

# Localized Effects of cAMP Mediated by Distinct Routes of Protein Kinase A

KJETIL TASKÉN AND EINAR MARTIN AANDAHL

*The Biotechnology Centre of Oslo, University of Oslo, Norway*

---

I. Introduction	138
II. Localized Pools of cAMP	139
A. Adenylyl cyclases	139
B. Phosphodiesterases	139
C. cAMP gradients	140
III. cAMP Effectors Other Than Protein Kinase A	140
A. CNG ion channels	140
B. cAMP-regulated GEFs	141
IV. Protein Kinase A	141
V. A Kinase Anchoring Proteins	143
VI. A Multitude of A Kinase Anchoring Proteins	145
A. AKAPs associated with ion channels	145
B. AKAPs associated with the cytoskeleton	146
C. Mitochondria-associated AKAPs	149
D. AKAPs involved in regulation of nuclear dynamics and chromatin condensation	149
VII. Signal Complexes Organized by A Kinase Anchoring Proteins	150
VIII. cAMP Signaling to the Nucleus and Gene Regulation	150
IX. Regulation of Cellular Processes and Organ Function by cAMP and Protein Kinase A	151
A. Regulation of cardiovascular function	151
B. Regulation of steroid biosynthesis	152
C. Regulation of reproductive function	153
D. Regulation of metabolism in adipocytes	154
E. Regulation of exocytotic processes	155
F. Regulation of immune function	155
X. Concluding Remarks	157

---

**Taskén, Kjetil, and Einar Martin Aandahl.** Localized Effects of cAMP Mediated by Distinct Routes of Protein Kinase A. *Physiol Rev* 84: 137–167, 2004; 10.1152/physrev.00021.2003.—More than 20% of the human genome encodes proteins involved in transmembrane and intracellular signaling pathways. The cAMP-protein kinase A (PKA) pathway is one of the most common and versatile signal pathways in eukaryotic cells and is involved in regulation of cellular functions in almost all tissues in mammals. Various extracellular signals converge on this signal pathway through ligand binding to G protein-coupled receptors, and the cAMP-PKA pathway is therefore tightly regulated at several levels to maintain specificity in the multitude of signal inputs. Ligand-induced changes in cAMP concentration vary in duration, amplitude, and extension into the cell, and cAMP microdomains are shaped by adenylyl cyclases that form cAMP as well as phosphodiesterases that degrade cAMP. Different PKA isozymes with distinct biochemical properties and cell-specific expression contribute to cell and organ specificity. A kinase anchoring proteins (AKAPs) target PKA to specific substrates and distinct subcellular compartments providing spatial and temporal specificity for mediation of biological effects channeled through the cAMP-PKA pathway. AKAPs also serve as scaffolding proteins that assemble PKA together with signal terminators such as phosphatases and cAMP-specific phosphodiesterases as well as components of other signaling pathways into multiprotein signaling complexes that serve as crossroads for different paths of cell signaling. Targeting of PKA and integration of a wide repertoire of proteins involved in signal transduction into complex signal networks further increase the specificity required for the precise regulation of numerous cellular and physiological processes.

## I. INTRODUCTION

The cAMP-protein kinase A (PKA) signaling pathway is characterized in detail in a number of cell types and organ systems. Activation of cAMP signaling involves binding of an extracellular ligand to a G protein-coupled receptor (GPCR) which through G proteins regulates one of several isoforms of adenylyl cyclase leading to generation of cAMP. Although other effectors of cAMP have been identified, the most common downstream effector system is PKA.

In this review, we discuss the different features of the cAMP-PKA pathway that provide specificity at the intracellular level and thereby convey tissue- and organ-specific effects. The question is how one single second messenger can be involved in regulation of such diverse cellular processes as regulation of the cell cycle, proliferation and differentiation and regulation of microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, as well as regulation of intracellular transport mechanisms and ion fluxes. The cAMP signaling pathway is further involved in controlling exocytotic events in polarized epithelial cells and is the primary intracellular pathway conveying  $\beta$ -adrenergic signaling in the cardiovascular system and in

adipose tissue. Also, cAMP pathways are involved in the regulation of steroidogenesis and reproductive function as well as in modulation of immune responses and a number of other effects elicited by hormones, neurotransmitters, and various paracrine ligands.

cAMP generation and degradation is regulated by the adenylyl cyclase and phosphodiesterase families of enzymes, respectively (305, 320). These enzymes are differentially expressed and regulated. cAMP-dependent protein kinase (PKA) is a heterotetramer composed of two regulatory and two catalytic subunits. Both the regulatory ( $RI\alpha$ ,  $RI\beta$ ,  $RII\alpha$ ,  $RII\beta$ ) and the catalytic ( $C\alpha$ ,  $C\beta$ ,  $C\gamma$ ) subunits possess distinct physical and biological properties, are differentially expressed, and are able to form different isoforms of PKA holoenzymes (reviewed in Refs. 300, 328). A kinase anchoring proteins (AKAPs) further contribute to the specificity as well as the versatility of the cAMP-PKA pathway by assembling multiprotein signal complexes allowing signal termination by phosphatases and cross-talk between different signaling pathways in close proximity to the substrates (Fig. 1) (84, 226). Integrating phosphodiesterases into these anchoring complexes further adds a temporal aspect to the spatial regulation of cAMP signals (303).

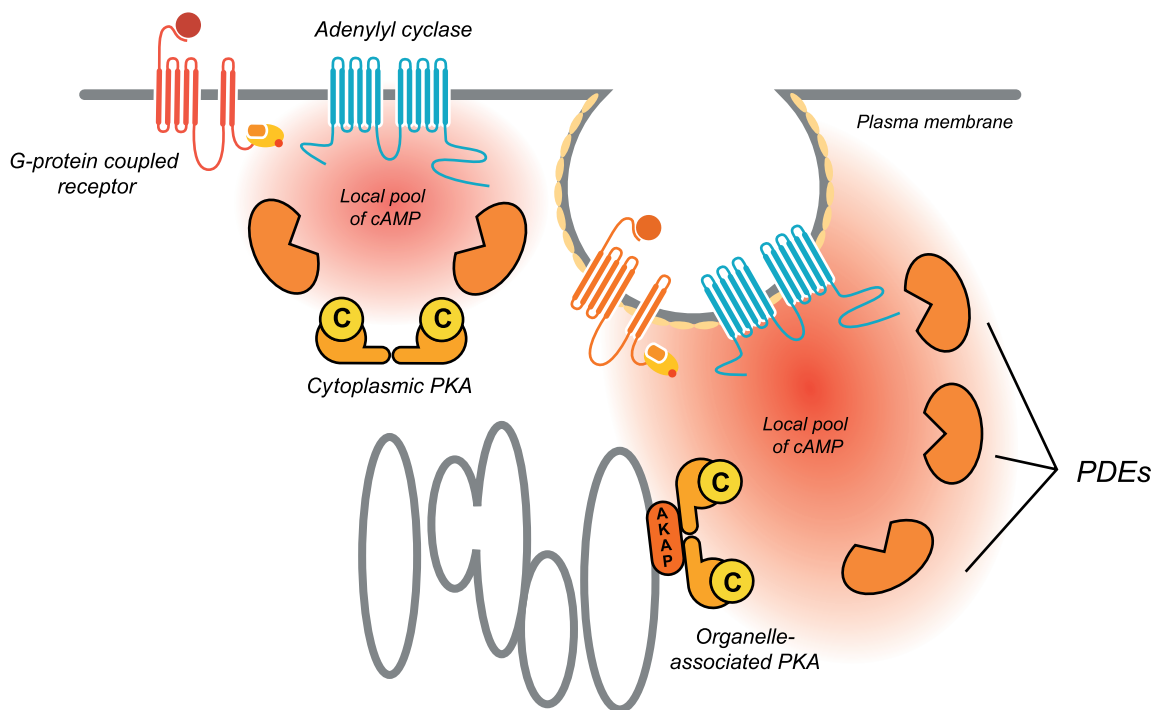


FIG. 1. Ligand binding to various G protein-coupled receptors activates adenylyl cyclases in their proximity and generates pools of cAMP. The local concentration and distribution of the cAMP gradient is limited by phosphodiesterases (PDEs). Particular G protein-coupled receptors are confined to specific domains of the cell membrane in association with intracellular organelles or cytoskeletal constituents. The subcellular structures may harbor specific isoforms of protein kinase A (PKA) that through anchoring via A kinase anchoring proteins (AKAPs) are localized in the vicinity of the receptor and the cyclase. These mechanisms serve to localize and limit the assembly of the pathway to a defined area of the cell close to the substrate.

## II. LOCALIZED POOLS OF cAMP

Ligands targeting G protein-coupled seven-span receptors that signal through cAMP all elicit positive or negative changes in cAMP concentration gradients via G proteins activating or inhibiting adenylyl cyclase. However, the pools of cAMP generated in response to a specific ligand are determined by the localization and availability of receptors and cyclases coupled to that response. Furthermore, such cAMP microdomains are shaped by phosphodiesterases and differ in amplitude as well as spatiotemporal dynamics. It is feasible that a cAMP gradient elicited by a distinct ligand is specifically organized to follow a distinct route of PKA signaling by reaching and activating a subset of or even a single PKA-AKAP complex to mediate a biological effect (Fig. 1). Similarly to the local domains of cAMP, localized  $\text{Ca}^{2+}$  gradients and spikes are well established and are generated by controlled release and reuptake (257, 272, 333, 366).

### A. Adenylyl Cyclases

In mammals, nine membrane-bound isoforms of adenylyl cyclase (AC1-AC9) and one soluble sperm-specific form have been identified, all of which have distinct regulatory properties (reviewed in Ref. 134). All the membrane-bound isoforms exhibit a basal activity that is enhanced upon binding of the stimulatory G protein  $\alpha$ -subunit ( $G_s\alpha$ ) and reduced upon binding of the inhibitory G protein  $\alpha$ -subunit ( $G_i\alpha$ ). In addition, regulatory mechanisms including various small molecules provide means to differentially regulate the members of the family.

The membrane-bound members of the AC enzyme family comprise glycoproteins of ~120 kDa with considerable sequence homology. The suggested structure based on the amino acid sequence includes a small, cytoplasmic domain (N), two hydrophobic transmembrane domains, and two large cytoplasmic domains (C). The cytoplasmic domains are the most homologous sequences and constitute the catalytic moiety of the enzyme.  $C_{1a}$  is the primary binding site for  $G_i\alpha$ , whereas  $C_{2a}$  is the primary binding site for  $G_s\alpha$  and potentially the G protein  $\beta\gamma$ -subunits.  $C_{2a}$  also contains phosphorylation sites for protein kinase C (PKC) and calmodulin (CaM) kinase II. The various isoforms of the membrane-bound ACs can be divided into groups based on structure and regulatory properties (for a recent review, see Ref. 251).

Whereas all the membrane-bound ACs are expressed in the brain, the expression has by in situ hybridization been shown to be specific for the various structures of the central nervous system. Some of the isoforms have also been linked to specific functions. AC1 and AC2 are both highly expressed in regions associated with learning and memory as cerebral cortex, hippocampus, and cerebel-

lum. Specifically, there is an enrichment of calcium-sensitive ACs in regions exposed to high intracellular free calcium induced by *N*-methyl-D-aspartate and voltage-gated  $\text{Ca}^{2+}$  channels, and AC1-mutated mice have affected long-term potentiation and spatial learning capabilities. Other tissues express AC isoforms at different stages of embryonic development, or in response to various stimuli such as nervous stimulation. Several tissues and cell types also display a sequential expression of AC isoforms during differentiation. Relatively little is known about the localization of various AC isoforms within subdomains of the plasma membrane. However, several AC isoforms (AC3–5) have been reported in lipid rafts and caveola and implicated in local cAMP microdomains at the membrane (289). This pertains also to G proteins and, for example,  $\beta_2$ -adrenoceptors in the heart (314). In olfactory neurons, AC3 has been shown to be exquisitely localized to cilia, providing a clear “point source” of cAMP and presumably an associated gradient within these cells (163).

### B. Phosphodiesterases

Cyclic nucleotide phosphodiesterases (PDEs) are enzymes responsible for the hydrolysis of cyclic nucleotides and play an important and highly regulated role in controlling the resting state levels of cAMP or cGMP intracellularly. Furthermore, they also contribute to establishing local gradients of cyclic nucleotides by being localized to subcellular compartments and by being recruited into multiprotein signaling complexes. This contributes to the temporal and spatial specificity of cyclic nucleotide signaling by regulating the availability of cAMP/cGMP to their effectors. The importance of the PDEs as regulators of signaling is evident from studies of PDE-deficient mice (157), and PDEs are also important drug targets in several diseases such as asthma and chronic obstructive pulmonary disease, cardiovascular diseases such as heart failure and atherosclerotic peripheral arterial disease, neurological disorders, and erectile dysfunction (69, 102, 130, 214, 310).

PDEs comprise a large superfamily of enzymes, and 11 families have been characterized on the basis of their amino acid sequences, substrate specificities, allosteric regulatory characteristics, and pharmacological properties (222, 305). In total, the superfamily of PDEs encompasses 25 genes in mammals giving rise to an estimate of more than 50 different PDE proteins (342). They share a modular architecture, with a conserved catalytic domain proximal to the COOH terminus, regulatory domains most often located at the  $\text{NH}_2$  terminus and targeting domains which we are only beginning to discover (67, 110, 147). The substrate specificities of the PDEs families include cAMP-specific, cGMP-specific, and dual-specific PDEs.

We will here briefly discuss the role of PDEs in the context of generating localized pools of cAMP.

### C. cAMP Gradients

The distribution of PDEs to different subcellular localizations was proposed early on by the observation that PDE activity was found in both the soluble and particulate fractions of the cell (316). Recent evidence further supports this notion and contributes to an emerging concept of a highly organized signal pathway where specific routes of cAMP signals are formed through the localized synthesis by cell- and tissue-specific adenylyl cyclases, and where the signal is delivered to targeted effectors and terminated in a spatial and temporal manner by specific PDEs establishing local pools of cAMP close to the effector molecules.

Putative or established targeting domains have now been identified for most of the PDE families (222). PDE3s are targeted to the endoplasmic reticulum by a transmembrane domain consisting of six transmembrane helices (78), and PDE4D5 interacts with RACK-1, a scaffold protein which binds certain PKC isoforms after activation by diacylglycerol (363). PDE4D3 is targeted to the Golgi/centrosomal region through anchoring by myomegalin (156, 343). Some PDE4D and PDE4A variants bind Src homology 3 (SH3) domains of, e.g., Src kinases (23, 24), and via their catalytic domain PDE4 isoforms bind to and are phosphorylated by Erk (211). PDE4A1 contains a novel lipid binding domain, TAPAS, with specificity for phosphatidic acid that serves to target this PDE to specific cellular membranes (16). Most recently, the PDE4 family is reported to be recruited to activated  $\beta$ -adrenoreceptors through interaction with  $\beta$ -arrestin (17, 252). Furthermore, direct interaction between a PDE and two different AKAPs has recently been reported. In rat Sertoli cells, AKAP450 targets PDE4D3 to the centrosomal region together with PKA type II in a ternary complex (329). In cardiomyocytes, muscle AKAP (mAKAP) binds and targets both PDE4D3 and PKA type II to the perinuclear region (87). These are the first examples of colocalized PKA/PDE complexes providing spatial control and termination of the cAMP signaling event by complexing PDE in the immediate vicinity. Furthermore, long PDE4 isoforms such as PDE4D3 are activated by PKA phosphorylation, effectively establishing a negative-feedback loop that terminates the cAMP signal locally (Fig. 2) (156, 212). In addition to the spatial control of PDEs by subcellular compartmentalization, PDE activity is also allosterically regulated, regulated by protein-protein interactions and by posttranslational modifications further contributing to the specificity in this signaling pathway (67, 222).

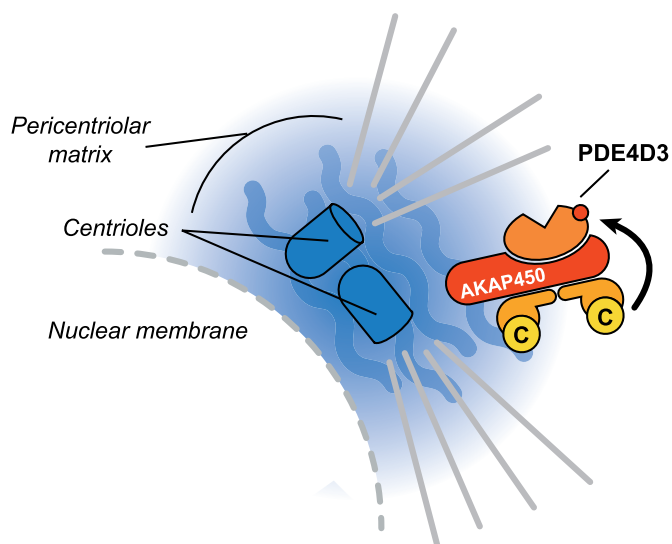


FIG. 2. AKAP450 targets PKA type II and PDE4D3 to the centrosomal region in Sertoli cells. A similar mechanism operates in cardiomyocytes, where mAKAP binds and targets both PDE4D3 and PKA type II to the perinuclear region. Colocalized PKA and PDE provides spatial control of PKA signaling via anchoring to the same AKAP and temporal control and termination of cAMP signaling by a sequence of events that involve the following: 1) the effect of cAMP is mediated by PKA phosphorylation of substrate proteins. 2) PKA phosphorylates and activates the PDE4D3 (PDE4D3 and other long PDE4 isoforms are PKA substrates, and phosphorylation leads to enhanced phosphodiesterase activity). 3) The colocalized and now activated PDE4D3 degrades cAMP and terminates the signal. This serves to establish a negative-feedback mechanism.

### III. cAMP EFFECTORS OTHER THAN PROTEIN KINASE A

Although PKA is generally recognized as being the primary effector of cAMP signaling, other effectors are known and encompass a class of cyclic nucleotide-gated (CNG) cation channels and a small family of guanine nucleotide exchange factors (GEFs) involved in the regulation of Ras-related proteins. The role of CNG channels appears to be specific to certain cell types where distinct ion fluxes are regulated. Functions of cAMP-regulated GEFs in various cellular contexts are currently being unravelled, and the biological significance of cAMP signaling to small G proteins is emerging and may prove increasingly important.

#### A. CNG Ion Channels

CNG ion channels have been found in a variety of cell types and tissues including kidney, testis, heart, and the central nervous system (reviewed in Refs. 42, 350, 364). These channels open in response to direct binding of intracellular cyclic nucleotides and contribute to cellular control of the membrane potential and intracellular  $\text{Ca}^{2+}$

levels. The first member of this family to be identified was the retinal rod photoreceptor, which is directly activated by cGMP (107, 365), and a similar channel was then subsequently identified in olfactory transduction able to bind both cAMP and cGMP (240). One of the most recently reported is the CatSper involved in cAMP-mediated sperm motility (259, 268).

The CNG ion channels are multi-subunit pore-forming channels. The different subunits are highly homologous and bear structural similarity to voltage-gated K<sup>+</sup> channels (368). The modulation of channel activity is through allosteric binding, and maximal activation typically requires four ligands bound (207, 277). The cyclic nucleotide binding domain is connected to the last transmembrane segment of the channel by 90 amino acids called the C-linker, which also has been shown to be important for the regulation of the channel activity (108, 123, 160).

## B. cAMP-Regulated GEFs

Ras-related proteins are monomeric GTPases. They cycle between an inactive GDP-bound state and an active GTP-bound state, which is achieved by the exchange of the tightly bound GDP for GTP. They then revert to the inactive state when the intrinsic GTPase activity again converts GTP to GDP (37, 38). Both these reactions are slow and are facilitated by GEFs and GTPase-activating proteins (GAPs), respectively. Ras proteins regulate downstream signaling proteins by recruitment to the plasma membrane and subsequent activation.

Rap-1, which is a small Ras-like GTPase (34, 254), was first identified as a protein that could suppress the oncogenic transformation of cells by Ras (176) and act as a suppressor of Ras (39, 68). A number of extracellular stimuli signal to Rap-1 and the more recently identified Rap-2 by induction of second messengers like cAMP, calcium, and diacylglycerol (DAG) that regulate Rap-specific GEFs. Two of these proteins called Epac (exchange protein activated by cAMP) 1 and 2 (or cAMP-GEFs) have raised considerable interest as their activities are directly regulated by cAMP, and thereby provide an additional effector system for cAMP signaling (80, 172). Epac1 has one cAMP binding site, whereas Epac2 contains two binding moieties (79). The cAMP binding domains in both Epac1 and Epac2 function as inhibitors of the COOH-terminal GEF domains in the absence of cAMP, whereas cAMP binding induces a conformational change exposing and activating the GEF domain (265). The recent use of a cell-permeable cAMP agonist that is selective for Epac has provided compelling evidence for a cAMP-Epac-Rap pathway (98).

## IV. PROTEIN KINASE A

In its inactive state, the PKA holoenzyme consists of two catalytic (C) subunits bound noncovalently to a regulatory (R) subunit dimer (186, 330). cAMP binds cooperatively to two sites termed A and B on each R subunit. In the inactive holoenzyme, only the B site is exposed and available for cAMP binding. When occupied, this enhances the binding of cAMP to the A site by an intramolecular steric change. Binding of four cAMP molecules, two to each R subunit, leads to a conformational change and dissociation into an R subunit dimer with four cAMP molecules bound and two C monomers (for review and references on cAMP binding domains, see Ref. 183). The C subunits then become catalytically active and phosphorylate serine and threonine residues on specific substrate proteins (for review and references on the C subunit, see Refs. 302, 331).

