]

Summary, conclusions and outlook

Due to non-causal and thus ineffective treatment, the medical need is huge in many diseases, including cancer and cardiovascular diseases. Heart failure alone affects 26 million people worldwide [256] and imposes costs for health care and lost productivity of an estimated 108 billion USD p.a [257]. In 2013, 382 million people worldwide suffered of diabetes and an increase of 55 % is estimated for the next 20 years [258]. These numbers stress the urgency for new therapeutic options in diseases affecting large parts of the populations of mainly Western countries.

Recent progress increases the understanding of the molecular mechanisms underlying the functioning of AKAPs and their protein-protein interactions. The discovery of new AKAPs, elucidation of the signaling processes they coordinate and of their physiological relevance and the detection of molecular aberrations related to AKAPs in diseases hint to new therapeutic concepts.

Due to their multiple protein-protein interactions individual AKAPs have various functions. As in the case of AKAP-Lbc's role in the development of cardiac hypertrophy these functions can be opposing, e.g. promote cardiac hypertrophy or be protective against it. This indicates first of all that therapeutic interventions at AKAPs require a detailed understanding of the AKAP of interest and secondly that strategies aiming at the elimination of a particular AKAP, e.g. by knockdown, need sceptical consideration beforehand, as loss of an AKAP causes loss of all of its functions. On the other hand, in conditions when an AKAP is downregulated such as the tumor suppressor gravin in solid tumors, measures to reconstitute its expression may be appropriate. Such strategies may include *in vitro* transcribed (IVT) mRNA, an approach that has reached clinical phase III trials in several indications including breast cancer [259].

The multiple protein-protein interactions of AKAPs each fulfilling different functions predestine them as targets for pharmacological interference. Generally, changes in the specificity and affinity of protein-protein interactions lead to malfunctions in cells and eventually to disease, making protein-protein interactions important drug targets for the next generation of therapies. Interference with intracellular protein-protein interaction is still a novel pharmacological concept, but proof-of-concept has been reached as first drugs have been approved (e.g. Tirobifan as cardiovascular drug, Maraviroc as anti-HIV drug) [260]. Tirobifan targets the glycoprotein IIb/IIIa complex on platelets reducing thrombotic cardiac events e.g. myocardial infarction while Maraviroc inhibits the interaction of HIV-1 gp120 with the chemokine coreceptor CCR5, a key step of HIV-1 entry into host cells. Peptide-based therapy is of high relevance also in other diseases, best exemplified by diabetes where insulin analogues are first line drugs for treatment, recently reviewed in [261].

In the case of AKAPs, interference with the 6 fold increased interactions of SPHKAP and AKAP2, the 2.4 fold increase of AKAP18 and the 12 fold increase of MAP2 with PKA in failing hearts [51] makes these interactions interesting targets. However, AKAP-PKA interactions are highly conserved and at present there are no agents available to selectively interfere with the interaction of a particular AKAP with PKA. Although attempts to develop peptides for inhibition of defined AKAP-PKA interactions are ongoing and some degree of specificity has been reached, i.e. peptides that discriminate between PKA types I and II (e.g. RIAD) and peptides that preferentially inhibit interactions of PKA with AKAP18 [241], it appears difficult to selectively target defined AKAP-PKA interactions due to the conserved mechanism underlying these interactions. This conservation also hampers the development of peptidomimetics and small molecules for inhibition of specific AKAP-PKA interactions. The first peptidomimetics [6, 153, 220] and a first small molecule, FMP-API-1, for the non-selective disruption of AKAP-PKA interactions are available [36, 247]. There are hints that allosteric sites such as the RI Specifier Region (RISR) in D-AKAPs [6] and a region C-terminally from the D/D domain of RII [36] are involved in AKAP-PKA interactions. Targeting such allosteric sites pharmacologically may reduce the affinity of the binding or even inhibit the interactions, as the sequences of e.g. RISRs are not highly conserved.

There are specific AKAP-dependent protein-protein interactions that appear relevant for disease such as the AKAP-Lbc-RhoA interaction seems relevant in cardiac hypertrophy and cancer. To validate

specific disease-relevant protein-protein interactions of AKAPs as drug targets, the development of small molecules for their inhibition is desirable. They are stable and membrane-permeant and thus suitable for validation procedures from cultured cells to clinical trials. Small molecules for targeting AKAP-dependent protein-protein interactions may be sought by high-throughput screening of libraries, a procedure by which FMP-API-1 was identified, or by rational ligand design. Rational design depends on high-resolution structural information on the interaction of interest. As more 3D structures of AKAP-dependent protein-protein interactions become available (e.g.[230, 234]) this is a likely strategy to be followed in the future. However, the prerequisite to make this a successful strategy is the development of novel approaches to obtain high-resolution structures of AKAPs that appear largely unfolded if generated recombinantly.

The examples chosen here to illustrate the involvement of AKAPs in disease are by far not exhaustive. AKAPs play roles in many more cellular processes whose dysregulation leads to disease. They are involved in complex mechanisms such as memory formation and blood pressure control [161, 262, 263] and tether different protein assemblies to various subcellular compartments [264]. Thus AKAPs are a rich source to be considered as pharmacological targets. The development of drug candidates targeting AKAPs and their testing in clinical trials remains a task and a goal for the future.

List of abbreviations

AC: adenylyl cyclase
AKAP: A-kinase anchoring protein
AKAP-IS: AKAP-*in silico*
AKAP-Lbc: AKAP-Lymphoid blast crisis
AKB:A-kinase binding domains
AQP2: Aquaporin-2
ASM: airway smooth muscle
ATP: adenosine triphosphate
AVP: arginine vasopressin
BAD: Bcl-2 antagonist of cell death
BCAM/Lu: basal cell adhesion molecule/ Lutheran
C: catalytic
cAMP: cyclic AMP
CD4: cluster of differentiation 4
COPD: chronic obstructive pulmonary disease
Crk: C-terminal Src
Csk: C-terminal Src kinase
D/D: dimerization and docking
D-AKAP: dual-specific AKAP
DH: Dbl-homologous
Epac: exchange proteins activated by phosphatases
ERK: extracellular signalling related kinase
FAK: Focal adhesion kinase
FMP-API-1: small molecule identified by high-throughput screen by Christian et al. [36]
GDP: guanosine diphosphate
GEF: guanine nucleotide exchange factor
GK: Glucokinase
GLP-1: glucagon-like peptide 1
GPCR: G-protein coupled receptor
GPER: G protein-coupled estrogen receptor 1
G_s: stimulatory G protein
GSK3β: Glycogen synthase kinase 3β
GSKIP: glycogen synthase kinase 3β (GSK3β) interaction protein
GTP: guanosine triphosphate
HEK: human embryonic kindey
HIV: human immunodeficiency virus
Ht31: peptide derived from RIIBD of AKAP-Lbc
IC50: mean inhibitory concentration
IL-8: Interleukin 8
KO: knockout
KSR: kinase suppressor of Raf
LPL: lipoprotein lipase
mAKAP: muscle-selective AKAP
MAP2D: microtubule-associated protein 2D

MAPK: mitogen activated protein kinase
MEK: mitogen activated protein kinase kinase (MAPKK)
mRNA: messenger RNA
MyBP: myosin-binding protein
NDI: nephrogenic diabetes insipidus
NFAT: nuclear factor activated in T-cells
NMR: nuclear magnetic resonance
OPA1: optic artrophy 1
PDE: phosphodiesterase
PGE_2: prostaglandin E_2
PH: pleckstrin homology
PKA: (cAMP-dependent) protein kinase A
PKC: protein kinase C
PKD: Polycystic kidney disease
PKNα: protein kinase Nα
PLN: phospholamban
PP1: protein phosphatase 1
PP2: protein phosphatase 2, synonym for calcineurin
R: regulatory
Raf: Rat fibrosarcoma
Ras: Rat sarcoma
RIAD: unnatural RI anchoring disruptor
RIIBD: RII binding domain
RyR2: type 2 ryanodine receptor
SERCA2: sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase 2
shRNA: short hairpin RNA
SIADH: syndrome of inappropriate antidiuretic hormone secretion
SR: sarcoplasmic reticulum
Src: Sarcoma
SSeCKS: Src suppressed
SSeCKs: Src-suppressed C kinase substrate
TAC: Thoracic aortic constriction
TCR: T cell receptor
TnI: Troponin I
TRPV1: Transient Receptor Potential Vanilloid 1
V2R: vasopressin V_2 receptor
WAVE-1: WASP-family verprolin homologous protein 1
Yotiao: synonym for AKAP9

Conflict of interest

The authors declare that they do not have conflicts of interest.

References

1. Wu, J., et al., *PKA type IIalpha holoenzyme reveals a combinatorial strategy for isoform diversity.* Science, 2007. **318**(5848): p. 274-9.
2. Kim, C., et al., *PKA-I holoenzyme structure reveals a mechanism for cAMP-dependent activation.* Cell, 2007. **130**(6): p. 1032-43.
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. 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.
7. Skroblin, P., et al., *Mechanisms of protein kinase a anchoring.* Int Rev Cell Mol Biol, 2010. **283**: p. 235-330.
8. 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.
9. 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.
10. Troger, J., et al., *A-kinase anchoring proteins as potential drug targets.* British journal of pharmacology, 2012. **166**(2): p. 420-33.
11. Appert-Collin, A., L. Baisamy, and D. Diviani, *Regulation of g protein-coupled receptor signaling by a-kinase anchoring proteins.* J Recept Signal Transduct Res, 2006. **26**(5-6): p. 631-46.
12. Dessauer, C.W., *Adenylyl cyclase--A-kinase anchoring protein complexes: the next dimension in cAMP signaling.* Mol Pharmacol, 2009. **76**(5): p. 935-41.
13. Cooper, D.M. and V.G. Tabbasum, *Adenylate cyclase-centred microdomains.* Biochem J, 2014. **462**(2): p. 199-213.
14. Nijholt, I.M., et al., *Neuronal AKAP150 coordinates PKA and Epac-mediated PKB/Akt phosphorylation.* Cell Signal, 2008. **20**(10): p. 1715-24.
15. Houslay, M.D., *Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown.* Trends Biochem Sci, 2010. **35**(2): p. 91-100.
16. Maurice, D.H., et al., *Advances in targeting cyclic nucleotide phosphodiesterases.* Nat Rev Drug Discov, 2014. **13**(4): p. 290-314.
17. Perez Lopez, I., et al., *A-kinase anchoring protein Lbc coordinates a p38 activating signaling complex controlling compensatory cardiac hypertrophy.* Mol Cell Biol, 2013. **33**(15): p. 2903-17.
18. Schillace, R.V., et al., *Multiple interactions within the AKAP220 signaling complex contribute to protein phosphatase 1 regulation.* J Biol Chem, 2001. **276**(15): p. 12128-34.
19. Oliveria, S.F., M.L. Dell'Acqua, and W.A. Sather, *AKAP79/150 anchoring of calcineurin controls neuronal L-type Ca2+ channel activity and nuclear signaling.* Neuron, 2007. **55**(2): p. 261-75.
20. Flynn, M.P., et al., *Luteinizing hormone receptor activation in ovarian granulosa cells promotes protein kinase A-dependent dephosphorylation of microtubule-associated protein 2D.* Mol Endocrinol, 2008. **22**(7): p. 1695-710.
21. Soni, S., et al., *Anchored protein kinase A signalling in cardiac cellular electrophysiology.* J Cell Mol Med, 2014.
22. Diviani, D., J. Soderling, and J.D. Scott, *AKAP-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation.* The Journal of biological chemistry, 2001. **276**(47): p. 44247-57.
23. Alto, N.M., J. Soderling, and J.D. Scott, *Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics.* J Cell Biol, 2002. **158**(4): p. 659-68.