Two classes of PKA isozymes, designated types I and II, were originally identified based on their order of elution by ion-exchange chromatography and shown to differ in the content of the R subunit, called RI or RII, respectively. Later further heterogeneity was unravelled by molecular cloning identifying RI $\alpha$ , RI $\beta$ , RII $\alpha$ , and RII $\beta$  as well as four C subunits C $\alpha$ , C $\beta$ , C $\gamma$ , and PRKX (reviewed in Ref. 300). PRKX (the human X chromosome-encoded protein kinase X) was recently described as a cAMP-dependent kinase that forms a catalytically inactive holoenzyme with RI, but does not bind to the RII subunit under physiological conditions (371). The R subunits exhibit different cAMP binding affinities giving rise to PKA holoenzymes with different thresholds for activation. Whereas PKA type II holoenzymes (RII $\alpha$ C $\alpha$ , RII $\beta$ C $\alpha$ ) typically activate with an activation constant ( $K_{act}$ ) of 200–400 nM of cAMP, type I holoenzymes (RI $\alpha$ C $\alpha$ , RI $\beta$ C $\alpha$ ) have higher affinity for cAMP and activate with  $K_{act}$  of 50–100 nM cAMP (89). In addition, the R subunits are differentially expressed in different cells and tissues and are able to form both homo- and heterodimers generating a large number of combinations, which further contribute to diversity and presumably specificity in the cAMP signal pathway.

Subcellular localization of PKA is mainly due to anchoring of the R subunits by AKAPs, which originally were seen as contaminants of purified PKA (208, 282, 332) and later understood to enhance the efficiency and specificity of the signaling events. While PKA type I is classically known to be biochemically soluble and was thus assumed to be mainly cytoplasmic, PKA type II is typically particulate and confined to subcellular structures and compartments anchored by cell- and tissue-specific AKAPs, a field largely pioneered by the Scott laboratory (reviewed in Refs. 63, 84, 86, 226). However, a few dual-specific AKAPs (D-AKAPs) anchoring both PKA type I and

TABLE 1. *A kinase anchoring proteins*

AKAP (Gene Nomenclature Committee Name)	Tissue	Subcellular Localization	Properties/Function	Reference Nos.
S-AKAP84/D-AKAP1/AKAP121/AKAP149 (AKAP1)	Testis, thyroid, heart, lung, liver, skeletal muscle, and kidney	Outer mitochondrial membrane/endoplasmatic reticulum/nuclear envelope/sperm midpiece	Dual-specific AKAP; binds lamin B and PP1; multiple splice variants	57, 150, 151, 205, 313, 337
AKAP-KL (AKAP2)	Kidney, lung, thymus, and cerebellum	Actin cytoskeleton/apical membrane of epithelial cells	Multiple splice variants	88
AKAP110 (AKAP3)	Testis	Axoneme	Binds G <sub>13</sub> α	213, 345
AKAP82/FSC1 (AKAP4)	Testis	Fibrous sheath of sperm tail	Potential role in sperm motility and capacitation; multiple splice variants; binds both RI and RII	52, 228, 229
AKAP75/79/150 (AKAP5)	Bovine/human/rat orthologs; brain	Plasma membrane/postsynaptic density	Polybasic domains target to plasma membrane and dendrites; binds PKC, calcineurin (PP2B), β-AR, SAP97, and PSD-95	41, 51, 60, 62, 282
mAKAP (AKAP6)	Heart, skeletal muscle, and brain	Nuclear membrane	Binds PDE4D3; spectrin repeat domains involved in subcellular targeting	87, 167, 217, 220, 361
AKAP15/18 α,β,γ,δ (AKAP7)	Brain, skeletal muscle, pancreas, and heart	Basolateral (α) and apical (β) plasma membrane, cytoplasm (γ), secretory vesicles (δ)	Targeted to plasma membrane via fatty acid modifications; modulation of Na <sup>+</sup> and L-type Ca <sup>2+</sup> channels (α); ADH-mediated translocation of AQP2 from vesicles to apical membrane in distal kidney tubules	114, 126, 127, 182, 338
AKAP95 (AKAP8)	Heart, liver, skeletal muscle, kidney, and pancreas	Nuclear matrix	Involved in initiation of chromosome condensation; binds Eg7/condensin; zinc-finger motif	59, 61, 95, 96, 312
AKAP450/AKAP350/Yotiao/CG-NAP/Hyperion (AKAP9)	Brain, pancreas, kidney, heart, skeletal muscle, thymus, spleen, placenta, lung, and liver	Postsynaptic density/neuromuscular junction/centrosomes/Golgi	Binds PDE4D3, PP1, PP2A, PKN, and PKC; targets PKA and PP1 to the NMDA receptor; multiple splice variants.	18, 19, 46, 103, 120, 173, 204, 287, 297, 321, 323, 329, 357, 359
D-AKAP2 (AKAP10)	Liver, lung, spleen, and brain		Dual-specific AKAP	132, 149
AKAP220/hAKAP220 (AKAP11)	Testis and brain	Vesicles/peroxisomes/centrosome	Binds PP1; dual-specific AKAP	196, 266, 284, 285
Gravin (AKAP12)	Endothelium	Actin cytoskeleton/cytoplasm	Binds PKC and β-AR; Xgravin-like (Xgl) is also a putative AKAP	124, 178, 241, 294
AKAP-Lbc/Ht31/Rt31 (AKAP13)	Ubiquitous	Cytoplasm	Ht31 RII binding site used in peptides to disrupt PKA anchoring; Rho-GEF that couples G <sub>12</sub> α to Rho	49, 85, 179
MAP2B	Ubiquitous	Microtubules	Binds tubulin; modulation of L-type Ca <sup>2+</sup> channels	76, 208, 282, 332
Ezrin/AKAP78 T-AKAP80	Secretory epithelia Testis	Actin cytoskeleton Fibrous sheath of sperm tail	Linked to CFTR via EBP50/NHERF	92, 318, 319, 223
SSECKS (Src-suppressed C kinase substrate)	Testis, elongating spermatids	Actin remodeling	Gravin-like	99
Pericentrin	Ubiquitous	Centrosome	Binds dynein and γ-tubulin; unique RII-binding domain	83
WAVE-1/Scar	Brain	Actin cytoskeleton	Binds Abl and Wrp; involved in sensorimotor and cognitive function	307, 356
Myosin VIIA PAP7	Ubiquitous Steroid-producing cells (adrenal gland and gonads)	Cytoskeleton Mitochondria	Hormonal regulation of cholesterol transport into mitochondria; binds RI in vivo	189 201
Neurobeachin AKAP28	Brain Primary airway cells	Golgi Ciliary axonemes	Modulation of ciliary beat frequency	348 188
Myeloid translocation gene (MTG) 8 and 16b	Lymphocytes	Golgi		115, 283
AKAP140	Granulosa cells and meiotic oocytes		Upregulated by FSH in granulosa cells; phosphorylated by CDK1 in oocytes; not cloned	47, 153, 184

TABLE 1. *Continued*

AKAP (Gene Nomenclature Committee Name)	Tissue	Subcellular Localization	Properties/Function	Reference Nos.
AKAP85	Lymphocytes	Golgi	Not cloned	273
BIG2 (brefeldin A-inhibited guanine nucleotide-exchange protein 2)		Cytosol and Golgi	GEF for ADP ribosylation factor GTPases; binds RI $\alpha$ /RI $\beta$ and RII $\alpha$ /RII $\beta$ through three separate PKA binding domains; cAMP regulated translocation of BIG from cytosol to Golgi	200
Rab32		Mitochondria	Regulation of mitochondrial dynamics and fission	7
AKAP <sub>CE</sub>	<i>Caenorhabditis elegans</i>		Binds to RI-like subunit; RING-finger protein with FYVE and TGF- $\beta$ receptor binding domain	11, 12, 142
DAKAP550	<i>Drosophila</i>	Plasma membrane/cytoplasm	Contains two RII-binding sites	133
DAKAP200	<i>Drosophila</i>	Plasma membrane	Binds F-actin and Ca calmodulin	202, 276
AKAP97/radial spoke protein 3 ( <i>RSP3</i> )	<i>Chlamydomonas</i>	Flagellar axonemes	Located near inner arm dyneins and possibly regulate flagellar motility	118

AKAP, A kinase anchoring protein; PKC, protein kinase C; PP, protein phosphatase;  $\beta$ -AR,  $\beta$ -adrenergic receptor; PDE, phosphodiesterase; PKA, protein kinase A; CFTR, cystic fibrosis transmembrane conductance regulator; FSH, follicle stimulating hormone; TGF- $\beta$ , transforming growth factor- $\beta$ ; ADH, antidiuretic hormone; NMDA, *N*-methyl-D-aspartate.

type II as well as some AKAPs that selectively bind PKA type I have more recently been identified (see Table 1).

As evident from solution of the NMR structure, the RII subunits dimerize at the NH<sub>2</sub> terminus in an antiparallel fashion forming an X-type, four-helix bundle that is necessary for both AKAP binding (NH<sub>2</sub>-terminal helix of both protomers) and dimerization (COOH-terminal helices of the bundle) through separate but overlapping regions involved in the two events (243–245) (Fig. 3). Dimerization is a prerequisite for AKAP binding, but deletion of residues 1–5 abolishes AKAP binding without disrupting dimer formation, and branched side chains at positions 3 and 5 are critical for the interaction with the AKAPs in a hydrophobic groove that is formed on top of the NH<sub>2</sub>-terminal helices (139, 140). The RI dimerization domain contains a similar helix-turn-helix motif recently solved by NMR which is shifted a little further from the NH<sub>2</sub> terminus and encompasses amino acids 12 to 61 (21, 22, 195). The extreme NH<sub>2</sub> terminus in RI is helical and believed to fold back onto the four-helix bundle and may thus contribute to differences in AKAP binding specificity between RII and RI.

## V. A KINASE ANCHORING PROTEINS

The intracellular targeting and compartmentalization of PKA is controlled through association with AKAPs. AKAPs are a structurally diverse family of functionally related proteins that now includes more than 50 members when splice variants with different targeting are included (Table 1, Fig. 4). They are defined on the basis of their ability to bind to PKA and coprecipitate catalytic activity. However, the functional importance further involves tar-

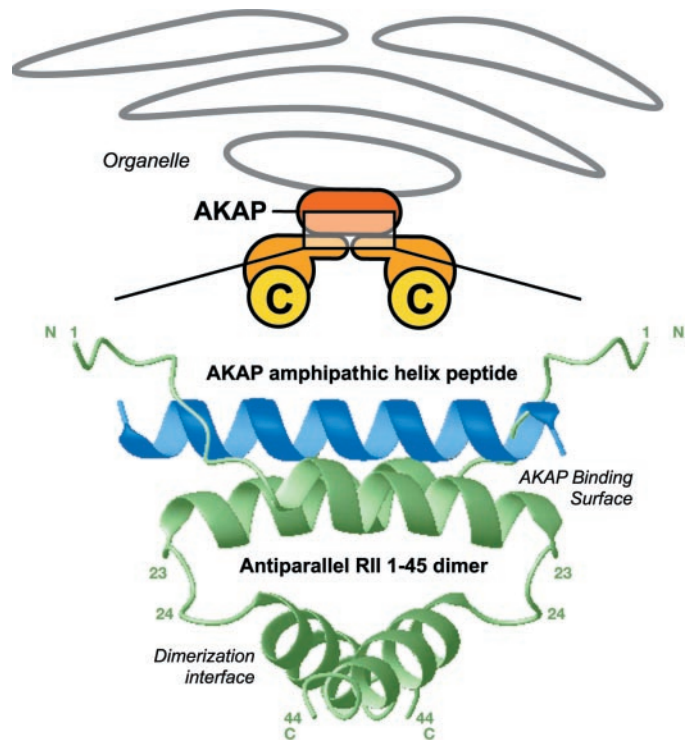


FIG. 3. The AKAP amphipathic helix binds to a hydrophobic groove in the dimerization domain of the PKA R subunit. Anchoring of PKA to AKAPs involves binding of the NH<sub>2</sub>-terminal dimerization and docking domain of the PKA R subunit to an amphipathic helix that constitutes the PKA binding domain of the AKAP. Solution of the NMR structure (*bottom*) reveals that the antiparallel RII dimer forms an X-type, 4-helix bundle where the two NH<sub>2</sub>-terminal helices form a hydrophobic groove that makes contact with the hydrophobic side of the AKAP amphipathic helix, whereas the COOH-terminal helices of the bundle appear to be involved mainly in dimerization (243–245). Furthermore, the ultimate NH<sub>2</sub> termini of the R subunit extend along the AKAP and may make additional contact points. (The ribbon diagram of the NMR structure was kindly provided by and reproduced with permission from Drs. John D. Scott, Vollum Institute, and Patricia A. Jennings, University of California San Diego.)

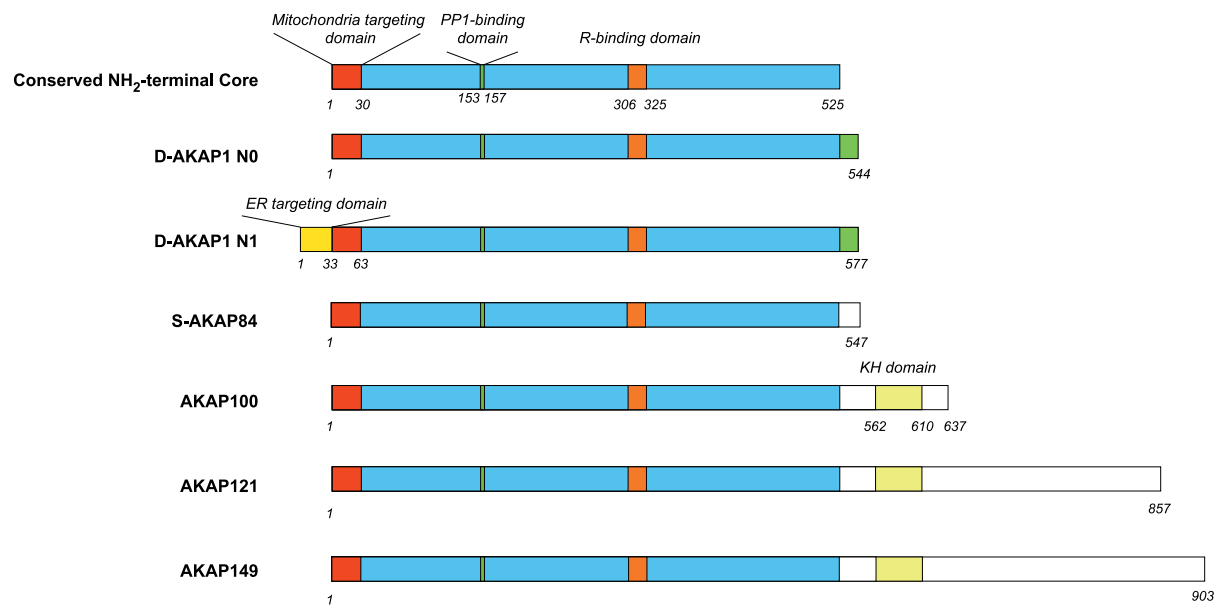


FIG. 4. Multiple splice variants originate from the AKAP1 gene. A total of 6 different splice products have been identified originating from the AKAP1 gene (151). D-AKAP1-N0 (150), S-AKAP84 (205), AKAP100 (151), AKAP121 (57), and AKAP149 (337) all have an NH<sub>2</sub>-terminal mitochondrial targeting domain but differ in their COOH termini. D-AKAP1-N1 has an additional NH<sub>2</sub>-terminally spliced endoplasmic reticulum (ER) targeting domain that presumably overrides the mitochondrial targeting signal (151). Although not identified by molecular cloning techniques, AKAP149 appears also to exist with an NH<sub>2</sub>-terminally spliced ER signal as AKAP149 is found in the ER and nuclear envelope (313).

getting the enzyme to specific subcellular compartments, thereby providing spatial and temporal regulation of the PKA signaling events. All the anchoring proteins contain a PKA-binding tethering domain and a unique targeting domain directing the PKA-AKAP complex to defined subcellular structures, membranes, or organelles. In addition to these two domains, several AKAPs are also able to form multivalent signal transduction complexes by interaction with phosphatases as well as other kinases and proteins involved in signal transduction. Through this central role in the spatial and temporal integration of effectors and substrates, AKAPs provide a high level of specificity and temporal regulation to the cAMP-PKA signaling pathway.

Typically, AKAPs anchor PKA type II holoenzymes (RII<sub>2</sub>C<sub>2</sub>) with high affinity (low nanomolar range). In contrast, their interaction with PKA type I (RI<sub>2</sub>C<sub>2</sub>) appears to occur at considerable lower affinity (49, 139–141). The availability of RII versus RI may determine which subunit is tethered by AKAPs in vivo (149, 150, 266, 201). However, the AKAP<sub>CE</sub> identified in *Caenorhabditis elegans* is demonstrated to specifically interact with the worm's RI-like subunit and does not bind mammalian RII (11, 12). Furthermore, the mammalian AKAP82 is shown to have an RI-specific PKA binding domain (228), and both PAP7 and hAKAP220 appear to anchor PKA type I inside cells, although mapping of the RI binding domains has not been conducted (201, 266). Thus RI also appears to be capable of forming physiologically relevant anchoring interactions.

The conserved PKA tethering domain in AKAPs forms an amphipathic helix of 14–18 residues that interacts with hydrophobic determinants located in the extreme NH<sub>2</sub> terminus of the regulatory subunit dimer (49, 50, 243, 245) (Fig. 3). The amphipathic helix of the AKAPs, with hydrophobic residues aligned along one face of the helix and charged residues along the other, binds to RII with high affinity (140, 141). Dual-specific AKAPs (149, 150) appear to bind to the RI dimerization and docking (DD) domain in a similar fashion (21, 22). Disruption of the amphipathic helix abolishes the binding to R both in vitro and in vivo, and the residues determining binding of RI and RII have been defined (8, 44, 50).

A peptide usually referred to as the Ht31 anchoring disruption peptide derived from the PKA tethering domain of the human thyroid AKAP Ht31, now called AKAP-Lbc, mimics the amphipathic helix that binds the extreme NH<sub>2</sub> terminus of the regulatory subunit of PKA and serve as a competitive anchoring inhibitor of PKA-AKAP interactions (49, 50). The Ht31 peptide has been used extensively as a tool to analyze the effects of disrupting PKA anchoring. Interestingly, recent analysis of the RII binding domain of AKAPs by bioinformatics and peptide array approaches unravelled high-affinity peptides with specificity for binding RII (AKAP-*is* peptide, Ref. 8). Similarly, isoform-specific peptide disruptors of PKA type I association with AKAPs have recently been developed (44). Use of such anchoring disruptors will greatly facilitate analysis of cellular effects of anchored PKA type I and II.



## VI. A MULTITUDE OF A KINASE ANCHORING PEPTIDES

### A. AKAPs Associated With Ion Channels

#### 1. AKAP79 and neuronal transmission

Protein phosphorylation and dephosphorylation by protein kinases and phosphatases play a key role in regulation of synaptic plasticity in the hippocampus (358). PKA-mediated phosphorylation potentiates the currents induced by activation of the excitatory AMPA receptor by phosphorylation of Ser-845 of the glutamate receptor 1 (GluR1) subunit (20, 128, 274, 347). The first demonstration that AKAP-mediated targeting of PKA is necessary for mediation of a biological effect of cAMP was shown by peptide-mediated disruption of a PKA-AKAP complex directing PKA toward the AMPA receptor leading to a significant reduction in the glutamate receptor activity measured by whole cell voltage clamping (275). The AKAP responsible for targeting PKA to the receptor was later identified as AKAP79 (AKAP150 and AKAP75 are murine and bovine orthologs, respectively) which is able to associate with both AMPA and NMDA receptors (41, 51, 60, 62, 122, 282). AKAP79 is targeted to the plasma membrane by three NH<sub>2</sub>-terminal basic regions that bind phosphatidylinositol 4,5-bisphosphate. Membrane-associated AKAP79 is then recruited to the NMDA and AMPA receptors by binding to the SH3 and guanylate kinase-like (GK) domains of the membrane-associated guanylate kinase (MAGUK) proteins, postsynaptic density (PSD)-95 and synapse-associated protein (SAP)-97, respectively (Fig. 5) (62). These processes are dependent on the actin cytoskeleton and recruit the AKAP79 to the NMDA and AMPA receptors localized in the postsynaptic densities of hippocampal synapses (122).

AKAP79 is also associated with  $\beta_2$ -adrenergic receptors ( $\beta_2$ -AR) and recruits PKA, PKC, and protein phosphatase (PP) 2B (Fig. 5) (113). The receptor undergoes cAMP-dependent desensitization after agonist stimulation by direct PKA phosphorylation and indirectly by PKA-mediated phosphorylation and enhancement of G protein-coupled receptor kinase 2 (GRK2) (25, 66, 113). PKA phosphorylation of the  $\beta_2$ -AR also induces a switch in the G protein coupling from G<sub>s</sub> to G<sub>i</sub> (74). This promotes a mitogenic signaling cascade mediated by G<sub>i</sub>,  $\beta$ -arrestin, and the Src-tyrosine kinase leading to mitogen-activated protein (MAP) kinase activation (210). Both receptor desensitization and MAP kinase activation can be disrupted by inhibition of PKA anchoring with Ht31 (113). Furthermore,  $\beta$ -arrestin recruits PDE4 which control  $\beta_2$ -AR phosphorylation by PKA and hence the G<sub>s</sub> to G<sub>i</sub> switch (17, 252).