24. Gelman, I.H., *The role of SSeCKS/gravin/AKAP12 scaffolding proteins in the spaciotemporal control of signaling pathways in oncogenesis and development.* Front Biosci, 2002. **7**: p. d1782-97.
25. Hundsrucker, C. and E. Klussmann, *Direct AKAP-mediated protein-protein interactions as potential drug targets.* Handb Exp Pharmacol, 2008(186): p. 483-503.
26. Carnegie, G.K., et al., *AKAP-Lbc mobilizes a cardiac hypertrophy signaling pathway.* Mol Cell, 2008. **32**(2): p. 169-79.
27. Mauban, J.R., et al., *AKAP-scaffolding proteins and regulation of cardiac physiology.* Physiology (Bethesda), 2009. **24**: p. 78-87.
28. Diviani, D., et al., *A-kinase anchoring proteins: molecular regulators of the cardiac stress response.* Biochim Biophys Acta, 2013. **1833**(4): p. 901-8.
29. Carr, D.W. and A.E. Newell, *The role of A-kinase anchoring proteins (AKaps) in regulating sperm function.* Soc Reprod Fertil Suppl, 2007. **63**: p. 135-41.
30. Luconi, M., et al., *Role of a-kinase anchoring proteins (AKAPs) in reproduction.* Front Biosci (Landmark Ed), 2011. **16**: p. 1315-30.
31. Vijayaraghavan, S., et al., *Protein kinase A-anchoring inhibitor peptides arrest mammalian sperm motility.* J Biol Chem, 1997. **272**(8): p. 4747-52.
32. Lester, L.B., L.K. Langeberg, and J.D. Scott, *Anchoring of protein kinase A facilitates hormone-mediated insulin secretion.* Proc Natl Acad Sci U S A, 1997. **94**(26): p. 14942-7.
33. Torgersen, K.M., E.M. Aandahl, and K. Tasken, *Molecular architecture of signal complexes regulating immune cell function.* Handb Exp Pharmacol, 2008(186): p. 327-63.
34. 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.
35. 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.* The Journal of biological chemistry, 2010. **285**(36): p. 27632-40.
36. 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.
37. 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.
38. Lygren, B., et al., *AKAP complex regulates Ca2+ re-uptake into heart sarcoplasmic reticulum.* EMBO Rep, 2007. **8**(11): p. 1061-7.
39. Lin, C., et al., *Cypher/ZASP is a novel A-kinase anchoring protein.* J Biol Chem, 2013. **288**(41): p. 29403-13.
40. 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.
41. 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.
42. 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.
43. Diviani, D., *Modulation of cardiac function by A-kinase anchoring proteins.* Curr Opin Pharmacol, 2008. **8**(2): p. 166-73.
44. del Vescovo, C.D., S. Cotecchia, and D. Diviani, *A-kinase-anchoring protein-Lbc anchors IkappaB kinase beta to support interleukin-6-mediated cardiomyocyte hypertrophy.* Mol Cell Biol, 2013. **33**(1): p. 14-27.
45. Cariolato, L., S. Cavin, and D. Diviani, *A-kinase anchoring protein (AKAP)-Lbc anchors a PKN-based signaling complex involved in alpha1-adrenergic receptor-induced p38 activation.* J Biol Chem, 2011. **286**(10): p. 7925-37.
46. Carnegie, G.K., et al., *AKAP-Lbc nucleates a protein kinase D activation scaffold.* Mol Cell, 2004. **15**(6): p. 889-99.

47. Taglieri, D.M., et al., *The C-terminus of the long AKAP13 isoform (AKAP-Lbc) is critical for development of compensatory cardiac hypertrophy.* J Mol Cell Cardiol, 2014. **66**: p. 27-40.
48. 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.
49. 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.
50. 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.
51. Aye, T.T., et al., *Reorganized PKA-AKAP associations in the failing human heart.* Journal of molecular and cellular cardiology, 2012. **52**(2): p. 511-8.
52. 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.
53. 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.
54. Lygren, B. and K. Tasken, *The potential use of AKAP18delta as a drug target in heart failure patients.* Expert Opin Biol Ther, 2008. **8**(8): p. 1099-108.
55. Klussmann, E. and W. Rosenthal, *Role and identification of protein kinase A anchoring proteins in vasopressin-mediated aquaporin-2 translocation.* Kidney Int, 2001. **60**(2): p. 446-9.
56. Nedvetsky, P.I., et al., *Regulation of aquaporin-2 trafficking.* Handb Exp Pharmacol, 2009(190): p. 133-57.
57. Szaszak, M., et al., *Compartmentalized cAMP signalling in regulated exocytic processes in non-neuronal cells.* Cell Signal, 2008. **20**(4): p. 590-601.
58. Sasaki, S., *Aquaporin 2: from its discovery to molecular structure and medical implications.* Mol Aspects Med, 2012. **33**(5-6): p. 535-46.
59. Verkman, A.S., M.O. Anderson, and M.C. Papadopoulos, *Aquaporins: important but elusive drug targets.* Nat Rev Drug Discov, 2014. **13**(4): p. 259-77.
60. Nedvetsky, P.I., et al., *Reciprocal regulation of aquaporin-2 abundance and degradation by protein kinase A and p38-MAP kinase.* J Am Soc Nephrol, 2010. **21**(10): p. 1645-56.
61. King, L.S., D. Kozono, and P. Agre, *From structure to disease: the evolving tale of aquaporin biology.* Nat Rev Mol Cell Biol, 2004. **5**(9): p. 687-98.
62. Klussmann, E., K. Maric, and W. Rosenthal, *The mechanisms of aquaporin control in the renal collecting duct.* Rev Physiol Biochem Pharmacol, 2000. **141**: p. 33-95.
63. Valenti, G., et al., *Minireview: aquaporin 2 trafficking.* Endocrinology, 2005. **146**(12): p. 5063-70.
64. Robben, J.H., N.V. Knoers, and P.M. Deen, *Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus.* Am J Physiol Renal Physiol, 2006. **291**(2): p. F257-70.
65. Chen, H.H. and R.W. Schrier, *Pathophysiology of volume overload in acute heart failure syndromes.* Am J Med, 2006. **119**(12 Suppl 1): p. S11-6.
66. Kwon, T.H., et al., *Aquaporins in the kidney.* Handb Exp Pharmacol, 2009(190): p. 95-132.
67. Schrier, R.W. and M.A. Cadnapaphornchai, *Renal aquaporin water channels: from molecules to human disease.* Prog Biophys Mol Biol, 2003. **81**(2): p. 117-31.
68. 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.
69. Henn, V., et al., *Compartmentalized cAMP signalling regulates vasopressin-mediated water reabsorption by controlling aquaporin-2.* Biochem Soc Trans, 2005. **33**(Pt 6): p. 1316-8.
70. Okutsu, R., et al., *AKAP220 colocalizes with AQP2 in the inner medullary collecting ducts.* Kidney Int, 2008. **74**(11): p. 1429-33.
71. 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.

72. Mekahli, D., et al., *Polycystins and cellular Ca2+ signaling.* Cell Mol Life Sci, 2013. **70**(15): p. 2697-712.
73. Torres, V.E. and P.C. Harris, *Strategies targeting cAMP signaling in the treatment of polycystic kidney disease.* J Am Soc Nephrol, 2014. **25**(1): p. 18-32.
74. Streets, A.J., et al., *Hyperphosphorylation of polycystin-2 at a critical residue in disease reveals an essential role for polycystin-1-regulated dephosphorylation.* Hum Mol Genet, 2013. **22**(10): p. 1924-39.
75. Choi, Y.H., et al., *Polycystin-2 and phosphodiesterase 4C are components of a ciliary A-kinase anchoring protein complex that is disrupted in cystic kidney diseases.* Proc Natl Acad Sci U S A, 2011. **108**(26): p. 10679-84.
76. Leech, C.A., et al., *Molecular physiology of glucagon-like peptide-1 insulin secretagogue action in pancreatic beta cells.* Prog Biophys Mol Biol, 2011. **107**(2): p. 236-47.
77. Dyachok, O., et al., *Oscillations of cyclic AMP in hormone-stimulated insulin-secreting beta-cells.* Nature, 2006. **439**(7074): p. 349-52.
78. Ni, Q., et al., *Signaling diversity of PKA achieved via a Ca2+-cAMP-PKA oscillatory circuit.* Nat Chem Biol, 2011. **7**(1): p. 34-40.
79. Conti, M. and J. Beavo, *Biochemistry and physiology of cyclic nucleotide phosphodiesterases: essential components in cyclic nucleotide signaling.* Annu Rev Biochem, 2007. **76**: p. 481-511.
80. Tengholm, A., *Cyclic AMP dynamics in the pancreatic beta-cell.* Ups J Med Sci, 2012. **117**(4): p. 355-69.
81. 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.
82. Lester, L.B., et al., *Targeted protein kinase A and PP-2B regulate insulin secretion through reversible phosphorylation.* Endocrinology, 2001. **142**(3): p. 1218-27.
83. Josefsen, K., et al., *AKAP 18 alpha and gamma have opposing effects on insulin release in INS-1E cells.* FEBS Lett, 2010. **584**(1): p. 81-5.
84. Hinke, S.A., et al., *Anchored phosphatases modulate glucose homeostasis.* EMBO J, 2012. **31**(20): p. 3991-4004.
85. Teo, A.K. and R.N. Kulkarni, *Setting sail for glucose homeostasis with the AKAP150-PP2B-anchor.* EMBO J, 2012. **31**(20): p. 3956-7.
86. Miller, R.A., et al., *Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP.* Nature, 2013. **494**(7436): p. 256-60.
87. Yang, J., et al., *Molecular modeling of BAD complex resided in a mitochondrion integrating glycolysis and apoptosis.* J Theor Biol, 2010. **266**(2): p. 231-41.
88. Danial, N.N., et al., *BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis.* Nature, 2003. **424**(6951): p. 952-6.
89. Vionnet, N., et al., *Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus.* Nature, 1992. **356**(6371): p. 721-2.
90. Grimsby, J., et al., *Allosteric activators of glucokinase: potential role in diabetes therapy.* Science, 2003. **301**(5631): p. 370-3.
91. DeFronzo, R.A., *Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM.* Diabetes, 1988. **37**(6): p. 667-87.
92. Sarabu, R., et al., *Discovery of piragliatin--first glucokinase activator studied in type 2 diabetic patients.* J Med Chem, 2012. **55**(16): p. 7021-36.
93. 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.
94. Ohsaki, Y., M. Suzuki, and T. Fujimoto, *Open questions in lipid droplet biology.* Chem Biol, 2014. **21**(1): p. 86-96.
95. Rogne, M. and K. Tasken, *Compartmentalization of cAMP Signaling in Adipogenesis, Lipogenesis, and Lipolysis.* Horm Metab Res, 2014.
96. Bridges, D., et al., *Identification and characterization of D-AKAP1 as a major adipocyte PKA and PP1 binding protein.* Biochem Biophys Res Commun, 2006. **346**(1): p. 351-7.

97. Marrades, M.P., et al., *A dysregulation in CES1, APOE and other lipid metabolism-related genes is associated to cardiovascular risk factors linked to obesity.* Obes Facts, 2010. **3**(5): p. 312-8.
98. Merrill, R.A. and S. Strack, *Mitochondria: a kinase anchoring protein 1, a signaling platform for mitochondrial form and function.* Int J Biochem Cell Biol, 2014. **48**: p. 92-6.
99. Ginsberg, M.D., et al., *PKA-dependent binding of mRNA to the mitochondrial AKAP121 protein.* J Mol Biol, 2003. **327**(4): p. 885-97.
100. Grozdanov, P.N. and D.M. Stocco, *Short RNA molecules with high binding affinity to the KH motif of A-kinase anchoring protein 1 (AKAP1): implications for the regulation of steroidogenesis.* Mol Endocrinol, 2012. **26**(12): p. 2104-17.
101. Oonishi, T., K. Sakashita, and N. Uyesaka, *Regulation of red blood cell filterability by Ca2+ influx and cAMP-mediated signaling pathways.* Am J Physiol, 1997. **273**(6 Pt 1): p. C1828-34.
102. Hines, P.C., et al., *Novel epinephrine and cyclic AMP-mediated activation of BCAM/Lu-dependent sickle (SS) RBC adhesion.* Blood, 2003. **101**(8): p. 3281-7.
103. Hebbel, R.P., et al., *Abnormal adherence of sickle erythrocytes to cultured vascular endothelium: possible mechanism for microvascular occlusion in sickle cell disease.* J Clin Invest, 1980. **65**(1): p. 154-60.
104. Ballas, S.K. and N. Mohandas, *Pathophysiology of vaso-occlusion.* Hematol Oncol Clin North Am, 1996. **10**(6): p. 1221-39.
105. Maciaszek, J.L., et al., *AKAP-dependent modulation of BCAM/Lu adhesion on normal and sickle cell disease RBCs revealed by force nanoscopy.* Biophys J, 2014. **106**(6): p. 1258-67.
106. Hillery, C.A., et al., *Hydroxyurea therapy decreases the in vitro adhesion of sickle erythrocytes to thrombospondin and laminin.* Br J Haematol, 2000. **109**(2): p. 322-7.
107. Davies, H., et al., *Mutations of the BRAF gene in human cancer.* Nature, 2002. **417**(6892): p. 949-54.
108. 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.
109. 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.
110. Lenoir, M., et al., *Structural Insights into the Activation of the RhoA GTPase by the Lbc Oncoprotein.* J Biol Chem, 2014.
111. 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.
112. Rogers, R., et al., *Mechanical homeostasis is altered in uterine leiomyoma.* Am J Obstet Gynecol, 2008. **198**(4): p. 474 e1-11.
113. Zheng, Y., et al., *Direct involvement of the small GTP-binding protein Rho in lbc oncogene function.* J Biol Chem, 1995. **270**(16): p. 9031-4.
114. Toksoz, D. and D.A. Williams, *Novel human oncogene lbc detected by transfection with distinct homology regions to signal transduction products.* Oncogene, 1994. **9**(2): p. 621-8.
115. Olson, M.F., et al., *Distinct roles for DH and PH domains in the Lbc oncogene.* Oncogene, 1997. **15**(23): p. 2827-31.
116. Ridley, A.J. and A. Hall, *The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors.* Cell, 1992. **70**(3): p. 389-99.
117. 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.
118. 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.
119. Ridley, A.J. and A. Hall, *Distinct patterns of actin organization regulated by the small GTP-binding proteins Rac and Rho.* Cold Spring Harb Symp Quant Biol, 1992. **57**: p. 661-71.
120. Caretta, A. and C. Mucignat-Caretta, *Protein kinase a in cancer.* Cancers (Basel), 2011. **3**(1): p. 913-26.