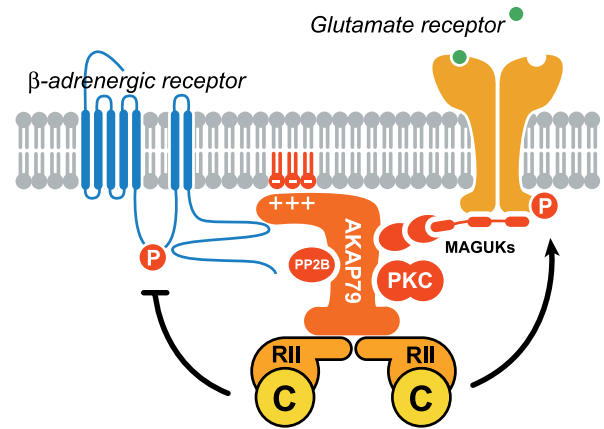


FIG. 5. AKAP79 is targeted to the plasma membrane by three NH<sub>2</sub>-terminal basic domains and is recruited to the AMPA receptor by binding to the membrane-associated guanylate kinase (MAGUK) proteins. AKAP79 also associates with  $\beta_2$ -adrenergic receptors ( $\beta_2$ -AR). This enhances  $\beta_2$ -AR-induced cAMP-PKA signaling by recruiting PKA close to the receptor and the site of adenylyl cyclase activation. PKA phosphorylation of the  $\beta_2$ -AR leads to desensitization of the receptor; however, PKA phosphorylation enhances glutamate receptor activity. Thus AKAP79 brings the cAMP-generating machinery, PKA, and the substrates into close proximity. In addition to anchor PKA, AKAP79 also recruits protein kinase C (PKC) and protein phosphatase 2B (PP2B) and thereby integrates several signaling pathways into a multiprotein complex.

#### 2. AKAP15/18

In skeletal muscle transverse tubules, L-type calcium channels initiate muscle contraction by directly interacting with ryanodine receptors to cause the release of calcium from the sarcoplasmic reticulum (SR) (53). The calcium channels function both as voltage sensors to initiate excitation-contraction coupling and as a slowly activating calcium entry pathway that regulates the force of the contraction (4, 53). Repetitive high-frequency depolarizing stimuli that mimic action potentials or single long depolarizing pulses greatly enhance the activity of the L-type calcium channels (291). This enhancement is voltage dependent and requires phosphorylation by PKA (291) and can be induced by  $\beta$ -adrenergic stimuli (Fig. 6) (14, 286).

The importance of PKA-mediated phosphorylation in the regulation of the calcium channel function in skeletal muscle is evident from experimental inhibition of the anchoring of PKA in the channel vicinity by Ht31 which leads to a 20-fold reduction in voltage-dependent potentiation of the calcium channel activity (126, 158, 159). The AKAP involved in this process has been identified as a 15- or 18-kDa protein that copurifies, coimmunoprecipitates, and colocalizes with the skeletal muscle calcium channel complex (114, 126, 127). AKAP15/18 (the  $\alpha$ -isoform) is an 81-residue protein containing an amphipathic helix that binds PKA and NH<sub>2</sub>-terminal myristoyl and palmitoyl lipid anchors that target the PKA-AKAP complex to the plasma

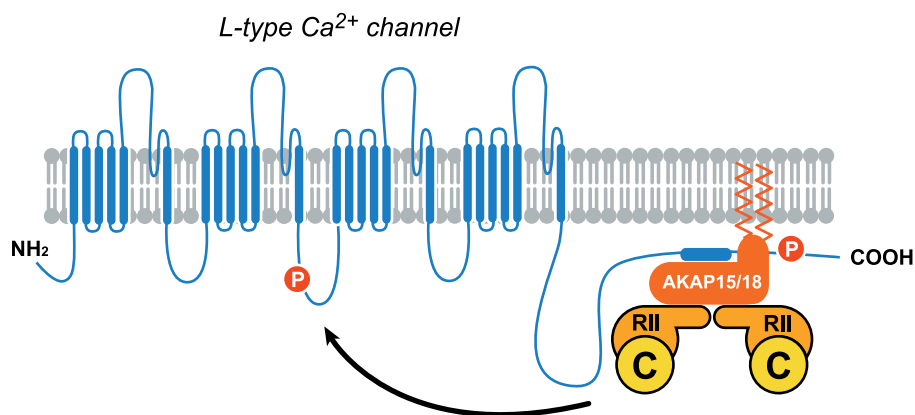


FIG. 6. AKAPs target PKA to ion channels to provide necessary proximity for phosphorylation to occur rapidly enough to regulate the channel in the short time frame of opening and closing of the channels. For example, the  $\alpha$ -isoform of AKAP15/18 anchors PKA to the COOH terminus of L-type  $\text{Ca}^{2+}$  channels in skeletal muscle. PKA phosphorylation enhances the activity of the channel.  $\text{NH}_2$ -terminal myristoyl and palmitoyl lipid modifications target the AKAP to the plasma membrane.

membrane (114, 126). The direct interaction between AKAP15/18 and the channel involves interaction with the COOH-terminal domain of the  $\alpha_1$ -subunit of the L-type calcium channel via a leucine zipperlike mechanism providing means of localizing PKA in close proximity to a major phosphorylation site located in the  $\alpha_1$ -subunit at serine-1854 (152). This ensures specific and rapid phosphorylation of the channel.

Further studies demonstrated that the first identified AKAP15/18 is one of several splice variants, now named AKAP18 $\alpha$ , and is localized to the basolateral membrane compartment in polarized cells. Furthermore, other splice variants from this gene have apical targeting (AKAP18 $\beta$ ) and localization to cytoplasm (AKAP18 $\gamma$ ) or to secretory granules (AKAP18 $\delta$ ) (182, 338).

### 3. Other ion channels regulated by PKA through AKAP interactions

Several other ion channels are also regulated by PKA through AKAP interactions. The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial  $\text{Cl}^-$  channel whose activity is enhanced by PKA-dependent phosphorylation (117, 260). More than 800 mutations in CFTR have been observed in patients with cystic fibrosis, and ~5% of these are in the regulatory domain of the channel containing 9 consensus sites for PKA phosphorylation. The interaction between CFTR and PKA involves targeting of PKA to CFTR by binding to the 78-kDa AKAP ezrin (92). Ezrin is the most studied member of the ezrin-moesin-radixin (ERM) family of proteins and plays structural and regulatory roles in the assembly and stabilization of specialized plasma membrane domains. Ezrin and related molecules are concentrated in surface projections such as microvilli and membrane ruffles where they link the microfilaments to the membrane. The interaction between ezrin and CFTR involves the  $\text{Na}^+/\text{H}^+$  exchanger (NHE) type 3 kinase A regulatory protein (E3KARP) which binds to CFTR via a PSD-95/Disc-large/zonula occludens-1 (PDZ) binding motif (318, 319). Thus E3KARP

acts as a scaffolding protein that links CFTR to ezrin. This is analogous to the targeting of ezrin to the  $\text{Na}^+/\text{H}^+$  exchanger in the renal brush border by the  $\text{Na}^+/\text{H}^+$  exchanger regulatory factor (NHERF or ezrin-binding phosphoprotein 50, EBP50) facilitating PKA-mediated phosphorylation and inhibition of the channel (264, 351, 352). Ezrin is also enriched in gastric parietal cells (91, 92) and plays an important role as a membrane-cytoskeletal linker in these cells (6, 135). When stimulated with gastrin, ezrin serves to recruit PKA to the secretory canaliculi (92).

PKA-mediated phosphorylation also enhances the activity of NMDA receptors (55, 262). The AKAP yotiao (splice variant from the AKAP9/AKAP450 gene) targets PKA to the receptor by binding to the NR1 subunit of the receptor (103, 204, 357). NMDA receptors are heteromultimers composed of an NR1 subunit and a variety of NR2 family members (190, 221, 232), and yotiao specifically interacts with the splice variant of NR1 that contains the C1 exon (204, 357). The functional relevance of yotiao-mediated anchoring of PKA has been demonstrated by whole cell current recording of transfected cells and by disruption of the anchoring by Ht31 (357). Yotiao also binds the PP1 which under resting conditions with low PKA activity dephosphorylates and deactivates the channel (32, 304, 346, 357). Thus yotiao coordinates the opposing kinase and phosphatase required for efficient regulation of the NMDA receptor function.

## B. AKAPs Associated With the Cytoskeleton

Phosphorylation of proteins associated with the cytoskeleton plays an important role in the dynamics and functional organization of the cytoskeleton. AKAPs are emerging as facilitators of cytoskeletal events as they target PKA to sites where it can phosphorylate substrates including actin, microtubules, the centrosome, and the sperm flagella.

### 1. Actin-associated AKAPs

Actin polymerization is an essential process in all eukaryotic cells, generating the basis for establishment of cell shape, polarity of cell constituents and membrane domains, motility, and cell division. The Rho family of small GTPases are key proteins in this process that link cell surface receptors to the organization of the actin cytoskeleton by regulating the activity of downstream effector molecules. The best studied members of the Rho family of small GTPases include Rho, Rac, and Cdc42 (31, 131). The cytoskeletal changes induced by these three molecules are associated with distinct integrin-based adhesion complexes and while Rho activation leads to assembly of stress fibers, activation of Rac and Cdc42 leads to generation of lamellipodia and filopodia, respectively (185, 246, 270, 271). The WASP family of proteins, consisting of WASP, N-WASP, and the Scar-1 orthologs WAVE1, WAVE2, and WAVE3, plays an important role in these molecular interactions by providing a molecular bridge that links Rho family members to the actin nucleation machinery, the Arp2/3 complex (143, 215, 324). Rac-1 interacts with WAVE1 to activate actin nucleation by releasing WAVE1 from a heterotetrameric complex (94, 227, 317). In addition, WAVE1 binds WRP, a Rac-selective GAP that specifically inhibits Rac function *in vivo* and functions as a signal termination factor for Rac (306). The WASP family members attach to the actin cytoskeleton through a verprolin homology (VPH) domain and a COOH-terminal acidic module that binds to the Arp2/3 complex.

WAVE1 was recently identified as an AKAP that is also able to bind the Abelson tyrosine kinase (Abl) (356). It was identified in a screen for brain AKAPs interacting with isolated SH3 domains from different signal transduction molecules. The two other WAVE isoforms, WAVE2 and WAVE3, which bear considerable sequence homology to WAVE1, lack certain key hydrophobic residues and do not bind RII. The RII-binding region of WAVE1 overlaps with a VPH domain (residue 493–510) that act as a binding site for G-actin. Although G-actin and RII recognize different determinants within the 493–510 sequence, RII and actin binding are mutually exclusive. Thus PKA anchoring by WAVE1 may be dynamically regulated by the actin concentration at sites of actin polymerization. Immunocytochemical analyses in Swiss 3T3 fibroblasts suggest that the WAVE1-kinase scaffold is assembled dynamically and translocates both PKA and Abl from focal adhesions to sites of actin reorganization such as lamellipodia and actin ring structures in response to platelet-derived growth factor treatment (356). The substrates for PKA and Abl are, however, not yet identified. Interestingly, targeted disruption of the WAVE1 gene generated mice with a complex psychomotoric and cognitive phenotype (307), and further genetic manipulation will determine the

extent to which PKA-signaling events are implicated in these varied cognitive processes.

Two other actin-binding proteins have been identified as AKAPs: gravin and ezrin. Whereas ezrin is discussed above, gravin is a multivalent 250-kDa scaffold protein that interacts with PKA, PKC, and actin (124, 241). It was identified as a cytoplasmic antigen recognized by sera from patients with myasthenia gravis. Gravin localizes to filopodia in endothelial and macrophage-like cells and shares significant sequence homology with SSeCKS (Src-suppressed C kinase substrate) which also binds PKA, PKC, and actin and mediates actin remodeling (99, 112, 119, 206, 242). In addition to playing a role in regulation of actin polymerization, gravin organizes PKA, PKC, and PP2B with the  $\beta_2$ -AR (294) in a complex that also includes the G protein-linked receptor kinase 2 (GRK2) and transiently  $\beta$ -arrestin and clathrin (203). Prolonged stimulation of G protein-linked receptors (GPLRs) leads to desensitization of the receptor-mediated signal and agonist-induced receptor sequestration (138). PKC and PP2B are important for the reversal of this process and thereby resensitization of the receptor, as both suppression of PKC and PP2B amplifies the agonist-induced desensitization of the receptor (294–296). Gravin is required for this event to occur (294). PKA, on the other hand, potentiates agonist-induced desensitization of the  $\beta_2$ -AR by causing its phosphorylation and switching from  $G_s$  to  $G_i$  coupling (138, 295).

AKAP-KL is a cytoskeletal-associated AKAP expressed in lung, kidney, and cerebellum (88). There are a total of six different isoforms of AKAP-KL showing tissue-specific expression. The intracellular localization of AKAP-KL is asymmetric with an apical distribution in polarized cells such as pulmonary alveolar epithelial cells and proximal renal tubular cells. It is not yet determined whether AKAP-KL directly interacts with the actin cytoskeleton, although AKAP-KL modulates actin structure in transfected HEK293 cells (88). AKAP-KL may be involved in establishing or maintaining cellular polarity, or facilitating transepithelial signaling processes.

### 2. Microtubule-associated AKAPs

The microtubule-associated protein 2 (MAP2) family of proteins stabilize microtubuli. The MAP2 proteins are predominantly expressed in neurons where they regulate microtubule nucleation, organelle transport within axons, and dendrites as well as anchoring of proteins involved in signal transduction (281). The association of MAP2 with microtubuli occurs through its tubulin binding domain, which binds to an acidic region in the COOH terminus of tubulin and is regulated by its phosphorylation status (70, 146, 292). In addition, MAP2 can also bind to and modify microfilament stability.

Numerous protein kinases and phosphatases are in-

volved in determining the phosphorylation status of MAP2, one of which is PKA. Interestingly, MAP2 was the first AKAP to be identified and tethers one-third of the cytosolic PKA to the microtubules in neurons (332). Several PKA phosphorylation sites have been identified in MAP2 which are also conserved in the closely related MAP tau, including the KXGS motifs located in the tubulin-binding domain. Phosphorylation of these motifs leads to detachment from tubulin (154, 288). The effects of PKA phosphorylation on MAP2 proteins include decreased binding of MAP2 to tubulin and actin, reduced microtubule polymerization, and reduced proteolytic degradation of MAP2 (281). Furthermore, mice with deletion of the MAP2 NH<sub>2</sub> terminus which includes the PKA binding site have decreased efficiency of MAP2 phosphorylation and impaired development of contextual memory (175).

### 3. Centrosome-associated AKAPs

The centrosome represents the major microtubule-organizing center of animal cells consisting of a pair of centrioles surrounded by the pericentriolar matrix composed of a pericentrin and  $\gamma$ -tubulin lattice (33, 370). With its crucial role in nucleation and organization of microtubules, the centrosome is important in cellular processes such as generating a microtubular framework for motor-protein based transport and positioning of vesicles and organelles (33, 168). In mitotic cells, centrosomes are important for the assembly and function of the mitotic spindles and thereby regulate the fidelity of chromosome segregation (65, 225). In addition, an increasing number of molecules that regulate cellular processes such as cell cycle progression and centrosome duplication are found to be localized to centrosomes.

Three AKAPs have been identified in centrosomes, AKAP450 (359), pericentrin (83), and hAKAP220, which is expressed in male germ cells (266). AKAP450 is also named AKAP350 (287) or CG-NAP (centrosome and Golgi localized PKN-associated protein) (323) and derives from the same gene as yotiao. AKAP450 is localized to the centrosome throughout the cell cycle, to the Golgi apparatus during interphase (323, 359), and in the cleavage furrow during anaphase and telophase (287). Although the roles of the pool of PKA anchored to centrosomal AKAPs are not well defined, it is possible that anchored pools of PKA participate in regulation of microtubule nucleation by targeting substrates such as stathmins (106, 125) (see Fig. 2). Moreover, the AKAP450 signal complex has a role in cell cycle progression. Displacement of endogenous AKAP450 and the molecules anchored to it by overexpression of the COOH-terminal AKAP450 targeting domain (PACT domain, pericentrin-AKAP40 centrosomal targeting domain, Ref. 120) results in cell cycle arrest and impaired cytokinesis and centriole duplication (173). In addition, the association between AKAP450 and RII $\alpha$

appears to be under direct regulation of the mitotic kinase CDK1 (46). At the onset of mitosis, CDK1 associated with the centrosome (18, 19) phosphorylates RII $\alpha$  on T54 leading to dissociation from its centrosomal site of anchoring (46, 174). This suggests the PKA-AKAP association in some cases may be dynamic and that CDK1 phosphorylation serves as a molecular switch that regulates RII $\alpha$  association with AKAP450, whereas AKAP95 has an opposite role and binds the CDK1-phosphorylated PKA as described below (Fig. 7) (46, 192).

AKAP450 interacts with several signal transduction enzymes in addition to PKA, including PKN, PP1, PP2A, and the immature nonphosphorylated form of PKC $\epsilon$  (321, 323). PKN is a serine/threonine kinase that associates with and phosphorylates intermediate filament proteins (218, 236), and AKAP450 targeting of PKN may thereby be important for cytoskeletal reorganization events. PKN is activated by Rho (9, 349) and unsaturated fatty acids such

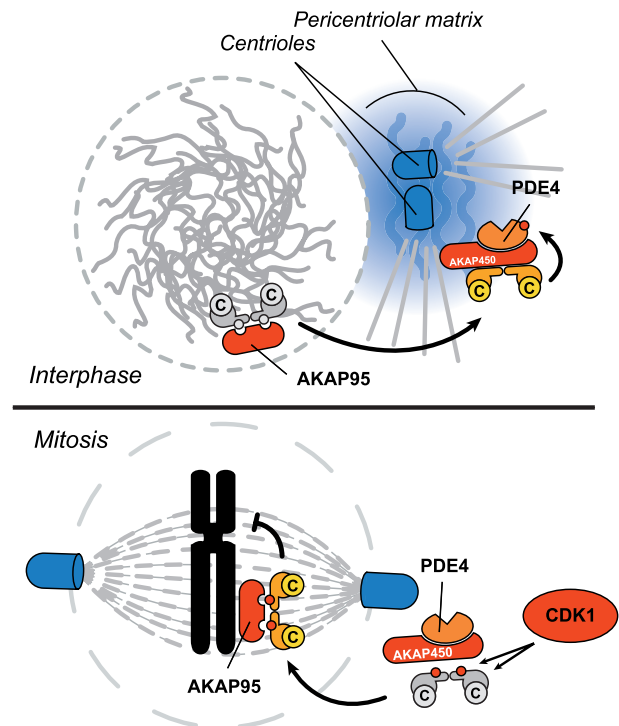


FIG. 7. PKA-AKAP associations may be dynamic. *Top*: during interphase, AKAP450 targets PKA to the pericentriolar matrix where PKA maintains the interphase microtubule network. AKAP450 anchors both PKA and PDE4, and PKA-mediated phosphorylation of PDE4 leads to increased phosphodiesterase activity, establishing a negative-feedback loop. In contrast, PKA holoenzyme cannot enter the nucleus, and the nuclear matrix-associated AKAP95 does not have PKA bound. *Bottom*: when entering mitosis, CDK1 accumulates at the centrosome and phosphorylates the RII $\alpha$  subunit of PKA. Phosphorylation of PKA releases it from AKAP450, allowing this pool of PKA to translocate to the condensing chromatin, since no nuclear envelope barrier exists during mitosis. Here, phosphorylated RII $\alpha$  binds to chromosome-associated AKAP95, and PKA prevents premature decondensation of chromatin during mitosis. At mitosis exit, an unknown phosphatase dephosphorylates RII $\alpha$ ; PKA then dissociates from AKAP95 and exits the nucleus before sealing of the nuclear envelope and reassociates with the pericentriolar matrix.

as arachidonic acid (235), or by truncation of its NH<sub>2</sub>-terminal regulatory region (322). Interaction between AKAP450 and the nonphosphorylated form of PKC $\epsilon$  is required for the phosphorylation-dependent maturation of PKC $\epsilon$ . Recently, AKAP450 was reported to anchor protein kinase CK1 $\delta$ , which is involved in control of cell cycle progression (297). In addition, AKAP450 also anchors PDE4D (329), which allows tight control of the phosphorylation state of proteins regulated by cAMP signaling. Spatial control is achieved by targeting of PKA by AKAP450, while temporal control and inactivation of the effect of cAMP on PKA is accomplished by complexing of PDE at the same site (see Fig. 2).

Pericentrin (full-length human protein named kendirin) is an integral component of the pericentriolar matrix (90) and forms a centrosomal macromolecular complex with  $\gamma$ -tubulin (82) and dynein (258) important in the dynamic organization of centrosomes and spindles (90). Binding to  $\gamma$ -tubulin is required for microtubule nucleation during mitosis and meiosis, and the association with dynein is necessary for the transport of pericentrin- $\gamma$ -tubulin complexes along microtubules to the centrosome. Pericentrin/kendirin shares a high degree of linear homology with AKAP450. This indicates a common origin, and pericentrin/kendirin also serves as an AKAP, although the pericentrin PKA anchoring domain composed of a 100-residue, hydrophobic binding region does not exhibit the structural characteristics of the RII-binding sites found in conventional AKAPs (83). AKAP450 and pericentrin also share the common PACT domain that targets both AKAPs to the centrosome (120). However, overexpression of the AKAP450 PACT domain displaces AKAP450, but not pericentrin, and vice versa, indicating specificity in the targeting of AKAP450 and pericentrin (173). The fact that both pericentrin and AKAP450 target PKA to centrosomes may indicate an important role in microtubule trafficking and centrosome nucleation where there is a need for redundancy or, as the displacement studies may indicate (173), that more than one AKAP is required to accurately position PKA versus substrates inside the centrosome. This indicates a much more sophisticated level of kinase compartmentalization than was originally conceived.

### C. Mitochondria-Associated AKAPs

Several mitochondrial AKAPs have been identified. S-AKAP84 (205), AKAP121 (57), D-AKAP1 (150), and AKAP149 (337) derive from the same gene by alternative splicing and are discussed below together with Rab32 (7). PBR-associated protein 7 (PAP7) is another mitochondrial AKAP that selectively binds RI $\alpha$  in vivo (201), discussed in section IXB.