121. Sechi, A.S. and J. Wehland, *ENA/VASP proteins: multifunctional regulators of actin cytoskeleton dynamics.* Front Biosci, 2004. **9**: p. 1294-310.
122. Troger, J., et al., *A-kinase anchoring proteins as potential drug targets.* Br J Pharmacol, 2012. **166**(2): p. 420-33.
123. Gold, M.G., T. Gonen, and J.D. Scott, *Local cAMP signaling in disease at a glance.* J Cell Sci, 2013. **126**(Pt 20): p. 4537-43.
124. 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.
125. 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.
126. 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.
127. 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.
128. 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.
129. Jo, U.H., et al., *Methylation of AKAP12{alpha} promoter in lung cancer.* Anticancer Res, 2010. **30**(11): p. 4595-600.
130. Liu, W., et al., *Quantitative assessment of AKAP12 promoter methylation in colorectal cancer using methylation-sensitive high resolution melting: Correlation with Duke's stage.* Cancer Biol Ther, 2010. **9**(11): p. 862-71.
131. Wu, W., et al., *Examination of AKAP12 promoter methylation in skin cancer using methylation-sensitive high-resolution melting analysis.* Clin Exp Dermatol, 2011. **36**(4): p. 381-5.
132. 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.
133. Su, B., et al., *SSeCKS/Gravin/AKAP12 inhibits cancer cell invasiveness and chemotaxis by suppressing a protein kinase C- Raf/MEK/ERK pathway.* J Biol Chem, 2010. **285**(7): p. 4578-86.
134. Mostafa, M.R., et al., *Gravin gene expression in acute myeloid leukemia.* Med Oncol, 2013. **30**(2): p. 548.
135. 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.
136. 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.
137. Chiriva-Internati, M., et al., *AKAP-4: a novel cancer testis antigen for multiple myeloma.* Br J Haematol, 2008. **140**(4): p. 465-8.
138. 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.
139. 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.
140. 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.
141. Hofmann, B., et al., *Human immunodeficiency virus proteins induce the inhibitory cAMP/protein kinase A pathway in normal lymphocytes.* Proc Natl Acad Sci U S A, 1993. **90**(14): p. 6676-80.
142. Aandahl, E.M., et al., *Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients.* FASEB J, 1998. **12**(10): p. 855-62.
143. 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.

144. Kammer, G.M., *The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response.* Immunol Today, 1988. **9**(7-8): p. 222-9.
145. 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.
146. Liang, W.S., et al., *Therapeutic targets for HIV-1 infection in the host proteome.* Retrovirology, 2005. **2**: p. 20.
147. Lemay, J., et al., *AKAP149 binds to HIV-1 reverse transcriptase and is involved in the reverse transcription.* J Mol Biol, 2008. **383**(4): p. 783-96.
148. Ruppelt, A., et al., *Inhibition of T cell activation by cyclic adenosine 5'-monophosphate requires lipid raft targeting of protein kinase A type I by the A-kinase anchoring protein ezrin.* J Immunol, 2007. **179**(8): p. 5159-68.
149. Lone, A.M. and K. Tasken, *Proinflammatory and immunoregulatory roles of eicosanoids in T cells.* Front Immunol, 2013. **4**: p. 130.
150. Rahmouni, S., et al., *Cyclo-oxygenase type 2-dependent prostaglandin E2 secretion is involved in retrovirus-induced T-cell dysfunction in mice.* Biochem J, 2004. **384**(Pt 3): p. 469-76.
151. Mosenden, R., et al., *Mice with disrupted type I protein kinase A anchoring in T cells resist retrovirus-induced immunodeficiency.* J Immunol, 2011. **186**(9): p. 5119-30.
152. Roy, N.H., et al., *Ezrin is a component of the HIV-1 virological presynapse and contributes to the inhibition of cell-cell fusion.* J Virol, 2014. **88**(13): p. 7645-58.
153. Singh, M., et al., *The RIAD peptidomimetic inhibits HIV-1 replication in humanized NSG mice.* Eur J Clin Invest, 2013.
154. Schillace, R.V., et al., *A-kinase anchoring in dendritic cells is required for antigen presentation.* PLoS One, 2009. **4**(3): p. e4807.
155. Bagley, K.C., et al., *Pertussis toxin and the adenylate cyclase toxin from Bordetella pertussis activate human monocyte-derived dendritic cells and dominantly inhibit cytokine production through a cAMP-dependent pathway.* J Leukoc Biol, 2002. **72**(5): p. 962-9.
156. Schillace, R.V., C.L. Miller, and D.W. Carr, *AKAPs in lipid rafts are required for optimal antigen presentation by dendritic cells.* Immunol Cell Biol, 2011. **89**(5): p. 650-8.
157. Hogg, J.C. and W. Timens, *The pathology of chronic obstructive pulmonary disease.* Annu Rev Pathol, 2009. **4**: p. 435-59.
158. Barnes, P.J., *Immunology of asthma and chronic obstructive pulmonary disease.* Nat Rev Immunol, 2008. **8**(3): p. 183-92.
159. Ito, K., et al., *Decreased histone deacetylase activity in chronic obstructive pulmonary disease.* N Engl J Med, 2005. **352**(19): p. 1967-76.
160. Oenema, T.A., et al., *Pro-inflammatory mechanisms of muscarinic receptor stimulation in airway smooth muscle.* Respir Res, 2010. **11**: p. 130.
161. Poppinga, W.J., et al., *A-kinase anchoring proteins: Cyclic AMP compartmentalization in neurodegenerative and obstructive pulmonary diseases.* Br J Pharmacol, 2014.
162. Oldenburger, A., et al., *Anti-inflammatory role of the cAMP effectors Epac and PKA: implications in chronic obstructive pulmonary disease.* PLoS One, 2012. **7**(2): p. e31574.
163. Roscioni, S.S., et al., *PKA and Epac cooperate to augment bradykinin-induced interleukin-8 release from human airway smooth muscle cells.* Respir Res, 2009. **10**: p. 88.
164. Roscioni, S.S., et al., *Protein kinase A and the exchange protein directly activated by cAMP (Epac) modulate phenotype plasticity in human airway smooth muscle.* Br J Pharmacol, 2011. **164**(3): p. 958-69.
165. Horvat, S.J., et al., *A-kinase anchoring proteins regulate compartmentalized cAMP signaling in airway smooth muscle.* FASEB J, 2012. **26**(9): p. 3670-9.
166. Bliss, T.V. and G.L. Collingridge, *A synaptic model of memory: long-term potentiation in the hippocampus.* Nature, 1993. **361**(6407): p. 31-9.
167. Martin, S.J. and R.G. Morris, *New life in an old idea: the synaptic plasticity and memory hypothesis revisited.* Hippocampus, 2002. **12**(5): p. 609-36.
168. Tunquist, B.J., et al., *Loss of AKAP150 perturbs distinct neuronal processes in mice.* Proc Natl Acad Sci U S A, 2008. **105**(34): p. 12557-62.

169. Rosenmund, C., et al., *Anchoring of protein kinase A is required for modulation of AMPA/kainate receptors on hippocampal neurons.* Nature, 1994. **368**(6474): p. 853-6.
170. Sanderson, J.L. and M.L. Dell'Acqua, *AKAP signaling complexes in regulation of excitatory synaptic plasticity.* Neuroscientist, 2011. **17**(3): p. 321-36.
171. Jayakar, S.S., et al., *PACAP induces plasticity at autonomic synapses by nAChR-dependent NOS1 activation and AKAP-mediated PKA targeting.* Mol Cell Neurosci, 2014. **63C**: p. 1-12.
172. Joiner, M.L., et al., *Assembly of a beta2-adrenergic receptor--GluR1 signalling complex for localized cAMP signalling.* EMBO J, 2010. **29**(2): p. 482-95.
173. Balijepalli, R.C., et al., *Localization of cardiac L-type Ca(2+) channels to a caveolar macromolecular signaling complex is required for beta(2)-adrenergic regulation.* Proc Natl Acad Sci U S A, 2006. **103**(19): p. 7500-5.
174. Davare, M.A., et al., *A beta2 adrenergic receptor signaling complex assembled with the Ca2+ channel Cav1.2.* Science, 2001. **293**(5527): p. 98-101.
175. Wang, D., et al., *Binding of amyloid beta peptide to beta2 adrenergic receptor induces PKA-dependent AMPA receptor hyperactivity.* FASEB J, 2010. **24**(9): p. 3511-21.
176. Schnizler, K., et al., *Protein kinase A anchoring via AKAP150 is essential for TRPV1 modulation by forskolin and prostaglandin E2 in mouse sensory neurons.* J Neurosci, 2008. **28**(19): p. 4904-17.
177. Zhang, X., L. Li, and P.A. McNaughton, *Proinflammatory mediators modulate the heat-activated ion channel TRPV1 via the scaffolding protein AKAP79/150.* Neuron, 2008. **59**(3): p. 450-61.
178. Jeske, N.A., et al., *A-kinase anchoring protein 150 controls protein kinase C-mediated phosphorylation and sensitization of TRPV1.* Pain, 2009. **146**(3): p. 301-7.
179. Por, E.D., et al., *PP2B/calcineurin-mediated desensitization of TRPV1 does not require AKAP150.* Biochem J, 2010. **432**(3): p. 549-56.
180. Efendiev, R., et al., *Scaffolding by A-kinase anchoring protein enhances functional coupling between adenylyl cyclase and TRPV1 channel.* J Biol Chem, 2013. **288**(6): p. 3929-37.
181. Kaminsky, E.B., et al., *An evidence-based approach to establish the functional and clinical significance of copy number variants in intellectual and developmental disabilities.* Genet Med, 2011. **13**(9): p. 777-84.
182. Anney, R., et al., *A genome-wide scan for common alleles affecting risk for autism.* Hum Mol Genet, 2010. **19**(20): p. 4072-82.
183. Prasad, A., et al., *A discovery resource of rare copy number variations in individuals with autism spectrum disorder.* G3 (Bethesda), 2012. **2**(12): p. 1665-85.
184. Wang, X., et al., *Neurobeachin: A protein kinase A-anchoring, beige/Chediak-higashi protein homolog implicated in neuronal membrane traffic.* J Neurosci, 2000. **20**(23): p. 8551-65.
185. Volders, K., K. Nuytens, and J.W. Creemers, *The autism candidate gene Neurobeachin encodes a scaffolding protein implicated in membrane trafficking and signaling.* Curr Mol Med, 2011. **11**(3): p. 204-17.
186. Nuytens, K., et al., *Platelets of mice heterozygous for neurobeachin, a candidate gene for autism spectrum disorder, display protein changes related to aberrant protein kinase A activity.* Mol Autism, 2013. **4**(1): p. 43.
187. Dahl, J.P., et al., *Characterization of the WAVE1 knock-out mouse: implications for CNS development.* J Neurosci, 2003. **23**(8): p. 3343-52.
188. Soderling, S.H., et al., *Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice.* Proc Natl Acad Sci U S A, 2003. **100**(4): p. 1723-8.
189. Ceglia, I., et al., *Signaling pathways controlling the phosphorylation state of WAVE1, a regulator of actin polymerization.* J Neurochem, 2010. **114**(1): p. 182-90.
190. Yokoyama, K., et al., *NYAP: a phosphoprotein family that links PI3K to WAVE1 signalling in neurons.* EMBO J, 2011. **30**(23): p. 4739-54.
191. Mack, T.G. and B.J. Eickholt, *New WAVEs in neuronal PI3K signalling.* EMBO J, 2011. **30**(23): p. 4693-5.