S-AKAP84 (205), AKAP121 (57), D-AKAP1 (150), and AKAP149 (337) share a 525-amino NH<sub>2</sub>-terminal core but

differ in the COOH-terminal domain as well as in their extreme NH<sub>2</sub> termini (Fig. 4). S-AKAP84, AKAP121, and the N0 isoform of D-AKAP1 share an identical targeting motif that anchors PKA type II to the outer mitochondrial membrane, whereas alternate splicing of the NH<sub>2</sub> terminus (N1 isoform) of D-AKAP1 directs the protein to the endoplasmic reticulum (ER) (N1) (151) and the nuclear envelope membrane network (discussed below) (313). The N1 isoform of D-AKAP1 contains an additional 30 residues responsible for anchoring this isoform to the ER (150, 151). The R-binding domains of S-AKAP84, AKAP121, and D-AKAP1 are also identical and anchor both RI and RII, although the binding affinity of RI $\alpha$  is lower than for RII $\alpha$  ( $K_d$  of 185 vs. 2 nM, respectively) (141).

Rab32 is a member of the Ras superfamily of small-molecular-weight G proteins that is targeted to mitochondria and involved in regulation of mitochondrial fission (7). PKA type II binds to the conserved  $\alpha_5$ -helix of Rab32 which indicates dual functions of Rab32 as an AKAP and regulator of mitochondrial dynamics (7).

### D. AKAPs Involved in Regulation of Nuclear Dynamics and Chromatin Condensation

Mitotic cell division requires that the DNA is properly condensed into chromosomes. This process involves topoisomerase II (3) and a family of proteins of highly conserved ATPases called SMCs (structural maintenance of chromosomes) (144, 145, 278). SMCs participate in multiprotein chromosome condensation complexes called condensins. Purification and characterization of condensins containing XCAP-C and XCAP-E, two *Xenopus* members of the SMC family, revealed two major forms of condensins, 8S and 13S (144). Condensins are targeted to the chromosomes during mitosis in a topoisomerase-independent manner, and the 13S subunit is required for chromatin condensation to take place. The 13S subunit contains both XCAP-C and XCAP-E and three other subunits including pEg7/XCAPD2, which is a required component of the complex for the condensation process (71).

AKAP95 is 95-kDa protein that harbors two zinc fingers (designated ZF1 and ZF2) in its COOH-terminal half, upstream of the PKA-binding domain (59, 96). In interphase, AKAP95 is localized exclusively in the nucleus and associates with the nuclear matrix but does not anchor RII $\alpha$  (59, 96, 61). At mitosis, AKAP95 redistributes from the nuclear matrix to chromatin and recruits the condensin complex once nuclear envelope breakdown has taken place (61, 312). Subsequently, AKAP95 anchors RII $\alpha$  onto, or in the vicinity of, the metaphase plate. Recruitment of RII $\alpha$  from a centrosome-Golgi localization during interphase to chromatin-bound AKAP95 at mitosis requires

phosphorylation of RII $\alpha$  on threonine-54 by CDK1 (192) (Fig. 7). Conversely, release of RII $\alpha$  from AKAP95 upon chromosome decondensation in vitro or mitosis exit correlates with threonine-54 dephosphorylation (192).

Distinct domains of AKAP95 are involved in binding to chromatin and in the recruitment of RII $\alpha$  and of the condensin complex (95). Chromatin binding of AKAP95 is required for condensation to take place, and the amount of Eg7/XCAPD2 recruited correlates with the extent of chromosome condensation in vitro (312). Furthermore, disruption of the ZF1-domain abrogates chromosome condensation, but not condensin recruitment. Thus AKAP95 is essential for chromosome condensation independently of condensin recruitment (95). Interestingly, mitotic chromosome condensation does not require anchoring of PKA to AKAP95 nor PKA activity. However, both PKA activity and binding to AKAP95 are required for the maintenance of condensed chromatin during mitosis, and blocking of PKA activity or disruption of anchoring leads to premature chromatin decondensation (61).

AKAP149 is not only targeted to mitochondria and ER but also associates with the nucleus as it is an integral protein of the ER/nuclear envelope membrane network (313). In addition to anchoring PKA, AKAP149 targets a fraction of chromatin-bound PP1 to the nuclear envelope upon nuclear reformation in vitro (313). The nuclear envelope is a dynamic structure that breaks down at mitosis and reforms in an ordered manner as a result of reversible phosphorylations of membrane, lamina, and chromatin proteins. The nuclear lamina consists of intermediate filaments called A/C- and B-type lamins. Lamins mediate the interactions between the inner nuclear membrane and chromatin, participate in DNA replication, and may provide a structural role for RNA splicing (97, 155, 309). Targeting of PP1 to the nuclear envelope correlates with the nuclear assembly of B-type lamins at the end of mitosis, and disruption of AKAP149 anchoring by a peptide containing the PP1-binding domain of AKAP149 leads to failure of B-type lamin assembly, caspase-dependent proteolysis, and apoptosis (311, 313). It is not yet determined whether AKAP149 anchors PKA and PP1 in distinct complexes or in one single complex. AKAP149 may position PKA and PP1 in close proximity where they can reversibly modulate the phosphorylation status of nuclear substrates such as NPP1 (29), DNA-binding cAMP response elements (269), B-type lamins (253), and inner nuclear membrane proteins harboring PKA phosphorylation sites.

mAKAP (originally cloned and characterized as AKAP100) is a 255-kDa scaffolding protein expressed in myocytes, skeletal muscle, and brain. mAKAP assembles a signal complex consisting of PKA and PDE4D3 at the nuclear envelope, the SR of cardiomyocytes, and intercalated discs in adult rat heart tissue (87, 167, 217, 220, 361). The assembly of the mAKAP signaling complex in the perinuclear region is induced by hypertrophic stimuli in

rat neonatal ventriculocytes and is thought to be associated with cellular differentiation and development of a ventricular hypertrophic phenotype (167). The induction of mAKAP expression also leads to redistribution of RII to the NE (167), which is interesting as PKA phosphorylation induces cAMP-responsive genes involved in propagation in cardiac hypertrophy (369), and the concurrent anchoring of PDE4D3 serves to establish a negative-feedback loop (87) (see discussion above and Fig. 2).

## VII. SIGNAL COMPLEXES ORGANIZED BY A KINASE ANCHORING PROTEINS

The highest level of specificity, and complexity, in cAMP-PKA signaling is accomplished by the assembly of multiprotein complexes by AKAPs. Several AKAPs with this property have been identified that provide precise spatiotemporal regulation of the cAMP-PKA pathway combined with the integration with other signaling pathways in one signal complex. AKAP79, AKAP450, AKAP220, gravin, WAVE, and mAKAP have been shown to scaffold signal complexes, and it is likely that we are still in the very beginning of understanding the role AKAPs play in the orchestration of intracellular signaling events in health and disease (for recent reviews, see Refs. 84, 86, 226).

In addition to its role in anchoring RII, studies of AKAP79 have contributed to the evolution of the model of AKAPs as scaffolding proteins able to bind and anchor multiple signal transduction proteins and also regulate their enzymatic function. From being discovered as proteins able to bind and anchor PKA, the capacity of AKAP79 to associate with other signal enzymes has led to the reevaluation of the original AKAP model. By coordinating the location of PKC and the calcium/CaM-dependent phosphatase PP2B (calcineurin) in addition to PKA, AKAP79 positions two second messenger-regulated kinases and a phosphatase near to neuronal substrates at the postsynaptic densities (see Fig. 5) (60, 177).

## VIII. cAMP SIGNALING TO THE NUCLEUS AND GENE REGULATION

A huge literature describes how cAMP via PKA regulates numerous genes through a wide range of different transcription factors either acting directly on a target gene by phosphorylation of an available transcription factor or indirectly through upregulation of a transcription factor or modulator that acts on second-generation target genes (reviewed in Refs. 75, 219, 230, 231). While most PKA substrates are phosphorylated by PKA anchored by an AKAP in close vicinity to the substrate, PKA signaling to the nucleus involves nuclear entry of the free C subunit. Size exclusion prevents the entry of the PKA holoen-

zyme complex or the R subunit dimer (224, 269, 308). When cAMP rises, the C subunit released from the holoenzyme enters the nucleus by passive diffusion (136), whereas termination of signaling to the nucleus involves an active mechanism. In the nucleus, the C subunit binds to the heat-stable protein kinase inhibitor (PKI), and this binding not only inactivates the C subunit but also by conformational change unveils a nuclear export signal in PKI which leads to export of the C-PKI complex from the nucleus (100, 101, 353–355). The pool of PKA that delivers C subunit for diffusion into the nucleus and gene regulation has been largely considered to be located to the cytoplasm. However, disruption of anchored PKA complexes by overexpression of soluble AKAP fragments affects cAMP signaling to the nucleus and gene regulation measured, e.g., as CREB phosphorylation (104). Furthermore, targeting of PKA via AKAP75/79/150 associated with the cytoskeleton enhances signaling to the nucleus apparently by delivering C subunit to the nucleus (105).

## IX. REGULATION OF CELLULAR PROCESSES AND ORGAN FUNCTION BY cAMP AND PROTEIN KINASE A

Targeting of PKA isozymes by AKAPs has been demonstrated to be important in an increasing number of physiological processes such as cAMP regulation of ion channels in the nervous system, regulation of the cell cycle which involves microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, and numerous intracellular transport mechanisms. The cAMP signaling pathway is further involved in controlling exocytotic events in polarized epithelial cells with implication for diabetes insipidus, hypertension, gastric ulcers, thyroid disease and diabetes mellitus, and asthma. Also  $\beta$ -adrenergic signaling in the heart and in the control of metabolism in adipose tissue requires localization of the cAMP signaling pathway. Finally, cAMP pathways are involved in the regulation of steroidogenesis, reproductive function, and immune responses. In the following sections, we discuss the role of localized pools of PKA in the context of some selected physiological processes where regulation by cAMP plays a major role.

### A. Regulation of Cardiovascular Function

Cardiac excitation-contraction coupling is the process from electrical excitation of the cardiomyocyte to contraction of the heart. The ubiquitous second messenger  $\text{Ca}^{2+}$  is essential in cardiac electrical activity and is the direct activator of the myofilaments, which cause contraction (27). Myocyte mishandling of  $\text{Ca}^{2+}$  is a cen-

tral cause of both contractile dysfunction and arrhythmias in pathophysiological conditions (256).

During the cardiac action potential,  $\text{Ca}^{2+}$  enters the cell through depolarization-activated L-type  $\text{Ca}^{2+}$  channels.  $\text{Ca}^{2+}$  entry triggers  $\text{Ca}^{2+}$  release from the SR. The combination of  $\text{Ca}^{2+}$  influx and release raises the free intracellular  $\text{Ca}^{2+}$  concentration allowing  $\text{Ca}^{2+}$  to bind to the myofilament protein troponin C, which then switches on the contractile machinery. For relaxation to occur, the concentration of  $\text{Ca}^{2+}$  must decline, allowing  $\text{Ca}^{2+}$  to dissociate from troponin. This requires  $\text{Ca}^{2+}$  transport out of the cytosol by several  $\text{Ca}^{2+}$  pumps, the most significant of which is the SERCA2  $\text{Ca}^{2+}$ -ATPase in the SR.

Sympathetic stimulation of the heart through  $\beta$ -adrenergic receptors increases both contraction force (inotropy) and heart rate (chronotropy). In order for the heart rate to increase, relaxation and  $\text{Ca}^{2+}$  decline must occur faster.  $\beta$ -AR stimulation activates a GTP-binding protein ( $G_s$ ), which stimulates adenylyl cyclase to produce cAMP, which in turn activates PKA. PKA then phosphorylates several proteins related to excitation-contraction coupling [L-type  $\text{Ca}^{2+}$  channels, ryanodine receptor (RyR), troponin I, and myosin binding protein C], thus regulating the  $\text{Ca}^{2+}$  flux from L-type  $\text{Ca}^{2+}$  channel and SR (Fig. 8). Furthermore, PKA phosphorylates phospholamban that regulates the activity of SERCA2 and leads to increased reuptake of  $\text{Ca}^{2+}$  into SR, a process which is affected in failing hearts (111, 234, 290). Localized signaling is clearly important in the regulation of  $\text{Ca}^{2+}$  in the heart. Considerable amounts of evidence exist showing that the cAMP increase in response to  $\beta$ -adrenergic stimuli is local (for review and references, see Refs. 28, 314) as well as controlled temporally as recently illustrated by use of fluorescence ratio energy transfer (FRET) with directly fluorescently labeled and microinjected PKA (121) and by the use of genetically encoded FRET probes for cAMP (367). Such pools of cAMP are shaped by phosphodiesterases localized in the vicinity of the SR (367). It is also clear from these studies that the GFP/YFP PKA probe for cAMP is targeted, indicating the presence of AKAPs. Both the  $\beta$ -AR and the L-type  $\text{Ca}^{2+}$  channel have known AKAPs associated that are present in heart (AKAP79, AKAP18 $\alpha$ , respectively). Furthermore, mAKAP have been shown to be colocalized with RyR (166, 216), although the majority of mAKAP is at the nuclear envelope of cardiomyocytes. With the presence of several additional substrates for PKA in this region of the cardiomyocyte, the possibility of additional AKAPs located in this region exists (Fig. 8). Finally, genetic analysis of single-nucleotide polymorphisms identified a mutant resulting in a single amino acid substitution in D-AKAP2 (I646V). The mutation lowers the affinity for R1 $\alpha$  and is associated with changes in electrocardiogram recordings and cardiac dysfunction, implicating D-AKAP2 in targeting of PKA, possibly to an ion

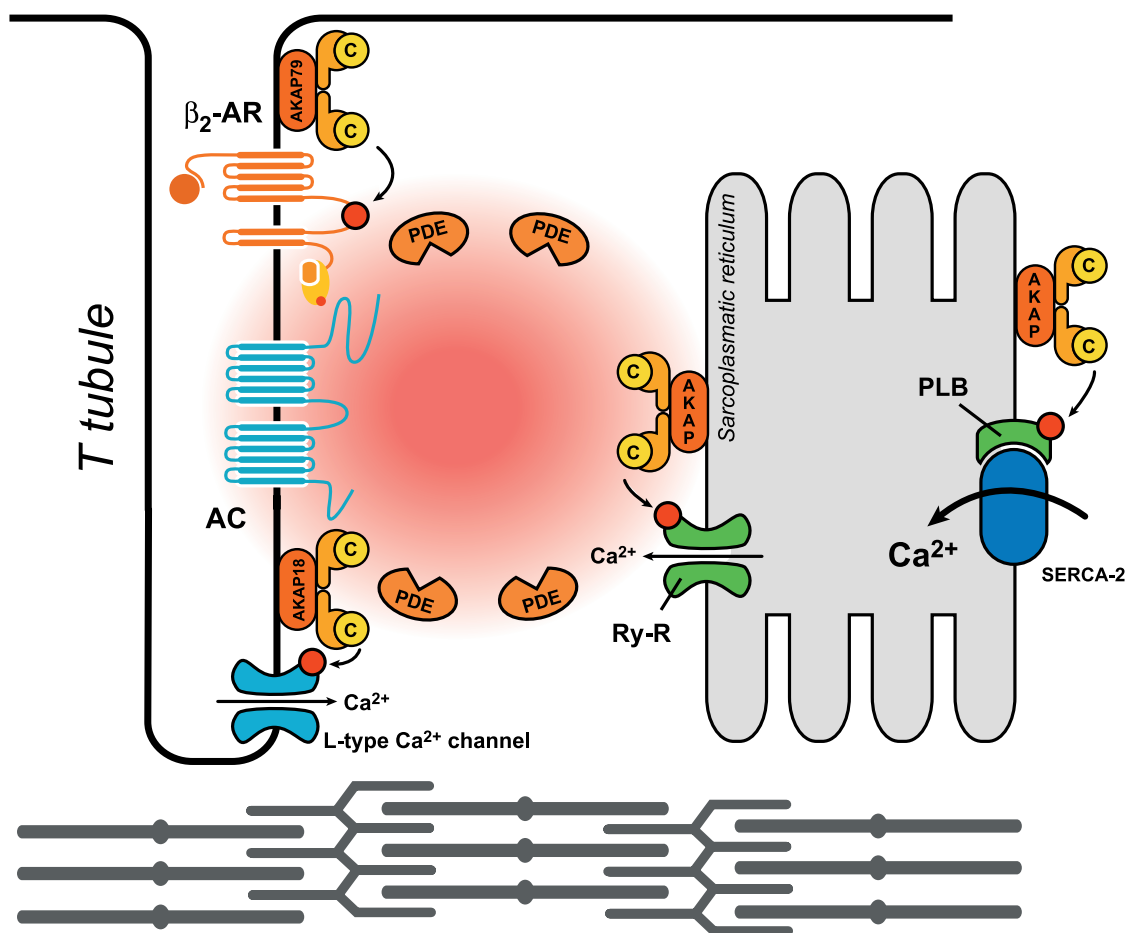


FIG. 8. Local gradients of cAMP and anchored pools of PKA mediate  $\beta$ -adrenergic regulation of cardiac function. Signaling through the  $\beta_2$ -adrenergic receptor localized in microdomains of the sarcolemma formed by t tubules generates a local gradient of cAMP that is also shaped by phosphodiesterases. cAMP activates PKA localized via AKAPs in the vicinity of its substrates such as the  $\beta_2$ -adrenergic receptor ( $\beta_2$ -AR), the L-type  $\text{Ca}^{2+}$  channel, and the ryanodine receptor (RyR) that regulates  $\text{Ca}^{2+}$  outflux from the sarcoplasmic reticulum (SR) and phospholamban (PLB) that through sarco(endo)plasmic reticulum (SERCA2) regulates  $\text{Ca}^{2+}$  reuptake into the SR, which is necessary for adrenergic regulation of heart rate and contraction.

channel, although the exact location of D-AKAP2 is not known (165).

## B. Regulation of Steroid Biosynthesis

Pituitary hormones such as ACTH, luteinizing hormone (LH), and follicle stimulating hormone (FSH) regulate steroid biosynthesis in the adrenal gland and gonads via cAMP and PKA. While ACTH target cells of the adrenal cortex, LH targets theca cells of the ovary and Leydig cells of the testis, and FSH targets granulosa cells of the ovary and Sertoli cells of the testis. ACTH increases the synthesis of cortisol from the adrenal gland, while LH and FSH induce production of estrogens and progesterone from the ovary and androgens from the testis in both an acute and a long-term fashion. While the long-term regulation of steroid biosynthesis involves upregulation of a number of

*P*-450 steroid hydroxylases at the transcriptional level, the acute regulation of steroid biosynthesis involves cAMP-mediated increase in cholesterol release from lipid droplets and cholesterol transport across the mitochondrial membrane as the rate-limiting step to provide substrate for the cholesterol side chain cleavage enzyme,  $p450^{\text{SCC}}$  (for review and references, see Refs. 293, 315). Although no PKA-AKAP complex has yet been reported in lipid droplets, recent evidence suggests that a pool of PKA type I is targeted to the mitochondrial membrane to regulate cholesterol transport (137).

PAP7 is a RI-binding AKAP of 52 kDa expressed in mouse, rat, and human tissues and at high levels in gonads, adrenal gland, and brain (201), which interacts with the peripheral-type benzodiazepine receptor (PBR), an 18-kDa protein localized in the mitochondrial outer membrane (13, 248). Although PBR is expressed in most tis-



sues, it has a particularly high expression level in steroid-producing tissues where PBR together with the steroid acute response protein (StAR) play an important role in steroid synthesis by mediating cholesterol delivery from the outer to the inner mitochondrial membrane (187, 250). Targeted disruption of PBR inhibits cholesterol transport and steroidogenesis in a Leydig tumor cell line (249), while human chorionic gonadotropin (hCG) stimulation of Leydig cells leads to an increase in steroids produced (35, 36). These effects of hCG can be blocked with H-89, a PKA inhibitor. The identification of PAP7 as a PBR and RI-anchoring protein provides a molecular basis for cAMP-regulated cholesterol transport across the mitochondrial membrane, which is one of the rate-limiting steps of steroid biosynthesis (137).

### C. Regulation of Reproductive Function

Spermatozoa represent the terminally differentiated stage of spermatogenesis. They are specialized for the task of fertilizing an egg, which is reflected in the compartmentalization of functions. 1) In the head of the sperm is the acrosomal vesicle that contains hydrolytic enzymes that facilitate the penetration of the egg's outer layer. 2) The head also contains the tightly packed haploid chromatin. 3) The energy production takes place in mitochondrial sheath in the midpiece of the tail, while 4) the motility is provided by a long flagellum whose central axoneme emanates from a basal body situated posterior to the nucleus. The axoneme consists of two central singlet microtubules surrounded by nine evenly spaced microtubule doublets. Bending of the flagellum is caused by sliding of adjacent microtubule doublets past one another driven by dynein motor proteins. The axoneme is surrounded by nine outer dense fibers mainly composed

of keratin and the fibrous sheath, which consists of two longitudinal columns interconnected by numerous transverse ribs.

The initiation and maintenance of sperm motility are thought to involve cAMP-dependent phosphorylation by PKA (43, 327). Although the target proteins are largely unknown, several AKAPs have been identified as being important in compartmentalizing the signaling events (233) (Fig. 9).