192. Waite, K. and B.J. Eickholt, *The neurodevelopmental implications of PI3K signaling.* Curr Top Microbiol Immunol, 2010. **346**: p. 245-65.
193. Newhall, K.J., et al., *Dynamic anchoring of PKA is essential during oocyte maturation.* Curr Biol, 2006. **16**(3): p. 321-7.
194. Aye, T.T., et al., *Reorganized PKA-AKAP associations in the failing human heart.* J Mol Cell Cardiol, 2012. **52**(2): p. 511-8.
195. Panza, E., et al., *The breakpoint identified in a balanced de novo translocation t(7;9)(p14.1;q31.3) disrupts the A-kinase (PRKA) anchor protein 2 gene (AKAP2) on chromosome 9 in a patient with Kallmann syndrome and bone anomalies.* Int J Mol Med, 2007. **19**(3): p. 429-35.
196. 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.
197. Bauman, A.L., et al., *The mAKAP signalosome and cardiac myocyte hypertrophy.* IUBMB Life, 2007. **59**(3): p. 163-9.
198. 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.
199. Yang, Y., et al., *Interaction between fidgetin and protein kinase A-anchoring protein AKAP95 is critical for palatogenesis in the mouse.* J Biol Chem, 2006. **281**(31): p. 22352-9.
200. 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.
201. Schimenti, K.J., et al., *AKAP9 is essential for spermatogenesis and sertoli cell maturation in mice.* Genetics, 2013. **194**(2): p. 447-57.
202. 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.
203. Tingley, W.G., et al., *Gene-trapped mouse embryonic stem cell-derived cardiac myocytes and human genetics implicate AKAP10 in heart rhythm regulation.* Proc Natl Acad Sci U S A, 2007. **104**(20): p. 8461-6.
204. Kammerer, S., et al., *Amino acid variant in the kinase binding domain of dual-specific A kinase-anchoring protein 2: a disease susceptibility polymorphism.* Proc Natl Acad Sci U S A, 2003. **100**(7): p. 4066-71.
205. Logue, J.S., et al., *AKAP220 protein organizes signaling elements that impact cell migration.* J Biol Chem, 2011. **286**(45): p. 39269-81.
206. Garnis, C., et al., *Alteration of AKAP220, an upstream component of the Rb pathway, in oral carcinogenesis.* Int J Cancer, 2005. **116**(5): p. 813-9.
207. Akakura, S., et al., *Loss of the SSeCKS/Gravin/AKAP12 gene results in prostatic hyperplasia.* Cancer Res, 2008. **68**(13): p. 5096-103.
208. 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.
209. Harada, A., et al., *MAP2 is required for dendrite elongation, PKA anchoring in dendrites, and proper PKA signal transduction.* J Cell Biol, 2002. **158**(3): p. 541-9.
210. Pescucci, C., et al., *Chromosome 2 deletion encompassing the MAP2 gene in a patient with autism and Rett-like features.* Clin Genet, 2003. **64**(6): p. 497-501.
211. Prasad, S.C., et al., *Branchial anomalies: diagnosis and management.* Int J Otolaryngol, 2014. **2014**: p. 237015.
212. Davies, V.J., et al., *Opa1 deficiency in a mouse model of autosomal dominant optic atrophy impairs mitochondrial morphology, optic nerve structure and visual function.* Hum Mol Genet, 2007. **16**(11): p. 1307-18.
213. Yu-Wai-Man, P., et al., *Multi-system neurological disease is common in patients with OPA1 mutations.* Brain, 2010. **133**(Pt 3): p. 771-86.