#### 1. Sperm/flagellar AKAPs

AKAP82 is the major structural protein of the fibrous sheath essential for sperm motility and fertility (52, 229). It is expressed only in testis (116) and is found throughout the longitudinal columns and transverse ribs (161). In vitro studies suggest that AKAP82 is a dual-specificity AKAP able to anchor both RI $\alpha$  and RII $\alpha$  (228). AKAP110 is also a testis-specific protein and is localized to the acrosomal region and the transverse ribs of the fibrous sheath (213, 345). T-AKAP80 is another AKAP identified in the fibrous sheath of epididymal sperm (223). S-AKAP84 is targeted to the mitochondrial sheath of elongating mouse spermatids, and de novo expression of S-AKAP84 during late spermatogenesis coincides with the maximal expression and subsequent anchoring of RII and PKA type II to mitochondria (205). Rat AKAP220 is a peroxisomal anchoring protein that is expressed in both testis and brain (196). Based on studies of the human ortholog hAKAP220, this protein in germ cells of the testis redistributes from a granular cytoplasmic pattern to a centrosomal localization in postmeiotic cells and to the midpiece/centrosome area in mature sperm (266). AKAP220 anchors both RI and RII (266) as well as PP1 (284), and thus regulates PP1 activity (285).

The fact that disruption of anchoring with Ht31 in-

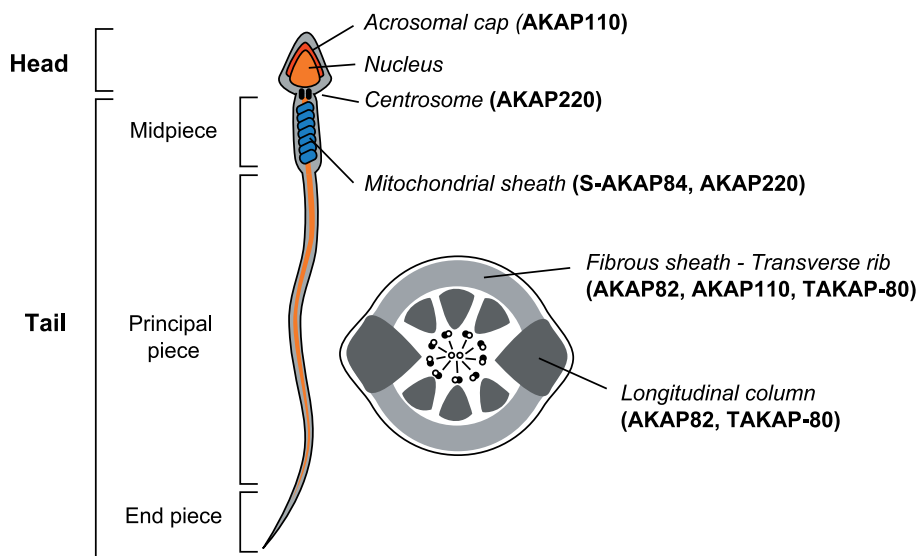


FIG. 9. Several AKAPs have been identified and localized to the different subcellular compartments of polarized mammalian sperm. The enlarged cross-section shows the flagellar structure and the localization of the various AKAPs in the midsection of the principal piece. [Adapted from Moss and Gerton (233).]

hibits sperm motility in a concentration-dependent manner indicates that compartmentalized PKA is implicated (344). However, inhibition of PKA catalytic activity has no effect on motility, and RII $\alpha$ -deficient mice are still fertile (45, 344). This may imply that the AKAPs involved anchor essential components other than PKA, such as ropporin (a protein previously shown to interact with the Rho signaling pathway) and AKAP-associated sperm protein, which are tentatively involved in regulation of motility (48). Despite this, RI $\alpha$  appears to rescue the phenotype in the RII $\alpha$  null-mutant mice (45). Furthermore, knockout of the C $\alpha$ -catalytic subunit of PKA in exon 2, which ablates both the constitutively expressed and the sperm-specific C $\alpha$ -isoforms (81, 267, 279, 280), renders the mice subfertile with severely reduced sperm motility (298). This argues a role for PKA in sperm motility, although the effect of anchoring is still elusive; AKAP-independent anchoring of the C-subunit, in addition to AKAP-dependent anchoring of the R-subunit, may explain some of the conflicting results.

#### D. Regulation of Metabolism in Adipocytes

During the last decades it has become clear that adipose tissue in addition to be a storage depot is also an endocrine organ that secretes several cytokines and growth factors, and may play significant roles in insulin resistance, cell differentiation, and growth (64). Two types of adipose tissue can be distinguished in the body, white adipose tissue (WAT) and brown adipose tissue (BAT), with quite opposite functions although they have the same “machinery” for lipogenic and lipolytic activity. While BAT is involved in adaptive thermogenesis, WAT stores energy as triglycerides (TG). Hydrolysis by hormone-sensitive lipase (HSL) of TG (the process called lipolysis) to free fatty acids (FFA) is the major gateway for the release of stored energy. This process is dramatically increased by PKA-mediated phosphorylation of HSL in adipocytes.

Both BAT and WAT are innervated by the sympathetic nervous system. Catecholamines stimulate lipolysis and thermogenesis in adipocytes, and this process is controlled largely by the  $\beta$ -ARs. Insulin is the most important physiological inhibitor of catecholamine-mediated lipolysis.  $\beta$ -ARs are members of the large family of G protein-coupled receptors. Three  $\beta$ -ARs subtypes ( $\beta_1$ -AR,  $\beta_2$ -AR,  $\beta_3$ -AR) exist. While  $\beta_1$ -AR and  $\beta_2$ -AR are broadly expressed throughout all tissues of the body,  $\beta_3$ -AR is found predominantly in adipocytes. They regulate several intracellular second messenger systems, among others cAMP/PKA and MAP kinase cascades through G $_s$  and G $_i$ , respectively. Phosphorylation of  $\beta_2$ -AR by PKA induces a switch in G protein coupling from G $_s$  to G $_i$ , and thus changing the mode of signaling from perturbation of the

cAMP/PKA signaling pathway to signaling through PKC/MAP kinases (74).

Stimulation of  $\beta$ -AR/cAMP signaling in BAT leads to transcriptional activation of the uncoupling protein 1 (UCP1) gene and increased UCP1 mRNA levels. Expression of UCP1 in brown adipocytes is the only gene product known to date that distinguishes BAT from WAT. UCP1 uncouples electron transport along the respiratory chain, which instead of generating ATP leads to production of heat [adaptive (nonshivering) thermogenesis] (for review, see Ref. 64).

Functional studies of PKA subunits revealed that mice lacking RII $\beta$  have markedly reduced deposits of white fat and are resistant to diet-induced obesity (72). The underlying cause for this phenotype appears to be a compensatory increase in RI $\alpha$  in the BAT. The RI $\alpha$  holoenzyme is more cAMP-responsive than the PKAII $\beta$  isozyme (the regulatory RI $\alpha$  subunit of PKAI $\alpha$  more readily dissociates from the catalytic subunit in response to cAMP), leading to increased activation of TG-depleting enzymes and synthesis of the uncoupling protein UCP1. As a consequence of the increased levels of RI $\alpha$ , thermogenesis in BAT is increased (209). Furthermore, in WAT, the compensatory increase in RI $\alpha$  in RII $\beta$ <sup>-/-</sup> animals is associated with increased basal kinase activity and increased basal rate of lipolysis.  $\beta$ -Adrenergic regulation of lipolysis, on the other hand, is markedly compromised, indicating that the R subunit isoform switch disrupts the subcellular localization of PKA required for  $\beta$ -AR-induced regulation of lipolysis (255).

A recent report shows that mice overexpressing the winged helix forkhead transcription factor *Foxc2* in WAT and BAT are lean and resistant to diet-induced obesity (54, 129). This is partly due to elevated levels of  $\beta$ -adrenergic receptors together with increased levels of the RI $\alpha$  subunit of PKA that lowers the threshold for activation by cAMP. This enhances signaling through the  $\beta$ -adrenergic cAMP-PKA signaling pathway, which in turn leads to increased levels and activity of HSL and UCP1. Subsequently, HSL metabolizes TGs to FFAs, and UCP1 dissipates energy through uncoupling of oxidative phosphorylation. According to this model, the energy content of FFA, released by HSL, will be dissipated through the induction of UCP1 in response to  $\beta$ -adrenergic stimuli. Thus these mice display a lean phenotype with lowered plasma levels of FFA, glucose, and insulin and increased oxygen consumption (54). Furthermore, *Foxc2* mRNA is upregulated in wild-type mice fed on a high-fat diet compared with standard diet, indicating that *Foxc2* is regulated in response to diet energy content. Following elevated *Foxc2* levels, the metabolic rate is increased in the sense that excess calories will have an increased tendency to dissipate heat rather than being stored as TG droplets.

Although cAMP plays a major role in regulation of

adipocyte metabolism, little is known about AKAPs in adipocytes. However, the observed differences in  $\beta$ -adrenergic regulation of metabolism in RII $\beta$ -containing adipocytes from wild-type mice compared with adipocytes from RII $\beta$  knock-out mice expressing RI $\alpha$  indicate that anchoring of PKA is crucial for the normal lipolytic response to adrenergic stimuli. This argues a putative role for a PKA-AKAP79 complex associated with the  $\beta$ -AR. Furthermore, D-AKAP1 has been shown in adipocyte mitochondria (56). We hypothesize the presence of yet unknown AKAPs, also in lipid droplets, involved in PKA regulation of HSL and perilipin (protein that controls release of fat) (326).

### E. Regulation of Exocytotic Processes

The gastric glands in the body of the stomach contain three main cell types: mucus-secreting cells, pepsin-secreting cells, and acid-secreting cells. The acid-secreting cells, called parietal cells, were the first system for which regulated recruitment and recycling of a transport protein, the proton pumping  $H^+K^+$ -ATPase, was proposed as a means for controlling secretion (109). Within the parietal cells, there is an extensive cytoplasmic canalicular membranous network called tubulovesicles. Stimulation of parietal cells with secretagogues leads to structural and functional changes that involve trafficking and fusion of cytoplasmic  $H^+K^+$ -ATPase-rich tubulovesicles with the apical surface recruiting the proton pumps to the surface of the glands leading to HCl secretion. The translocation process is cAMP/PKA dependent, and both  $H_2$  receptor antagonists and H-89, which is a PKA inhibitor, block this event (5, 362). The substrate for PKA has not been identified, but it is likely that PKA anchoring is required and occurs via ezrin (5, 92). Ezrin colocalizes with F-actin constituting the cytoskeleton underlying the tubulovesicular membrane, and anchors RII.

Insulin secretion from pancreatic  $\beta$ -cells is regulated by reversible phosphorylation of  $\beta$ -cell substrates. Protein phosphorylation by PKA and PKC enhances insulin secretion, while protein phosphatases inhibit this process (10, 162). The effect of cAMP is dependent on targeting of PKA by AKAP79 (197, 198). In addition to targeting PKA, AKAP79 also anchors the calcium/CaM-dependent phosphatase PP2B. Transient inhibition of PP2B by cyclosporin A (CsA) leads to increased insulin secretion (93); thus PP2B negatively regulates insulin secretion by dephosphorylation. Furthermore, PKA and PP2B share a common substrate, namely, synapsin 1 (197). AKAP79 may therefore coordinate the reversible phosphorylation events involved in PKA-mediated insulin secretion. In addition, PKA activation enhances the activity of PP2B, thereby establishing a negative-feedback loop terminating the PKA signal. AKAP79 assembles and targets a similar

signaling complex consisting of PKA and PP2B in neurons (60, 169).

Targeting of PKA phosphorylation events is also important in cAMP-dependent regulation of water reabsorption in renal principal cells. Antidiuretic hormone (ADH) initiates its action by binding to receptors located in the basolateral membrane of the principal cells in the collecting ducts and induces cAMP production. PKA activation leads to direct phosphorylation of the water channel (aquaporin-2, AQP2). Phosphorylation of the channel does not alter the permeability but leads to subsequent translocation of the channel from intracellular vesicles into the apical membrane (170, 181, 191). PKA colocalizes with the AQP2-containing vesicles, and the translocation process can be blocked by Ht31, suggesting an important role for PKA anchoring (180, 181). Several AKAPs have been identified in principal cells, and although the AKAP involved in AQP2 exocytosis has not yet been precisely identified, AKAP18 $\delta$  is a potential candidate (182) (Fig. 10).

### F. Regulation of Immune Function

Engagement of the T-cell receptor/CD3 (TCR/CD3) complex can lead to a wide range of responses spanning from anergy and apoptosis to T-cell activation with cytokine production, cytotoxic activity, and proliferation. An optimal immune response requires the antigen to be presented to the T cell by an antigen presenting cell (the primary stimulus) in conjunction with a costimulatory stimulus (the secondary stimulus, e.g., CD28). Engagement of the TCR/CD3 complex elicits a signaling cascade in the T cell that involves numerous signaling molecules including protein tyrosine kinases (PTKs), protein tyrosine phosphatases (PTPs), G proteins, GEFs and adaptor molecules (194, 239). The earliest event is activation of the Src family PTKs Lck and Fyn, which subsequently leads to phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) present in the  $\zeta$  and CD3  $\epsilon$ ,  $\delta$  and  $\gamma$  subunits of the TCR (30, 193). The phosphorylation of the ITAMs promotes recruitment and subsequent activation of the Syk-PTK ZAP-70. The activation of Src and Syk/ZAP-70 PTKs leads to phosphorylation of adaptor molecules and enzymes facilitating the activation of downstream signaling pathways (194). These events take place in specialized microdomains of the plasma membrane termed lipid rafts that have a high constituency of cholesterol and glycosphingolipids (360). The activation cascade culminates in gene transcription, cytoskeletal rearrangement, cytokine production, and proliferation.

There are several inhibitory mechanisms that negatively regulate the activation process and mount a threshold for the activation process (reviewed in Ref. 341). The inhibitory mechanisms prevent inappropriate or exagger-

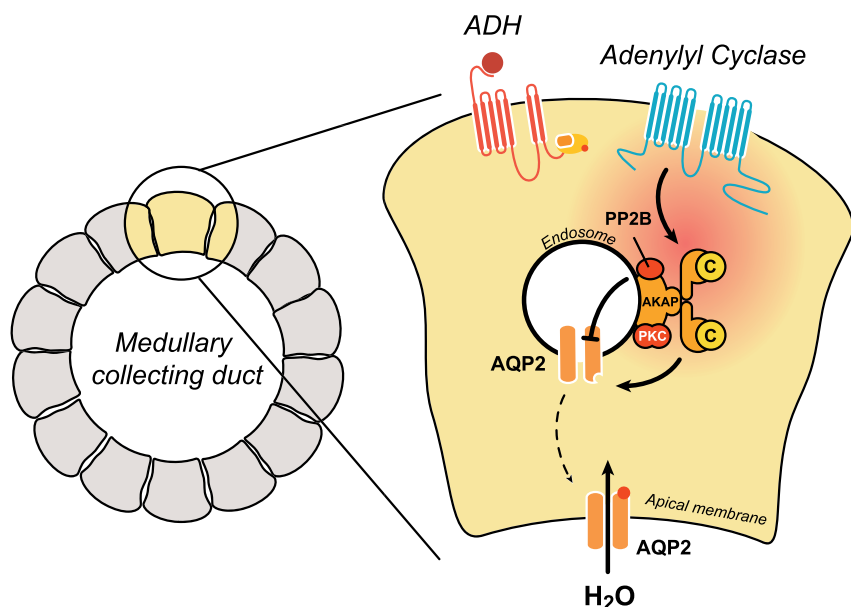


FIG. 10. Anchored PKA controls exocytotic processes. Antidiuretic hormone (ADH) elicits cAMP production by binding to the vasopressin V<sub>2</sub> receptors on the basolateral membrane surface of the principal cells in the renal collecting ducts. cAMP/PKA signaling is required for the translocation of aquaporin-2 (AQP2) from endosomes into the apical membrane in order for water reabsorption to occur. Several AKAPs have been identified in renal collecting ducts (181), and both PKA anchoring and catalytic activity are required for ADH-mediated AQP2 translocation. The specific AKAP involved has not yet been identified, but a potential candidate is AKAP18 $\delta$  (182).

ated immune activation and autoimmunity. The Src-family kinases are regulated by intramolecular interaction between the SH2 domain and the COOH-terminal phosphotyrosine that keeps the kinase in its inactive state. The COOH-terminal inhibitory site (Y505 in Lck) is phosphorylated by COOH-terminal Src kinase (Csk) and dephosphorylated by the protein tyrosine phosphatase CD45 (26, 58, 237, 238, 247). CD45 also inactivates Lck by dephosphorylation of its autophosphorylation site Y394 (88a). Csk thereby plays a key role in the negative regulation of TCR-mediated signal transduction and intersects the activation cascade at a very early level.

PGE<sub>2</sub> and other ligands elevating cAMP by binding to GPCRs inhibit TCR-induced T-cell activation and thereby exert important immunoregulatory functions (164). Based on studies with selective agonists, activation of PKA type I (RI $\alpha$ C<sub>2</sub>) has been shown to be necessary and sufficient for mediating these effects of cAMP (299, 301). Similarly, PKA type I negatively regulates activation of B cells through the B-cell antigen receptor (199) and natural killer (NK) cell cytotoxicity elicited through specific NK cell receptors (334). Although PKA can modulate TCR signaling at multiple levels (reviewed in Ref. 336), the observed inhibitory effects of cAMP on TCR-induced  $\zeta$ -chain phosphorylation point toward an important role for Csk, which is the most upstream PKA target reported so far. PKA phosphorylates S364 in Csk and induces a two- to fourfold increase in phosphotransferase activity of Csk in lipid rafts of T cells (340).

Analyses of lipid raft purifications from normal resting T cells for the presence of different subunits of PKA revealed that both the catalytic subunit and the regulatory subunit RI $\alpha$  (but no RII subunits) are constitutively associated with the lipid rafts (340). This suggests that the

observed colocalization of PKA type I and TCR in capped T cells (301) occurs in lipid rafts and that there are mechanisms for specific targeting of PKA type I to these areas involving interaction with an AKAP in lipid rafts (unpublished results). However, additional possibilities include anchoring of the PKA catalytic subunit, e.g., via the NH<sub>2</sub>-terminal myristyl group into rafts, or via interactions with a caveolin-like protein in T-cell rafts, similar to the PKA C $\alpha$  interaction with caveolin in other cell types (263).

Studies of the organization of G proteins in the plasma membrane revealed that in addition to G proteins, lipid rafts also contain adenylyl cyclase activity (148). In fact, a substantial fraction of the total isoproterenol or forskolin-stimulated adenylyl cyclase in S49 lymphoma cells is present in these fractions, strongly suggesting that the receptor-G protein and G protein-adenylyl cyclase coupling occur in lipid rafts, and similar data have been obtained for normal T cells and HEK293 cells (339). This implies targeting of the molecular machinery necessary for the generation of cAMP and activation of PKA type I after engagement of GPCRs to lipid rafts.

So far, two different mechanisms are reported to regulate Csk activity. PKA, through phosphorylation of Ser-364, increases Csk kinase activity two- to fourfold leading to reduced Lck activity and  $\zeta$ -chain phosphorylation. The other mechanism involves the adaptor molecule Cbp/PAG. Cbp/PAG recruits Csk to the site of action in lipid rafts (40, 171), and the interaction between Csk-SH2 and Cbp/PAG through phosphorylated Y314/Y317 (rat/human Cbp/PAG) increases Csk activity (325). Addition of either recombinant Cbp/PAG or peptides corresponding to the Csk-SH2 binding site significantly increased Csk kinase activity toward a Src substrate *in vitro*. Thus PKA

phosphorylation of Csk and interaction with Cbp/PAG may act together in turning on Csk activity, providing a powerful mechanism for terminating activation through receptors eliciting Src kinase signaling (Fig. 11).

Interestingly, the cAMP inhibitory pathway has also been shown to be implicated in several disease conditions. T cells from human immunodeficiency virus (HIV)-infected patients have elevated levels of cAMP and hyperactivation of PKA. Targeting of the cAMP-PKA type I pathway by selective antagonists reverses T-cell dysfunction in HIV T cells *ex vivo* (1, 2). A similar mechanism contributes to the T-cell dysfunction in a subset of patients with common variable immunodeficiency (15), and to the severe T-cell anergy in a murine immunodeficiency model termed MAIDS (mouse AIDS) (261).