214. Gold, M.G., et al., *Molecular basis of AKAP specificity for PKA regulatory subunits.* Mol Cell, 2006. **24**(3): p. 383-95.
215. 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.
216. Taylor, S.S., et al., *Signaling through cAMP and cAMP-dependent protein kinase: diverse strategies for drug design.* Biochim Biophys Acta, 2008. **1784**(1): p. 16-26.
217. 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.
218. 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.
219. 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.
220. 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.
221. Sarma, G.N., et al., *Structure of D-AKAP2:PKA RI complex: insights into AKAP specificity and selectivity.* Structure, 2010. **18**(2): p. 155-66.
222. Vijayaraghavan, S., et al., *Isolation and molecular characterization of AKAP110, a novel, sperm-specific protein kinase A-anchoring protein.* Mol Endocrinol, 1999. **13**(5): p. 705-17.
223. 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.
224. Burgers, P.P., et al., *A Systematic Evaluation of Protein Kinase A - A-kinase Anchoring Protein Interaction Motifs.* Biochemistry, 2014.
225. McSorley, T., et al., *Spatial organisation of AKAP18 and PDE4 isoforms in renal collecting duct principal cells.* European journal of cell biology, 2006. **85**(7): p. 673-8.
226. Gold, M.G., et al., *AKAP18 contains a phosphoesterase domain that binds AMP.* J Mol Biol, 2008. **375**(5): p. 1329-43.
227. Horner, A., et al., *Mechanism for targeting the A-kinase anchoring protein AKAP18delta to the membrane.* J Biol Chem, 2012. **287**(51): p. 42495-501.
228. Smith, F.D., et al., *Intrinsic disorder within an AKAP-protein kinase A complex guides local substrate phosphorylation.* Elife, 2013. **2**: p. e01319.
229. Gold, M.G., et al., *Architecture and dynamics of an A-kinase anchoring protein 79 (AKAP79) signaling complex.* Proc Natl Acad Sci U S A, 2011. **108**(16): p. 6426-31.
230. Li, H., et al., *Balanced interactions of calcineurin with AKAP79 regulate Ca2+-calcineurin-NFAT signaling.* Nat Struct Mol Biol, 2012. **19**(3): p. 337-45.
231. Aittaleb, M., C.A. Boguth, and J.J. Tesmer, *Structure and function of heterotrimeric G protein-regulated Rho guanine nucleotide exchange factors.* Mol Pharmacol, 2010. **77**(2): p. 111-25.
232. Zheng, Y., *Dbl family guanine nucleotide exchange factors.* Trends Biochem Sci, 2001. **26**(12): p. 724-32.
233. Cook, D.R., K.L. Rossman, and C.J. Der, *Rho guanine nucleotide exchange factors: regulators of Rho GTPase activity in development and disease.* Oncogene, 2013.
234. Abdul Azeez, K.R., et al., *The Crystal Structure of the RhoA : AKAP-Lbc DH-PH Domain Complex.* Biochem J, 2014.
235. Schott, M.B. and B. Grove, *Receptor-mediated Ca2+ and PKC signaling triggers the loss of cortical PKA compartmentalization through the redistribution of gravin.* Cell Signal, 2013. **25**(11): p. 2125-35.
236. Rawe, V.Y., et al., *WAVE1 intranuclear trafficking is essential for genomic and cytoskeletal dynamics during fertilization: cell-cycle-dependent shuttling between M-phase and interphase nuclei.* Dev Biol, 2004. **276**(2): p. 253-67.
237. Smith, K.E., E.S. Gibson, and M.L. Dell'Acqua, *cAMP-dependent protein kinase postsynaptic localization regulated by NMDA receptor activation through translocation of an A-kinase anchoring protein scaffold protein.* J Neurosci, 2006. **26**(9): p. 2391-402.

238. 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.
239. 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.
240. 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.
241. 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.
242. Hulme, J.T., et al., *A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle Ca2+ channel and modulates its function.* J Biol Chem, 2002. **277**(6): p. 4079-87.
243. Craik, D.J., et al., *The future of peptide-based drugs.* Chem Biol Drug Des, 2013. **81**(1): p. 136-47.
244. Jin, L., W. Wang, and G. Fang, *Targeting protein-protein interaction by small molecules.* Annu Rev Pharmacol Toxicol, 2014. **54**: p. 435-56.
245. Higueruelo, A.P., H. Jubb, and T.L. Blundell, *Protein-protein interactions as druggable targets: recent technological advances.* Curr Opin Pharmacol, 2013. **13**(5): p. 791-6.
246. Schachterle, C., et al., *Screening for Small Molecule Disruptors of AKAP-PKA Interactions.* Methods Mol Biol, 2015. **1294**: p. 151-66.
247. Yu, X., et al., *G protein-coupled estrogen receptor 1 mediates relaxation of coronary arteries via cAMP/PKA-dependent activation of MLCP.* Am J Physiol Endocrinol Metab, 2014. **307**(4): p. E398-407.
248. 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.
249. McConnell, B.K., et al., *Disruption of protein kinase A interaction with A-kinase-anchoring proteins in the heart in vivo: effects on cardiac contractility, protein kinase A phosphorylation, and troponin I proteolysis.* J Biol Chem, 2009. **284**(3): p. 1583-92.
250. 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.
251. Burton, K.A., et al., *Type II regulatory subunits are not required for the anchoring-dependent modulation of Ca2+ channel activity by cAMP-dependent protein kinase.* Proc Natl Acad Sci U S A, 1997. **94**(20): p. 11067-72.
252. Faruque, O.M., et al., *Cell-permeable peptide-based disruption of endogenous PKA-AKAP complexes: a tool for studying the molecular roles of AKAP-mediated PKA subcellular anchoring.* Am J Physiol Cell Physiol, 2009. **296**(2): p. C306-16.
253. Wang, Y., et al., *Isoform-selective disruption of AKAP-localized PKA using hydrocarbon stapled peptides.* ACS Chem Biol, 2014. **9**(3): p. 635-42.
254. Wang, Y., et al., *PKA-type I Selective Constrained Peptide Disruptors of AKAP Complexes.* ACS Chem Biol, 2015.
255. Yu, X., et al., *G protein-coupled estrogen receptor 1 (GPER) mediates relaxation of coronary arteries via cAMP/PKA-dependent activation of MLCP.* Am J Physiol Endocrinol Metab, 2014.
256. Ambrosy, A.P., et al., *The global health and economic burden of hospitalizations for heart failure: lessons learned from hospitalized heart failure registries.* J Am Coll Cardiol, 2014. **63**(12): p. 1123-33.
257. Cook, C., et al., *The annual global economic burden of heart failure.* Int J Cardiol, 2014. **171**(3): p. 368-76.
258. Guariguata, L., et al., *Global estimates of diabetes prevalence for 2013 and projections for 2035.* Diabetes Res Clin Pract, 2014. **103**(2): p. 137-49.

259. Sahin, U., K. Kariko, and O. Tureci, *mRNA-based therapeutics - developing a new class of drugs.* Nat Rev Drug Discov, 2014. **13**(10): p. 759-80.
260. Ivanov, A.A., F.R. Khuri, and H. Fu, *Targeting protein-protein interactions as an anticancer strategy.* Trends Pharmacol Sci, 2013. **34**(7): p. 393-400.
261. Bavec, A., *(Poly)peptide-based therapy for diabetes mellitus: insulins versus incretins.* Life Sci, 2014. **99**(1-2): p. 7-13.
262. Nystoriak, M.A., et al., *AKAP150 contributes to enhanced vascular tone by facilitating large-conductance Ca2+-activated K+ channel remodeling in hyperglycemia and diabetes mellitus.* Circ Res, 2014. **114**(4): p. 607-15.
263. Sonkusare, S.K., et al., *AKAP150-dependent cooperative TRPV4 channel gating is central to endothelium-dependent vasodilation and is disrupted in hypertension.* Sci Signal, 2014. **7**(333): p. ra66.
264. Lefkimmiatis, K. and M. Zaccolo, *cAMP signaling in subcellular compartments.* Pharmacol Ther, 2014. **143**(3): p. 295-304.