## X. CONCLUDING REMARKS

Although a number of early studies indicated possibilities of compartmentalization of cAMP, the predominating view only little more than a decade ago was still that cAMP would be raised throughout the cell in response to many ligands. Detailed studies of compartmentalization of specific receptors and ACs to distinct membrane subdomains as well as live cell imaging of cAMP and unravelling of the subcellular targeting of PDEs has

now made clear that physiological increases in cAMP occur in discrete microdomains. Similarly, although PKA type II was well known to be biochemically particulate and several AKAPs were known 10 years ago, the prevailing view was still that many effects of cAMP would be mediated by *en bloc* activation of PKA over large areas of the cell and/or that the C subunit would be released from a PKA holoenzyme complex and travel some distance to find its substrate. However, since then it has become clear that a large spectrum of AKAP proteins is available (>50 AKAPs per date when differentially targeted splice variants are included, Table 1). Furthermore, new AKAPs for PKA type I, long thought to be primarily cytoplasmic and freely diffusible, are now increasingly reported. In addition, the requirement for anchoring of PKA to regulate specific substrates as well as to mediate a number of physiological effects has been extensively studied over the past decade, and with few exceptions it has been shown that most cAMP/PKA-regulated physiological processes require an anchored kinase. Thus the concept described in this review that has emerged over the past 10–15 years and that is now fairly well established is that a ligand normally will elicit a characteristic and local pool of cAMP that will follow a distinct route to reach and activate a single PKA-AKAP complex close to the substrate to mediate a distinct biological effect (Fig. 1). Ac-

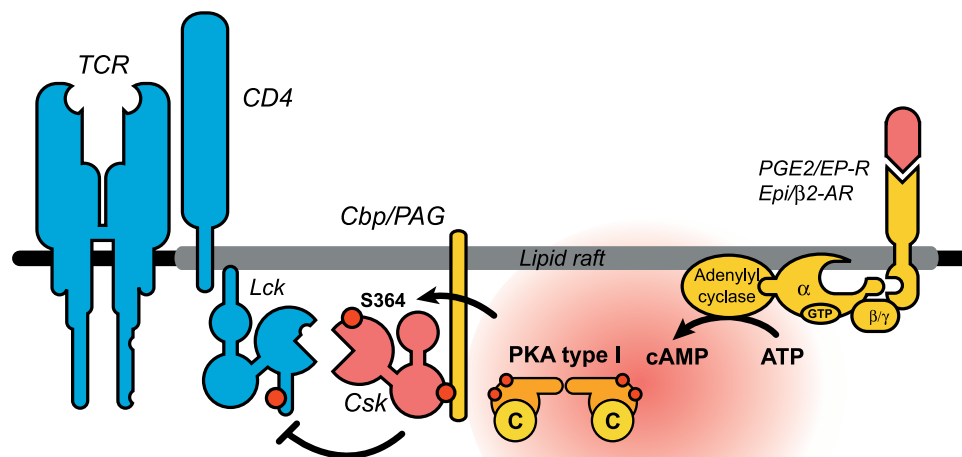


FIG. 11. cAMP inhibits T-cell activation through a PKA type I-Csk-Lck inhibitory pathway in lipid rafts. Proteins involved in proximal TCR signaling events are localized in lipid rafts, representing small regions of detergent-resistant lipid domains of the membrane. Both the cAMP-generating machinery (adenylyl cyclase) and the effectors (PKA type I and Csk) are localized in the lipid rafts. The mechanism involved in targeting  $R1\alpha$  to lipid rafts has not yet been fully elucidated, but is likely to involve an AKAP. PKA type I inhibits T-cell activation by phosphorylating Csk on S364, leading to a 2- to 4-fold increase in Csk kinase activity. Csk is recruited to lipid rafts by binding to Y317-phosphorylated Cbp/PAG through its SH2 domain and inhibits TCR signaling and Lck activity by phosphorylation of a COOH-terminal inhibitory tyrosine residue (Lck-Y505). Csk is constitutively localized to lipid rafts in resting T cells but is transiently displaced to the cytosol during T-cell activation (335) to allow the activation cascade to proceed. The phosphatase responsible for the dephosphorylation of Cbp/PAG and the release of Csk was recently identified as CD45 (77), whereas Lck-mediated phosphorylation of Cbp/PAG (40) leads to rerecruitment of Csk and reestablishment of the inhibitory pathway.

cordingly, each substrate appears to have its own, private anchored pool of PKA and its own local gradient of cAMP. Future studies will determine if this holds true for all ligands that signal through cAMP and substrates phosphorylated by PKA. Further studies will presumably unravel yet new AKAPs and substrates for PKA types I and II and determine how signaling through the cAMP-PKA pathway integrates with the complex signaling networks within the cell.

We are grateful to Dr. Philippe Collas and members of the Taskén laboratory for critical reading of the manuscript.

Address for reprint requests and other correspondence: K. Taskén, The Biotechnology Centre of Oslo, Univ. of Oslo, PO Box 1125 Blindern, N-0317 Oslo, Norway (E-mail: kjetil.tasken@biotek.uio.no).

## REFERENCES

- Aandahl EM, Aukrust P, Muller F, Hansson V, Tasken K, and Froland SS. Additive effects of IL-2 and protein kinase A type I antagonist on function of T cells from HIV-infected patients on HAART. *AIDS* 13: F.109–F.114, 1999.
- Aandahl EM, Aukrust P, Skalhegg BS, Muller F, Froland SS, Hansson V, and Tasken K. Protein kinase A type I antagonist restores immune responses of T cells from HIV-infected patients. *FASEB J* 12: 855–862, 1998.
- Adachi Y, Luke M, and Laemmli UK. Chromosome assembly in vitro: topoisomerase II is required for condensation. *Cell* 64: 137–148, 1991.
- Adams BA and Beam KG. Muscular dysgenesis in mice: a model system for studying excitation-contraction coupling. *FASEB J* 4: 2809–2816, 1990.
- Agnew BJ, Duman JG, Watson CL, Coling DE, and Forte JG. Cytological transformations associated with parietal cell stimulation: critical steps in the activation cascade. *J Cell Sci* 112: 2639–2646, 1999.
- Algrain M, Turunen O, Vaheri A, Louvard D, and Arpin M. Ezrin contains cytoskeleton and membrane binding domains accounting for its proposed role as a membrane-cytoskeletal linker. *J Cell Biol* 120: 129–139, 1993.
- Alto NM, Soderling J, and Scott JD. Rab32 is an A-kinase anchoring protein and participates in mitochondrial dynamics. *J Cell Biol* 158: 659–668, 2002.
- Alto NM, Soderling SH, Hoshi N, Langeberg LK, Fayos MR, Jennings PA, and Scott JD. Bioinformatic design of AKAP “in-silico”: a potent and selective peptide antagonist of type II protein kinase A anchoring. *Proc Natl Acad Sci USA* 100: 4445–4450, 2003.
- Amano M, Mukai H, Ono Y, Chihara K, Matsui T, Hamajima Y, Okawa K, Iwamatsu A, and Kaibuchi K. Identification of a putative target for Rho as the serine-threonine kinase protein kinase N. *Science* 271: 648–650, 1996.
- Ammala C, Eliasson L, Bokvist K, Berggren PO, Honkanen RE, Sjöholm A, and Rorsman P. Activation of protein kinases and inhibition of protein phosphatases play a central role in the regulation of exocytosis in mouse pancreatic beta cells. *Proc Natl Acad Sci USA* 91: 4343–4347, 1994.
- Angelo R and Rubin CS. Molecular characterization of an anchor protein (AKAPCE) that binds the RI subunit (RCE) of type I protein kinase A from *Caenorhabditis elegans*. *J Biol Chem* 273: 14633–14643, 1998.
- Angelo RG and Rubin CS. Characterization of structural features that mediate the tethering of *Caenorhabditis elegans* protein kinase A to a novel A kinase anchor protein. Insights into the anchoring of PKAI isoforms. *J Biol Chem* 275: 4351–4362, 2000.
- Anholt RR, Pedersen PL, De Souza EB, and Snyder SH. The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane. *J Biol Chem* 261: 576–583, 1986.
- Arreola J, Calvo J, Garcia MC, and Sanchez JA. Modulation of calcium channels of twitch skeletal muscle fibres of the frog by adrenaline and cyclic adenosine monophosphate. *J Physiol* 393: 307–330, 1987.
- Aukrust P, Aandahl EM, Skalhegg BS, Nordoy I, Hansson V, Tasken K, Froland SS, and Muller F. Increased activation of protein kinase A type I contributes to the T cell deficiency in common variable immunodeficiency. *J Immunol* 162: 1178–1185, 1999.
- Baillie GS, Huston E, Scotland G, Hodgkin M, Gall I, Peden AH, Mackenzie C, Houslay ES, Currie R, Pettitt TR, Walmsley AR, Wakelam MJ, Warwick J, and Houslay MD. Tapas-1, a novel microdomain within the unique N-terminal region of the P.D.E.4A.1 cAMP specific phosphodiesterase that allows rapid, Ca<sup>2+</sup>-triggered membrane association with selectivity for interaction with phosphatidic acid. *J Biol Chem* 277: 28298–28309, 2002.
- Baillie GS, Sood A, McPhee I, Gall I, Perry SJ, Lefkowitz RJ, and Houslay MD. beta-Arrestin-mediated PDE4 cAMP phosphodiesterase recruitment regulates beta-adrenoceptor switching from G<sub>s</sub> to G<sub>i</sub>. *Proc Natl Acad Sci USA* 100: 940–945, 2003.
- Bailey E, Doree M, Nurse P, and Bornens M. p34cdc2 is located in both nucleus and cytoplasm: part is centrosomally associated at G<sub>2</sub>/M and enters vesicles at anaphase. *EMBO J* 8: 3985–3995, 1989.
- Bailey E, Pines J, Hunter T, and Bornens M. Cytoplasmic accumulation of cyclin B1 in human cells: association with a detergent-resistant compartment and with the centrosome. *J Cell Sci* 101: 529–545, 1992.
- Banke TG, Bowie D, Lee H, Huganir RL, Schousboe A, and Traynelis SF. Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *J Neurosci* 20: 89–102, 2000.
- Banky P, Huang LJ, and Taylor SS. Dimerization/docking domain of the type Ialpha regulatory subunit of cAMP-dependent protein kinase. Requirements for dimerization and docking are distinct but overlapping. *J Biol Chem* 273: 35048–35055, 1998.
- Banky P, Newlon MG, Roy M, Garrod S, Taylor SS, and Jennings PA. Isoform-specific differences between the type Ialpha and IIalpha cyclic AMP-dependent protein kinase anchoring domains revealed by solution NMR. *J Biol Chem* 275: 35146–35152, 2000.
- Beard MB, Huston E, Campbell L, Gall I, McPhee I, Yarwood S, Scotland G, and Houslay MD. In addition to the SH3 binding region, multiple regions within the N-terminal noncatalytic portion of the cAMP-specific phosphodiesterase, PDE4A5, contribute to its intracellular targeting. *Cell Signal* 14: 453–465, 2002.
- Beard MB, O’Connell JC, Bolger GB, and Houslay MD. The unique N-terminal domain of the cAMP phosphodiesterase PDE4D4 allows for interaction with specific SH3 domains. *FEBS Lett* 460: 173–177, 1999.
- Benovic JL, Pike LJ, Cerione RA, Staniszewski C, Yoshimasa T, Codina J, Caron MG, and Lefkowitz RJ. Phosphorylation of the mammalian beta-adrenergic receptor by cyclic AMP-dependent protein kinase. Regulation of the rate of receptor phosphorylation and dephosphorylation by agonist occupancy and effects on coupling of the receptor to the stimulatory guanine nucleotide regulatory protein. *J Biol Chem* 260: 7094–7101, 1985.
- Bergman M, Mustelin T, Oetken C, Partanen J, Flint NA, Amrein KE, Autero M, Burn P, and Alitalo K. The human p50csk tyrosine kinase phosphorylates p56lck at Tyr-505 and down regulates its catalytic activity. *EMBO J* 11: 2919–2924, 1992.
- Bers DM. Cardiac excitation-contraction coupling. *Nature* 415: 198–205, 2002.
- Bers DM and Ziolo MT. When is cAMP not cAMP? Effects of compartmentalization. *Circ Res* 89: 373–375, 2001.
- Beullens M, Van Eynde A, Bollen M, and Stalmans W. Inactivation of nuclear inhibitory polypeptides of protein phosphatase-1 (NIPP-1) by protein kinase A. *J Biol Chem* 268: 13172–13177, 1993.
- Billadeau DD and Leibson PJ. ITAMS versus ITIMs: striking a balance during cell regulation. *J Clin Invest* 109: 161–168, 2002.
- Bishop AL and Hall A. Rho GTPases and their effector proteins. *Biochem J* 348: 241–255, 2000.
- Blank T, Nijholt I, Teichert U, Kugler H, Behrsing H, Fienberg A, Greengard P, and Spiess J. The phosphoprotein DARPP32

- mediates cAMP-dependent potentiation of striatal *N*-methyl-D-aspartate responses. *Proc Natl Acad Sci USA* 94: 14859–14864, 1997.
33. **Bornens M.** Centrosome composition and microtubule anchoring mechanisms. *Curr Opin Cell Biol* 14: 25–34, 2002.
  34. **Bos JL.** All in the family? New insights and questions regarding interconnectivity of Ras, Rap1 and Ral. *EMBO J* 17: 6776–6782, 1998.
  35. **Boujrad N, Gaillard JL, Garnier M, and Papadopoulos V.** Acute action of choriogonadotropin on Leydig tumor cells: induction of a higher affinity benzodiazepine-binding site related to steroid biosynthesis. *Endocrinology* 135: 1576–1583, 1994.
  36. **Boujrad N, Vidic B, and Papadopoulos V.** Acute action of choriogonadotropin on Leydig tumor cells: changes in the topography of the mitochondrial peripheral-type benzodiazepine receptor. *Endocrinology* 137: 5727–5730, 1996.
  37. **Bourne HR, Sanders DA, and McCormick F.** The GTPase Superfamily: a conserved switch for diverse cell functions. *Nature* 348: 125–132, 1990.
  38. **Bourne HR, Sanders DA, and McCormick F.** The GTPase Superfamily: conserved structure and molecular mechanism. *Nature* 349: 117–127, 1991.
  39. **Boussiotis VA, Freeman GJ, Berezovskaya A, Barber DL, and Nadler LM.** Maintenance of human T cell anergy: blocking of IL-2 gene transcription by activated Rap1. *Science* 278: 124–128, 1997.
  40. **Brdicka T, Pavlistova D, Leo A, Bruyns E, Korinek V, Angelisova P, Scherer J, Shevchenko A, Hilgert I, Cerny J, Drbal K, Kuramitsu Y, Kornacker B, Horejsi V, and Schraven B.** Phosphoprotein associated with glycosphingolipid-enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase csk and is involved in regulation of T cell activation. *J Exp Med* 191: 1591–1604, 2000.
  41. **Bregman DB, Bhattacharyya N, and Rubin CS.** High affinity binding protein for the regulatory subunit of cAMP-dependent protein kinase II. B. Cloning, characterization, and expression of cDNAs for rat brain P150. *J Biol Chem* 264: 4648–4656, 1989.
  42. **Broillet MC and Firestein S.** Cyclic nucleotide-gated channels. Molecular mechanisms of activation. *Ann NY Acad Sci* 868: 730–740, 1999.
  43. **Brokaw CJ.** Regulation of sperm flagellar motility by calcium and cAMP-dependent phosphorylation. *J Cell Biochem* 35: 175–184, 1987.
  44. **Burns-Hamuro LL, Ma Y, Kammerer S, Reineke U, Self C, Cook C, Olson G, Cantor CR, Braun A, and Taylor SS.** Designing isoform-specific peptide disruptors of protein kinase A localization. *Proc Natl Acad Sci USA* 100: 4072–4077, 2003.
  45. **Burton KA, Treash-Osio B, Muller CH, Dunphy EL, and McKnight GS.** Deletion of type II $\alpha$  regulatory subunit delocalizes protein kinase A in mouse sperm without affecting motility or fertilization. *J Biol Chem* 274: 24131–24136, 1999.
  46. **Carlson CR, Wiczak O, Vossebein L, Labbe JC, Skalhegg BS, Keryer G, Herberg FW, Collas P, and Tasken K.** Cdk1-mediated phosphorylation of the RII $\alpha$  regulatory subunit of PKA works as a molecular switch that promotes dissociation of RII $\alpha$  from centrosomes at mitosis. *J Cell Sci* 114: 3243–3254, 2001.
  47. **Carr DW, Demanno DA, Atwood A, Hunzicker-Dunn M, and Scott JD.** Follicle-stimulating hormone regulation of A-kinase anchoring proteins in granulosa cells. *J Biol Chem* 268: 20729–20732, 1993.
  48. **Carr DW, Fujita A, Stentz CL, Liberty GA, Olson GE, and Narumiya S.** Identification of sperm-specific proteins that interact with A-kinase anchoring proteins in a manner similar to the type II regulatory subunit of PKA. *J Biol Chem* 276: 17332–17338, 2001.
  49. **Carr DW, Hausken ZE, Fraser ID, Stofko-Hahn RE, and Scott JD.** Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein. Cloning and characterization of the RII-binding domain. *J Biol Chem* 267: 13376–13382, 1992.
  50. **Carr DW, Stofko-Hahn RE, Fraser ID, Bishop SM, Acott TS, Brennan RG, and Scott JD.** 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* 266: 14188–14192, 1991.
  51. **Carr DW, Stofko-Hahn RE, Fraser ID, Cone RD, and Scott JD.** Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins. Characterization of AKAP 79. *J Biol Chem* 267: 16816–16823, 1992.
  52. **Carrera A, Gerton GL, and Moss SB.** The major fibrous sheath polypeptide of mouse sperm: structural and functional similarities to the A-kinase anchoring proteins. *Dev Biol* 165: 272–284, 1994.
  53. **Catterall WA.** Excitation-contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels. *Cell* 64: 871–874, 1991.
  54. **Cederberg A, Gronning LM, Ahren B, Tasken K, Carlsson P, and Enerback S.** Foxc2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106: 563–573, 2001.
  55. **Cerne R, Rusin KI, and Randic M.** Enhancement of the *N*-methyl-D-aspartate response in spinal dorsal horn neurons by cAMP-dependent protein kinase. *Neurosci Lett* 161: 124–128, 1993.
  56. **Chaudhry A, Zhang C, and Granneman JG.** Characterization of RII(beta) and D-AKAP1 in differentiated adipocytes. *Am J Physiol Cell Physiol* 282: C205–C212, 2002.
  57. **Chen Q, Lin RY, and Rubin CS.** Organelle-specific targeting of protein kinase AII (PKAII). Molecular and in situ characterization of murine A kinase anchor proteins that recruit regulatory subunits of PKAII to the cytoplasmic surface of mitochondria. *J Biol Chem* 272: 15247–15257, 1997.
  58. **Chow LM, Fournel M, Davidson D, and Veillette A.** Negative regulation of T-cell receptor signalling by tyrosine protein kinase p50csk. *Nature* 365: 156–160, 1993.
  59. **Coghlan VM, Langeberg LK, Fernandez A, Lamb NJ, and Scott JD.** Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. *J Biol Chem* 269: 7658–7665, 1994.
  60. **Coghlan VM, Perrino BA, Howard M, Langeberg LK, Hicks JB, Gallatin WM, and Scott JD.** Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science* 267: 108–111, 1995.
  61. **Collas P, Le Guellec K, and Tasken K.** The A kinase-anchoring protein AKAP95 is a multivalent protein with a key role in chromatin condensation at mitosis. *J Cell Biol* 147: 1167–1180, 1999.
  62. **Colledge M, Dean RA, Scott GK, Langeberg LK, Haganir RL, and Scott JD.** Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* 27: 107–119, 2000.
  63. **Colledge M and Scott JD.** Akaps: from structure to function. *Trends Cell Biol* 9: 216–221, 1999.
  64. **Collins S and Surwit RS.** The beta-adrenergic receptors and the control of adipose tissue metabolism and thermogenesis. *Recent Prog Horm Res* 56: 309–328, 2001.
  65. **Compton DA.** Focusing on spindle poles. *J Cell Sci* 111: 1477–1481, 1998.
  66. **Cong M, Perry SJ, Lin FT, Fraser ID, Hu LA, Chen W, Pitcher JA, Scott JD, and Lefkowitz RJ.** Regulation of membrane targeting of the G protein-coupled receptor kinase 2 by protein kinase A and its anchoring protein AKAP79. *J Biol Chem* 276: 15192–15199, 2001.
  67. **Conti M.** Phosphodiesterases and cyclic nucleotide signaling in endocrine cells. *Mol Endocrinol* 14: 1317–1327, 2000.
  68. **Cook SJ, Rubinfeld B, Albert I, and McCormick F.** RapV12 antagonizes Ras-dependent activation of ERK1 and ERK2 by LPA and EGF in Rat-1 fibroblasts. *EMBO J* 12: 3475–3485, 1993.
  69. **Corbin JD, Francis SH, and Webb DJ.** Phosphodiesterase type 5 as a pharmacologic target in erectile dysfunction. *Urology* 60: 4–11, 2002.
  70. **Cross D, Dominguez J, Maccioni RB, and Avila J.** Map-1 and Map-2 binding sites at the C-terminus of beta-tubulin. Studies with synthetic tubulin peptides. *Biochemistry* 30: 4362–4366, 1991.
  71. **Cubizolles F, Legagneux V, Le Guellec R, Chartrain I, Uzbekov R, Ford C, and Le Guellec K.** pEg7, a new *Xenopus* protein required for mitotic chromosome condensation in egg extracts. *J Cell Biol* 143: 1437–1446, 1998.
  72. **Cummings DE, Brandon EP, Planas JV, Motamed K, Idzerda RL, and McKnight GS.** Genetically lean mice result from targeted disruption of the RII beta subunit of protein kinase A. *Nature* 382: 622–626, 1996.
  74. **Daaka Y, Luttrell LM, and Lefkowitz RJ.** Switching of the

- coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 390: 88–91, 1997.
75. Daniel PB, Walker WH, and Habener JF. Cyclic AMP signaling and gene regulation. *Annu Rev Nutr* 18: 353–383, 1998.
  76. Davare MA, Dong F, Rubin CS, and Hell JW. The A-kinase anchor protein MAP2B and cAMP-dependent protein kinase are associated with class C L-type calcium channels in neurons. *J Biol Chem* 274: 30280–30287, 1999.
  77. Davidson D, Bakinowski M, Thomas ML, Horejsi V, and Veillette A. Phosphorylation-dependent regulation of T-cell activation by PAG/Cbp, a lipid raft-associated transmembrane adaptor. *Mol Cell Biol* 23: 2017–2028, 2003.
  78. Degerman E, Belfrage P, and Manganiello VC. Structure, localization, and regulation of cGMP-inhibited phosphodiesterase (PDE3). *J Biol Chem* 272: 6823–6826, 1997.
  79. De Rooij J, Rehmann H, Van Triest M, Cool RH, Wittinghofer A, and Bos JL. Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. *J Biol Chem* 275: 20829–20836, 2000.
  80. De Rooij J, Zwartkruis FJ, Verheijen MH, Cool RH, Nijman SM, Wittinghofer A, and Bos JL. Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* 396: 474–477, 1998.
  81. Desseyn JL, Burton KA, and McKnight GS. Expression of a nonmyristylated variant of the catalytic subunit of protein kinase A during male germ-cell development. *Proc Natl Acad Sci USA* 97: 6433–6438, 2000.
  82. Dichtenberg JB, Zimmerman W, Sparks CA, Young A, Vidair C, Zheng Y, Carrington W, Fay FS, and Doxsey SJ. Pericentrin and gamma-tubulin form a protein complex and are organized into a novel lattice at the centrosome. *J Cell Biol* 141: 163–174, 1998.
  83. Diviani D, Langeberg LK, Doxsey SJ, and Scott JD. Pericentrin anchors protein kinase A at the centrosome through a newly identified RII-binding domain. *Curr Biol* 10: 417–420, 2000.
  84. Diviani D and Scott JD. Akap signaling complexes at the cytoskeleton. *J Cell Sci* 114: 1431–1437, 2001.
  85. Diviani D, Soderling J, and Scott JD. Akap-Lbc anchors protein kinase A and nucleates Galpha 12-selective Rho-mediated stress fiber formation. *J Biol Chem* 276: 44247–44257, 2001.
  86. Dodge K and Scott JD. Akap79 and the evolution of the AKAP model. *FEBS Lett* 476: 58–61, 2000.
  87. Dodge KL, Khuangathiene S, Kapiloff MS, Mouton R, Hill EV, Houslay MD, Langeberg LK, and Scott JD. mAkap assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. *EMBO J* 20: 1921–1930, 2001.
  88. Dong F, Feldmesser M, Casadevall A, and Rubin CS. Molecular characterization of a cDNA that encodes six isoforms of a novel murine A kinase anchor protein. *J Biol Chem* 273: 6533–6541, 1998.
  - 88a. D'oro U and Ashwell JD. Cutting edge: the CD45 tyrosine phosphatase is an inhibitor of Lck activity in thymocytes. *J Immunol* 162: 1879–1883, 1999.
  89. Dostmann WR and Taylor SS. Identifying the molecular switches that determine whether (Rp)-cAMPs functions as an antagonist or an agonist in the activation of cAMP-dependent protein kinase I. *Biochemistry* 30: 8710–8716, 1991.
  90. Doxsey SJ, Stein P, Evans L, Calarco PD, and Kirschner M. Pericentrin, a highly conserved centrosome protein involved in microtubule organization. *Cell* 76: 639–650, 1994.
  91. Dransfield DT, Bradford AJ, and Goldenring JR. Distribution of A-kinase anchoring proteins in parietal cells. *Biochim Biophys Acta* 1269: 215–220, 1995.
  92. Dransfield DT, Bradford AJ, Smith J, Martin M, Roy C, Mangeat PH, and Goldenring JR. Ezrin is a cyclic AMP-dependent protein kinase anchoring protein. *EMBO J* 16: 35–43, 1997.
  93. Ebihara K, Fukunaga K, Matsumoto K, Shichiri M, and Miyamoto E. Cyclosporin A stimulation of glucose-induced insulin secretion in MIN6 cells. *Endocrinology* 137: 5255–5263, 1996.
  94. Eden S, Rohatgi R, Podtelejnikov AV, Mann M, and Kirschner MW. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature* 418: 790–793, 2002.
  95. Eide T, Carlson C, Tasken K, Hirano T, Tasken K, and Collas P. Distinct but overlapping domains of AKAP95 are implicated in chromosome condensation and condensin targeting. *EMBO Reports* 3: 426–432, 2002.
  96. Eide T, Coghlan V, Orstavik S, Holsve C, Solberg R, Skalhegg BS, Lamb NJ, Langeberg L, Fernandez A, Scott JD, Jahnsen T, and Tasken K. Molecular cloning, chromosomal localization and cell cycle-dependent subcellular distribution of the A-kinase anchoring protein, AKAP95. *Exp Cell Res* 238: 305–316, 1998.
  97. Ellis DJ, Jenkins H, Whitfield WG, and Hutchison CJ. G-stimulin fusion proteins act as dominant negative mutants in *Xenopus* egg extract and reveal the function of the lamina in DNA replication. *J Cell Sci* 110: 2507–2518, 1997.
  98. Enserink JM, Christensen AE, De Rooij J, Van Triest M, Schwede F, Genieser HG, Doskeland SO, Blank JL, and Bos JL. A novel Epac-specific cAMP analogue demonstrates independent regulation of Rap1 and ERK. *Nat Cell Biol* 4: 901–906, 2002.
  99. Erlichman J, Gutierrez-Juarez R, Zucker S, Mei X, and Orr GA. Developmental expression of the protein kinase C substrate-binding protein (clone 72/SSeCKS) in rat testis identification as a scaffolding protein containing an A-kinase-anchoring domain which is expressed during late-stage spermatogenesis. *Eur J Biochem* 263: 797–805, 1999.
  100. Fantozzi DA, Harootunian AT, Wen W, Taylor SS, Feramisco JR, Tsien RY, and Meinkoth JL. Thermostable inhibitor of cAMP-dependent protein kinase enhances the rate of export of the kinase catalytic subunit from the nucleus. *J Biol Chem* 269: 2676–2686, 1994.
  101. Fantozzi DA, Taylor SS, Howard PW, Maurer RA, Feramisco JR, and Meinkoth JL. Effect of the thermostable protein kinase inhibitor on intracellular localization of the catalytic subunit of cAMP-dependent protein kinase. *J Biol Chem* 267: 16824–16828, 1992.
  102. Feldman AM and McNamara DM. Reevaluating the role of phosphodiesterase inhibitors in the treatment of cardiovascular disease. *Clin Cardiol* 25: 256–262, 2002.
  103. Feliciello A, Cardone L, Garbi C, Ginsberg MD, Varrone S, Rubin CS, Avvedimento EV, and Gottesman ME. Yotiao protein, a ligand for the NMDA receptor, binds and targets cAMP-dependent protein kinase II(1). *FEBS Lett* 464: 174–178, 1999.
  104. Feliciello A, Giuliano P, Porcellini A, Garbi C, Obici S, Mele E, Angotti E, Grieco D, Amabile G, Cassano S, Li Y, Musti AM, Rubin CS, Gottesman ME, and Avvedimento EV. The v-Ki-Ras oncogene alters cAMP nuclear signaling by regulating the location and the expression of cAMP-dependent protein kinase IIbeta. *J Biol Chem* 271: 25350–25359, 1996.
  105. Feliciello A, Li Y, Avvedimento EV, Gottesman ME, and Rubin CS. A-kinase anchor protein 75 increases the rate and magnitude of cAMP signaling to the nucleus. *Curr Biol* 7: 1011–1014, 1997.
  106. Fernandez A, Cavadore JC, Demaille J, and Lamb N. Implications for cAMP-dependent protein kinase in the maintenance of the interphase state. *Prog Cell Cycle Res* 1: 241–253, 1995.
  107. Fesenko EE, Kolesnikov SS, and Lyubarsky AL. Induction by cyclic GMP of cationic conductance in plasma membrane of retinal rod outer segment. *Nature* 313: 310–313, 1985.
  108. Flynn GE, Johnson JP Jr, and Zagotta WN. Cyclic nucleotide-gated channels: shedding light on the opening of a channel pore. *Nat Rev Neurosci* 2: 643–651, 2001.
  109. Forte TM, Machen TE, and Forte JG. Ultrastructural changes in oxyntic cells associated with secretory function: a membrane-recycling hypothesis. *Gastroenterology* 73: 941–955, 1977.
  110. Francis SH, Turko IV, and Corbin JD. Cyclic nucleotide phosphodiesterases: relating structure and function. *Prog Nucleic Acid Res Mol Biol* 65: 1–52, 2001.
  111. Frank K and Kranias EG. Phospholamban and cardiac contractility. *Ann Med* 32: 572–578, 2000.
  112. Frankfort BJ and Gelman IH. Identification of novel cellular genes transcriptionally suppressed by v-src. *Biochem Biophys Res Commun* 206: 916–926, 1995.
  113. Fraser ID, Cong M, Kim J, Rollins EN, Daaka Y, Lefkowitz RJ, and Scott JD. Assembly of an A kinase-anchoring protein-beta(2)-adrenergic receptor complex facilitates receptor phosphorylation and signaling. *Curr Biol* 10: 409–412, 2000.
  114. Fraser ID, Tavalin SJ, Lester LB, Langeberg LK, Westphal AM, Dean RA, Marrion NV, and Scott JD. A novel lipid-anchored A-kinase anchoring protein facilitates cAMP-responsive membrane events. *EMBO J* 17: 2261–2272, 1998.



115. Fukuyama T, Sueoka E, Sugio Y, Otsuka T, Niho Y, Akagi K, and Koza T. Mtg8 proto-oncoprotein interacts with the regulatory subunit of type II cyclic AMP-dependent protein kinase in lymphocytes. *Oncogene* 20: 6225–6232, 2001.
116. Fulcher KD, Mori C, Welch JE, O'Brien DA, Klapper DG, and Eddy EM. Characterization of Fsc1 cDNA for a mouse sperm fibrous sheath component. *Biol Reprod* 52: 41–49, 1995.
117. Gadsby DC and Nairn AC. Control of CFTR channel gating by phosphorylation and nucleotide hydrolysis. *Physiol Rev* 79: S77–S107, 1999.
118. Gaillard AR, Diener DR, Rosenbaum JL, and Sale WS. Flagellar radial spoke protein 3 is an A-kinase anchoring protein (AKAP). *J Cell Biol* 153: 443–448, 2001.
119. Gelman IH, Lee K, Tomblor E, Gordon R, and Lin X. Control of cytoskeletal architecture by the src-suppressed C kinase substrate, SSeCKS. *Cell Motil Cytoskeleton* 41: 1–17, 1998.
120. Gillingham AK and Munro S. The PACT domain, a conserved centrosomal targeting motif in the coiled-coil proteins AKAP450 and pericentrin. *EMBO Reports* 1: 524–529, 2000.
121. Goillard JM, Vincent PV, and Fischmeister R. Simultaneous measurements of intracellular cAMP and L-type  $Ca^{2+}$  current in single frog ventricular myocytes. *J Physiol* 530: 79–91, 2001.
122. Gomez LL, Alam S, Smith KE, Horne E, and Dell'acqua ML. Regulation of A-kinase anchoring protein 79/150-cAMP-dependent protein kinase postsynaptic targeting by NMDA receptor activation of calcineurin and remodeling of dendritic actin. *J Neurosci* 22: 7027–7044, 2002.
123. Gordon SE and Zagotta WN. Localization of regions affecting an allosteric transition in cyclic nucleotide-activated channels. *Neuron* 14: 857–864, 1995.
124. Gordon T, Grove B, Loftus JC, O'Toole T, McMillan R, Lindstrom J, and Ginsberg MH. Molecular cloning and preliminary characterization of a novel cytoplasmic antigen recognized by myasthenia gravis sera. *J Clin Invest* 90: 992–999, 1992.
125. Gradin HM, Larsson N, Marklund U, and Gullberg M. Regulation of microtubule dynamics by extracellular signals: cAMP-dependent protein kinase switches off the activity of oncoprotein 18 in intact cells. *J Cell Biol* 140: 131–141, 1998.
126. Gray PC, Johnson BD, Westenbroek RE, Hays LG, Yates JR III, Scheuer T, Catterall WA, and Murphy BJ. Primary structure and function of an A kinase anchoring protein associated with calcium channels. *Neuron* 20: 1017–1026, 1998.
127. Gray PC, Tibbs VC, Catterall WA, and Murphy BJ. Identification of a 15-kDa cAMP-dependent protein kinase-anchoring protein associated with skeletal muscle L-type calcium channels. *J Biol Chem* 272: 6297–6302, 1997.
128. Greengard P, Jen J, Nairn AC, and Stevens CF. Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. *Science* 253: 1135–1138, 1991.
129. Gronning LM, Cederberg A, Miura N, Enerback S, and Tasken K. Insulin and TNF alpha induce expression of the forkhead transcription factor gene Foxc2 in 3T3-L1 adipocytes via PI3K and ERK 1/2-dependent pathways. *Mol Endocrinol* 16: 873–883, 2002.
130. Grouse JR III, Allan MC, and Elam MB. Clinical manifestation of atherosclerotic peripheral arterial disease and the role of cilostazol in treatment of intermittent claudication. *J Clin Pharmacol* 42: 1291–1298, 2002.
131. Hall A. Rho GTPases and the actin cytoskeleton. *Science* 279: 509–514, 1998.
132. Hamuro Y, Burns L, Canaves J, Hoffman R, Taylor S, and Woods V. Domain organization of D-AKAP2 revealed by enhanced deuterium exchange-mass spectrometry (DXMS). *J Mol Biol* 321: 703–714, 2002.
133. Han JD, Baker NE, and Rubin CS. Molecular characterization of a novel A kinase anchor protein from *Drosophila melanogaster*. *J Biol Chem* 272: 26611–26619, 1997.
134. Hanoune J and Defer N. Regulation and role of adenylyl cyclase isoforms. *Annu Rev Pharmacol Toxicol* 41: 145–174, 2001.
135. Hanzel D, Reggio H, Bretscher A, Forte JG, and Mangeat P. The secretion-stimulated 80K phosphoprotein of parietal cells is ezrin, and has properties of a membrane cytoskeletal linker in the induced apical microvilli. *EMBO J* 10: 2363–2373, 1991.
136. Harootunian AT, Adams SR, Wen W, Meinkoth JL, Taylor SS, and Tsien RY. Movement of the free catalytic subunit of cAMP-dependent protein kinase into and out of the nucleus can be explained by diffusion. *Mol Biol Cell* 4: 993–1002, 1993.
137. Huet T, Liu J, Li H, Gazouli M, Culty M, and Papadopoulos V. PBR, StAR, and PKA: partners in cholesterol transport in steroidogenic cells. *Endocr Res* 28: 395–401, 2002.
138. Hausdorff WP, Caron MG, and Lefkowitz RJ. Turning off the signal: desensitization of beta-adrenergic receptor function. *FASEB J* 4: 2881–2889, 1990.
139. Hausken ZE, Coghlan VM, Hastings CA, Reimann EM, and Scott JD. Type II regulatory subunit (RII) of the cAMP-dependent protein kinase interaction with A-kinase anchor proteins requires isoleucines 3 and 5. *J Biol Chem* 269: 24245–24251, 1994.
140. Hausken ZE, Dell'acqua ML, Coghlan VM, and Scott JD. Mutational analysis of the A-kinase anchoring protein (AKAP)-binding site on RII classification of side chain determinants for anchoring and isoform selective association with AKAPs. *J Biol Chem* 271: 29016–29022, 1996.
141. Herberg FW, Maleszka A, Eide T, Vossebein L, and Tasken K. Analysis of A kinase anchoring protein (AKAP) interaction with protein kinase A (PKA) regulatory subunits: PKA isoform specificity in AKAP binding. *J Mol Biol* 298: 329–339, 2000.
142. Herrgard S, Jambeck P, Taylor SS, and Subramaniam S. Domain architecture of a *Caenorhabditis elegans* AKAP suggests a novel AKAP function. *FEBS Lett* 486: 107–111, 2000.
143. Higgs HN and Pollard TD. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. *Annu Rev Biochem* 70: 649–676, 2001.
144. Hirano T, Kobayashi R, and Hirano M. Condensins, chromosome condensation protein complexes containing XCAP-C, XCAP-E, and a *Xenopus* homolog of the *Drosophila* Barren protein. *Cell* 89: 511–521, 1997.
145. Hirano T and Mitchison TJ. A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. *Cell* 79: 449–458, 1994.
146. Hirokawa N. Microtubule organization and dynamics dependent on microtubule-associated proteins. *Curr Opin Cell Biol* 6: 74–81, 1994.
147. Houslay MD and Adams DR. PDE4 cAMP phosphodiesterases: modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochem J* 370: 1–18, 2003.
148. Huang C, Hepler JR, Chen LT, Gilman AG, Anderson RG, and Mumby SM. Organization of G proteins and adenylyl cyclase at the plasma membrane. *Mol Biol Cell* 8: 2365–2378, 1997.
149. Huang LJ, Durick K, Weiner JA, Chun J, and Taylor SS. D-Akap2, a novel protein kinase A anchoring protein with a putative RGS domain. *Proc Natl Acad Sci USA* 94: 11184–11189, 1997.
150. Huang LJ, Durick K, Weiner JA, Chun J, and Taylor SS. Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits. *J Biol Chem* 272: 8057–8064, 1997.
151. Huang LJ, Wang L, Ma Y, Durick K, Perkins G, Deerinck TJ, Ellisman MH, and Taylor SS. NH<sub>2</sub>-terminal targeting motifs direct dual specificity A-kinase-anchoring protein 1 (D-AKAP1) to either mitochondria or endoplasmic reticulum. *J Cell Biol* 145: 951–959, 1999.
152. Hulme JT, Ahn M, Hauschka SD, Scheuer T, and Catterall WA. A novel leucine zipper targets AKAP15 and cyclic AMP-dependent protein kinase to the C terminus of the skeletal muscle  $Ca^{2+}$  channel and modulates its function. *J Biol Chem* 277: 4079–4087, 2002.
153. Hunzicker-Dunn M, Scott JD, and Carr DW. Regulation of expression of A-kinase anchoring proteins in rat granulosa cells. *Biol Reprod* 58: 1496–1502, 1998.
154. Illenberger S, Drewes G, Trinczek B, Biernat J, Meyer HE, Olmsted JB, Mandelkow EM, and Mandelkow E. Phosphorylation of microtubule-associated proteins MAP2 and MAP4 by the protein kinase p110mark. Phosphorylation sites and regulation of microtubule dynamics. *J Biol Chem* 271: 10834–10843, 1996.
155. Jagatheesan G, Thanumalayan S, Muralikrishna B, Rangaraj N, Karande AA, and Parnaik VK. Colocalization of intranuclear lamin foci with RNA splicing factors. *J Cell Sci* 112: 4651–4661, 1999.

156. **Jin SL, Bushnik T, Lan L, and Conti M.** Subcellular localization of rolipram-sensitive, cAMP-specific phosphodiesterases. Differential targeting and activation of the splicing variants derived from the PDE4D gene. *J Biol Chem* 273: 19672–19678, 1998.
157. **Jin SL, Richard FJ, Kuo WP, D'Ercole AJ, and Conti M.** Impaired growth and fertility of cAMP-specific phosphodiesterase PDE4D-deficient mice. *Proc Natl Acad Sci USA* 96: 11998–12003, 1999.
158. **Johnson BD, Brousal JP, Peterson BZ, Gallombardo PA, Hockerman GH, Lai Y, Scheuer T, and Catterall WA.** Modulation of the cloned skeletal muscle L-type  $Ca^{2+}$  channel by anchored cAMP-dependent protein kinase. *J Neurosci* 17: 1243–1255, 1997.
159. **Johnson BD, Scheuer T, and Catterall WA.** Voltage-dependent potentiation of L-type  $Ca^{2+}$  channels in skeletal muscle cells requires anchored cAMP-dependent protein kinase. *Proc Natl Acad Sci USA* 91: 11492–11496, 1994.
160. **Johnson JP Jr and Zagotta WN.** Rotational movement during cyclic nucleotide-gated channel opening. *Nature* 412: 917–921, 2001.
161. **Johnson LR, Foster JA, Haig-Ladewig L, Vanscoy H, Rubin Cs, Moss Sb, and Gerton GI.** Assembly of AKAP82, a protein kinase A anchor protein, into the fibrous sheath of mouse sperm. *Dev Biol* 192: 340–350, 1997.
162. **Jones PM and Persaud SJ.** Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic beta-cells. *Endocr Rev* 19: 429–461, 1998.
163. **Juilfs DM, Fulle HJ, Zhao AZ, Houslay MD, Garbers DL, and Beavo JA.** A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase (PDE2) and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc Natl Acad Sci USA* 94: 3388–3395, 1997.
164. **Kammer GM.** The adenylate cyclase-cAMP-protein kinase A pathway and regulation of the immune response. *Immunol Today* 9: 222–229, 1988.
165. **Kammerer S, Hamuro LI, Ma Y, Hamon Sc, Canaves Jm, Shi Mm, Nelson Mr, Cantor Cr, Taylor Ss, and Braun A.** Amino acid variant in the kinase binding domain of D-AKAP2: a disease susceptibility polymorphism. *Proc Natl Acad Sci USA* 100: 4066–4071, 2003.
166. **Kapiloff MS, Jackson N, and Airhart N.** mA-KAP and the ryanodine receptor are part of a multi-component signaling complex on the cardiomyocyte nuclear envelope. *J Cell Sci* 114: 3167–3176, 2001.
167. **Kapiloff MS, Schillace RV, Westphal AM, and Scott JD.** mA-KAP: an A kinase anchoring protein targeted to the nuclear membrane of differentiated myocytes. *J Cell Sci* 112: 2725–2736, 1999.
168. **Karki S and Holzbaur EL.** Cytoplasmic dynein and dynactin in cell division and intracellular transport. *Curr Opin Cell Biol* 11: 45–53, 1999.
169. **Kashishian A, Howard M, Loh C, Gallatin WM, Hoekstra MF, and Lai Y.** Akap79 inhibits calcineurin through a site distinct from the immunophilin-binding region. *J Biol Chem* 273: 27412–27419, 1998.
170. **Katsura T, Gustafson CE, Ausiello DA, and Brown D.** Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. *Am J Physiol Renal Physiol* 272: F817–F822, 1997.
171. **Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K, Tarkhovskiy A, and Okada M.** Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. *Nature* 404: 999–1003, 2000.
172. **Kawasaki H, Springett GM, Mochizuki N, Toki S, Nakaya M, Matsuda M, Housman DE, and Graybiel AM.** A family of cAMP-binding proteins that directly activate Rap1. *Science* 282: 2275–2279, 1998.
173. **Keryer G, Witzak O, Delouvee A, Kemmner WA, Rouillard D, Tasken K, and Bornens M.** Dissociating the centrosomal matrix protein AKAP450 from centrosomes impairs centriole duplication and cell cycle progression. *Mol Biol Cell*. In press.
174. **Keryer G, Yassenko M, Labbe JC, Castro A, Lohmann SM, Evain-Brion D, and Tasken K.** Mitosis-specific phosphorylation and subcellular redistribution of the RIIalpha regulatory subunit of cAMP-dependent protein kinase. *J Biol Chem* 273: 34594–34602, 1998.
175. **Khuchua Z, Wozniak Df, Bardgett Me, McDonald M, Boero J, Hartman Re, Sims S, and Strauss AW.** Deletion of the N-terminus of murine MAP2 by gene targeting disrupts hippocampal cal neuron architecture and alters contextual memory. *Neuroscience* 119: 101–111, 2003.
176. **Kitayama H, Sugimoto Y, Matsuzaki T, Ikawa Y, and Noda MA.** Ras-related gene with transformation suppressor activity. *Cell* 56: 77–84, 1989.
177. **Klauck TM, Faux MC, Labudda K, Langeberg LK, Jaken S, and Scott JD.** Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. *Science* 271: 1589–1592, 1996.
178. **Klingbeil P, Frazzetto G, and Bouwmeester T.** Xgravin-like (Xgl), a novel putative a-kinase anchoring protein (AKAP) expressed during embryonic development in *Xenopus*. *Mech Dev* 100: 323–326, 2001.
179. **Klussmann E, Edemir B, Pepperle B, Tamma G, Henn V, Klauschenz E, Hundsrucker C, Maric K, and Rosenthal W.** Ht31: the first protein kinase A anchoring protein to integrate protein kinase A and Rho signaling. *FEBS Lett* 507: 264–268, 2001.
180. **Klussmann E, Maric K, and Rosenthal W.** The mechanisms of aquaporin control in the renal collecting duct. *Rev Physiol Biochem Pharmacol* 141: 33–95, 2000.
181. **Klussmann E, Maric K, Wiesner B, Beyermann M, and Rosenthal W.** Protein kinase A anchoring proteins are required for vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. *J Biol Chem* 274: 4934–4938, 1999.
182. **Klussmann E and Rosenthal W.** Role and identification of protein kinase A anchoring proteins in vasopressin-mediated aquaporin-2 translocation. *Kidney Int* 60: 446–449, 2001.
183. **Kopperud R, Christensen AE, Kjarland E, Viste K, Kleivdal H, and Doskeland SO.** Formation of inactive cAMP-saturated holoenzyme of cAMP-dependent protein kinase under physiological conditions. *J Biol Chem* 277: 13443–13448, 2002.
184. **Kovo M, Schillace RV, Galiani D, Josefsberg LB, Carr DW, and Dekel N.** Expression and modification of PKA and AKAPs during meiosis in rat oocytes. *Mol Cell Endocrinol* 192: 105–113, 2002.
185. **Kozma R, Ahmed S, Best A, and Lim L.** The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol Cell Biol* 15: 1942–1952, 1995.
186. **Krebs EG and Beavo JA.** Phosphorylation-dephosphorylation of enzymes. *Annu Rev Biochem* 48: 923–959, 1979.
187. **Krueger KE and Papadopoulos V.** Peripheral-type benzodiazepine receptors mediate translocation of cholesterol from outer to inner mitochondrial membranes in adrenocortical cells. *J Biol Chem* 265: 15015–15022, 1990.
188. **Kultgen PL, Byrd SK, Ostrowski LE, and Milgram SL.** Characterization of an a-kinase anchoring protein in human ciliary axonemes. *Mol Biol Cell* 13: 4156–4166, 2002.
189. **Kussel-Andermann P, El Amraoui A, Safieddine S, Hardelin JP, Nouaille S, Camonis J, and Petit C.** Unconventional myosin VIIA is a novel A-kinase-anchoring protein. *J Biol Chem* 275: 29654–29659, 2000.
190. **Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, and Arakawa M.** Molecular diversity of the NMDA receptor channel. *Nature* 358: 36–41, 1992.
191. **Lande MB, Jo I, Zeidel ML, Somers M, and Harris HW Jr.** Phosphorylation of aquaporin-2 does not alter the membrane water permeability of rat papillary water channel-containing vesicles. *J Biol Chem* 271: 5552–5557, 1996.
192. **Landsverk HB, Carlson CR, Steen RL, Vossebein L, Herberg FW, Tasken K, and Collas P.** Regulation of anchoring of the RIIalpha regulatory subunit of PKA to AKAP95 by threonine phosphorylation of RIIalpha: implications for chromosome dynamics at mitosis. *J Cell Sci* 114: 3255–3264, 2001.
193. **Latour S and Veillette A.** Proximal protein tyrosine kinases in immunoreceptor signaling. *Curr Opin Immunol* 13: 299–306, 2001.
194. **Leo A and Schraven B.** Adapters in lymphocyte signalling. *Curr Opin Immunol* 13: 307–316, 2001.

195. **Leon DA, Herberg FW, Banky P, and Taylor SS.** A stable alpha-helical domain at the N terminus of the R1alpha subunits of cAMP-dependent protein kinase is a novel dimerization/docking motif. *J Biol Chem* 272: 28431–28437, 1997.
196. **Lester LB, Coghlan VM, Nauert B, and Scott JD.** Cloning and characterization of a novel A-kinase anchoring protein AKAP 220, association with testicular peroxisomes. *J Biol Chem* 271: 9460–9465, 1996.
197. **Lester LB, Faux MC, Nauert JB, and Scott JD.** Targeted protein kinase A and PP-2B regulate insulin secretion through reversible phosphorylation. *Endocrinology* 142: 1218–1227, 2001.
198. **Lester LB, Langeberg LK, and Scott JD.** Anchoring of protein kinase A facilitates hormone-mediated insulin secretion. *Proc Natl Acad Sci USA* 94: 14942–14947, 1997.
199. **Levy FO, Rasmussen AM, Tasken K, Skalhogg BS, Huitfeldt HS, Funderud S, Smeland EB, and Hansson V.** Cyclic AMP dependent protein kinase (cAK) in human B cells: co-localization of type I cAK (RI alpha 2 C2) with the antigen receptor during anti-immunoglobulin-induced B cell activation. *Eur J Immunol* 26: 1290–1296, 1996.
200. **Li H, Adamik R, Pacheco-Rodriguez G, Moss J, and Vaughan M.** Protein kinase A anchoring (AKAP) domains in brefeldin A-inhibited guanine nucleotide-exchange protein 2 (BIG2). *Proc Natl Acad Sci USA* 100: 1627–1632, 2003.
201. **Li H, Degenhardt B, Tobin D, Yao ZX, Tasken K, and Papadopoulos V.** Identification, localization and function in steroidogenesis of PAP7: a peripheral-type benzodiazepine receptor and PKA (R1alpha)-associated protein. *Mol Endocrinol* 15: 2211–2228, 2001.
202. **Li Z, Rossi EA, Hoheisel JD, Kalderon D, and Rubin CS.** Generation of a novel A kinase anchor protein and a myristoylated alanine-rich C kinase substrate-like analog from a single gene. *J Biol Chem* 274: 27191–27200, 1999.
203. **Lin F, Wang H, and Malbon CC.** Gravin-mediated formation of signaling complexes in beta 2-adrenergic receptor desensitization and resensitization. *J Biol Chem* 275: 19025–19034, 2000.
204. **Lin JW, Wyszynski M, Madhavan R, Sealock R, Kim JU, and Sheng M.** Yotiao, a novel protein of neuromuscular junction and brain that interacts with specific splice variants of NMDA receptor subunit NR1. *J Neurosci* 18: 2017–2027, 1998.
205. **Lin RY, Moss SB, and Rubin CS.** Characterization of SAKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells. *J Biol Chem* 270: 27804–27817, 1995.
206. **Lin X, Tomblor E, Nelson PJ, Ross M, and Gelman IH.** A novel src- and ras-suppressed protein kinase C substrate associated with cytoskeletal architecture. *J Biol Chem* 271: 28430–28438, 1996.
207. **Liu DT, Tibbs GR, Paoletti P, and Siegelbaum SA.** Constraining ligand-binding site stoichiometry suggests that a cyclic nucleotide-gated channel is composed of two functional dimers. *Neuron* 21: 235–248, 1998.
208. **Lohmann SM, Decamilli P, Einig I, and Walter U.** High-affinity binding of the regulatory subunit (RII) of cAMP-dependent protein kinase to microtubule-associated and other cellular proteins. *Proc Natl Acad Sci USA* 81: 6723–6727, 1984.
209. **Lowell BB.** Fat metabolism. Slimming with leaner enzyme. *Nature* 382: 585–586, 1996.
210. **Luttrell LM, Ferguson SS, Daaka Y, Miller WE, Maudsley S, Della Rocca GJ, Lin F, Kawakatsu H, Owada K, Luttrell DK, Caron MG, and Lefkowitz RJ.** Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science* 283: 655–661, 1999.
211. **Mackenzie SJ, Baillie GS, McPhee I, Bolger GB, and Houslay MD.** Erk2 mitogen-activated protein kinase binding, phosphorylation, and regulation of the PDE4D cAMP-specific phosphodiesterases. The involvement of COOH-terminal docking sites and NH<sub>2</sub>-terminal UCR regions. *J Biol Chem* 275: 16609–16617, 2000.
212. **Mackenzie SJ, Baillie GS, McPhee I, Mackenzie C, Seamons R, McSorley T, Millen J, Beard MB, Van Heeke G, and Houslay MD.** Long PDE4 cAMP specific phosphodiesterases are activated by protein kinase A-mediated phosphorylation of a single serine residue in Upstream Conserved Region I (UCR1). *Br J Pharmacol* 136: 421–433, 2002.
213. **Mandal A, Naaby-Hansen S, Wolkowicz MJ, Klotz K, Shetty J, Retief JD, Coonrod SA, Kinter M, Sherman N, Cesar F, Flickinger CJ, and Herr JC.** Fsp95, a testis-specific 95-kilodalton fibrous sheath antigen that undergoes tyrosine phosphorylation in capacitated human spermatozoa. *Biol Reprod* 61: 1184–1197, 1999.
214. **Manji HK, Quiroz JA, Sporn J, Payne JL, Denicoff K, Gray NA, Zarate CA, and Charney DS.** Enhancing neuronal plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. *Biol Psych* 53: 707–742, 2003.
215. **Marchand JB, Kaiser DA, Pollard TD, and Higgs HN.** Interaction of WASP/Scar proteins with actin and vertebrate Arp2/3 complex. *Nat Cell Biol* 3: 76–82, 2001.
216. **Marks AR.** Ryanodine receptors/calcium release channels in heart failure and sudden cardiac death. *J Mol Cell Cardiol* 33: 615–624, 2001.
217. **Marx SO, Reiken S, Hisamatsu Y, Jayaraman T, Burkhoff D, Roseblit N, and Marks AR.** PKA phosphorylation dissociates FKBP126 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101: 365–376, 2000.
218. **Matsuzawa K, Kosako H, Inagaki N, Shibata H, Mukai H, Ono Y, Amano M, Kaibuchi K, Matsuura Y, Azuma I, and Inagaki M.** Domain-specific phosphorylation of vimentin and glial fibrillary acidic protein by PKN. *Biochem Biophys Res Commun* 234: 621–625, 1997.
219. **Mayr B and Montminy M.** Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol* 2: 599–609, 2001.
220. **McCartney S, Little BM, Langeberg LK, and Scott JD.** Cloning and characterization of A-kinase anchor protein 100 (AKAP100) A protein that targets A-kinase to the sarcoplasmic reticulum. *J Biol Chem* 270: 9327–9333, 1995.
221. **Meguro H, Mori H, Araki K, Kushiya E, Kutsuwada T, Yamazaki M, Kumanishi T, Arakawa M, Sakimura K, and Mishina M.** Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature* 357: 70–74, 1992.
222. **Mehats C, Andersen CB, Filipanti M, Jin SL, and Conti M.** Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends Endocrinol Metab* 13: 29–35, 2002.
223. **Mei X, Singh IS, Erlichman J, and Orr GA.** Cloning and characterization of a testis-specific, developmentally regulated A-kinase-anchoring protein (TAKAP-80) present on the fibrous sheath of rat sperm. *Eur J Biochem* 246: 425–432, 1997.
224. **Meinkoth JL, Ji Y, Taylor SS, and Feramisco JR.** Dynamics of the distribution of cyclic AMP-dependent protein kinase in living cells. *Proc Natl Acad Sci USA* 87: 9595–9599, 1990.
225. **Merdes A and Cleveland DW.** Pathways of spindle pole formation: different mechanisms: conserved components. *J Cell Biol* 138: 953–956, 1997.
226. **Michel JJ and Scott JD.** Akap mediated signal transduction. *Annu Rev Pharmacol Toxicol* 42: 235–257, 2002.
227. **Miki H, Suetsugu S, and Takenawa T.** Wave, a novel WASP family protein involved in actin reorganization induced by Rac. *EMBO J* 17: 6932–6941, 1998.
228. **Miki K and Eddy EM.** Identification of tethering domains for protein kinase A type Ialpha regulatory subunits on sperm fibrous sheath protein FSC1. *J Biol Chem* 273: 34384–34390, 1998.
229. **Miki K, Willis WD, Brown PR, Goulding EH, Fulcher KD, and Eddy EM.** Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. *Dev Biol* 248: 331–342, 2002.
230. **Monaco L, Lamas M, Tamai K, Lalli E, Zazopoulos E, Penna L, Nantel F, Foulkes NS, Mazzucchelli C, and Sassone-Corsi P.** Coupling transcription to signaling pathways: cAMP and nuclear factor cAMP-responsive element modulator. *Adv Second Messenger Phosphoprotein Res* 31: 63–74, 1997.
231. **Montminy M.** Transcriptional regulation by cyclic AMP. *Annu Rev Biochem* 66: 807–822, 1997.
232. **Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, and Seeburg PH.** Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* 256: 1217–1221, 1992.
233. **Moss SB and Gerton GL.** A-kinase anchor proteins in endocrine systems and reproduction. *Trends Endocrinol Metab* 12: 434–440, 2001.
234. **Movsesian MA.** cAMP-mediated signal transduction and sarco-

- plasmic reticulum function in heart failure. *Ann NY Acad Sci* 853: 231–239, 1998.
235. Mukai H, Kitagawa M, Shibata H, Takanaga H, Mori K, Shimakawa M, Miyahara M, Hirao K, and Ono Y. Activation of PKN, a novel 120-kDa protein kinase with leucine zipper-like sequences, by unsaturated fatty acids and by limited proteolysis. *Biochem Biophys Res Commun* 204: 348–356, 1994.
  236. Mukai H, Toshimori M, Shibata H, Kitagawa M, Shimakawa M, Miyahara M, Sunakawa H, and Ono Y. PKN associates and phosphorylates the head-rod domain of neurofilament protein. *J Biol Chem* 271: 9816–9822, 1996.
  237. Mustelin T, Coggeshall KM, and Altman A. Rapid activation of the T-cell tyrosine protein kinase pp56lck by the CD45 phosphotyrosine phosphatase. *Proc Natl Acad Sci USA* 86: 6302–6306, 1989.
  238. Mustelin T, Pessa-Morikawa T, Autero M, Gassmann M, Andersson LC, Gahrberg CG, and Burn P. Regulation of the p59fyn protein tyrosine kinase by the CD45 phosphotyrosine phosphatase. *Eur J Immunol* 22: 1173–1178, 1992.
  239. Mustelin T, and Tasken K. Positive and negative regulation of T cell activation through kinases and phosphatases. *Biochem J* 371: 15–27, 2003.
  240. Nakamura T and Gold GH. A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* 325: 442–444, 1987.
  241. Nauert JB, Klauack TM, Langeberg LK, and Scott JD. Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein. *Curr Biol* 7: 52–62, 1997.
  242. Nelson PJ and Gelman IH. Cell-cycle regulated expression and serine phosphorylation of the myristylated protein kinase C substrate, SSeCKS: correlation with culture confluency, cell cycle phase and serum response. *Mol Cell Biochem* 175: 233–241, 1997.
  243. Newlon MG, Roy M, Hausken ZE, Scott JD, and Jennings PA. The A-kinase anchoring domain of type IIalpha cAMP-dependent protein kinase is highly helical. *J Biol Chem* 272: 23637–23644, 1997.
  244. Newlon MG, Roy M, Morikis D, Carr DW, Westphal R, Scott JD, and Jennings PA. A novel mechanism of PKA anchoring revealed by solution structures of anchoring complexes. *EMBO J* 20: 1651–1662, 2001.
  245. Newlon MG, Roy M, Morikis D, Hausken ZE, Coghlan V, Scott JD, and Jennings PA. The molecular basis for protein kinase A anchoring revealed by solution NMR. *Nat Struct Biol* 6: 222–227, 1999.
  246. Nobes Cd and Hall A. Rho, rac and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 81: 53–62, 1995.
  247. Okada M, Nada S, Yamanashi Y, Yamamoto T, and Nakagawa H. Csk: a protein-tyrosine kinase involved in regulation of src family kinases. *J Biol Chem* 266: 24249–24252, 1991.
  248. Papadopoulos V. Peripheral-type benzodiazepine/diazepam binding inhibitor receptor: biological role in steroidogenic cell function. *Endocr Rev* 14: 222–240, 1993.
  249. Papadopoulos V, Amri H, Li H, Boujrad N, Vidic B, and Garnier M. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. *J Biol Chem* 272: 32129–32135, 1997.
  250. Papadopoulos V, Mukhin AG, Costa E, and Krueger KE. The peripheral-type benzodiazepine receptor is functionally linked to Leydig cell steroidogenesis. *J Biol Chem* 265: 3772–3779, 1990.
  251. Patel TB, Du Z, Pierre S, Cartin L, and Scholich K. Molecular biological approaches to unravel adenylyl cyclase signaling and function. *Gene* 269: 13–25, 2001.
  252. Perry SJ, Bailie GS, Kohout TA, McPhee I, Magiera MM, Ang KL, Miller WE, McLean AJ, Conti M, Houslay MD, and Lefkowitz RJ. Targeting of cyclic AMP degradation to beta 2-adrenergic receptors by beta-arrestins. *Science* 298: 834–836, 2002.
  253. Peter M, Nakagawa J, Doree M, Labbe JC, and Nigg EA. In vitro disassembly of the nuclear lamina and M phase-specific phosphorylation of lamins by cdc2 kinase. *Cell* 61: 591–602, 1990.
  254. Pizon V, Chardin P, Lerosey I, Olofsson B, and Tavittian A. Human Cdnas Rap1 and Rap2 homologous to the *Drosophila* gene Dras3 encode proteins closely related to Ras in the effector region. *Oncogene* 3: 201–204, 1988.
  255. Planas JV, Cummings DE, Idzerda RL, and McKnight GS. Mutation of the RIIBeta subunit of protein kinase A differentially affects lipolysis but not gene induction in white adipose tissue. *J Biol Chem* 274: 36281–36287, 1999.
  256. Pogwizd SM, Schlotthauer K, Li L, Yuan W, and Bers DM. Arrhythmogenesis and contractile dysfunction in heart failure: roles of sodium-calcium exchange, inward rectifier potassium current, and residual beta-adrenergic responsiveness. *Circ Res* 88: 1159–1167, 2001.
  257. Pozzan T, Rizzuto R, Volpe P, and Meldolesi J. Molecular and cellular physiology of intracellular calcium stores. *Physiol Rev* 74: 595–636, 1994.
  258. Purohit A, Tynan SH, Vallee R, and Doxsey SJ. Direct interaction of pericentrin with cytoplasmic dynein light intermediate chain contributes to mitotic spindle organization. *J. Cell Biol* 147: 481–492, 1999.
  259. Quill TA, Ren D, Clapham DE, and Garbers DL. A voltage-gated ion channel expressed specifically in spermatozoa. *Proc Natl Acad Sci USA* 98: 12527–12531, 2001.
  260. Quinton PM. Physiological basis of cystic fibrosis: a historical perspective. *Physiol Rev* 79 Suppl: S3–S22, 1999.
  261. Rahmouni S, Aandahl EM, Trebak M, Boniver J, Tasken K, and Moutschen M. Increased cAMP levels and protein kinase (PKA) type I activation in CD4+ T cells and B cells contribute to retrovirus-induced immunodeficiency of mice (MAIDS): a useful in vivo model for drug testing. *FASEB J* 15: 1466–1468, 2001.
  262. Raman IM, Tong G, and Jahr CE. Beta-adrenergic regulation of synaptic NMDA receptors by cAMP-dependent protein kinase. *Neuron* 16: 415–421, 1996.
  263. Razani B, Rubin CS, and Lisanti MP. Regulation of cAMP-mediated signal transduction via interaction of caveolins with the catalytic subunit of protein kinase A. *J Biol Chem* 274: 26353–26360, 1999.
  264. Reczek D, Berryman M, and Bretscher A. Identification of EBP50: a PDZ containing phosphoprotein that associates with members of the ezrin-radixin-moesin family. *J Cell Biol* 139: 169–179, 1997.
  265. Rehmann H, Prakash B, Wolf E, Rueppel A, De Rooij J, Bos JL, and Wittinghofer A. Structure and regulation of the cAMP-binding domains of Epac2. *Nat Struct Biol* 10: 26–32, 2003.
  266. Reinton N, Collas P, Haugen TB, Skalhegg BS, Hansson V, Jahnsen T, and Tasken K. Localization of a novel human A-kinase-anchoring protein, hAKAP220, during spermatogenesis. *Dev Biol* 223: 194–204, 2000.
  267. Reinton N, Orstavik S, Haugen TB, Jahnsen T, Tasken K, and Skalhegg BS. A novel isoform of human cyclic 3',5'-adenosine monophosphate-dependent protein kinase, c alpha-s, localizes to sperm midpiece. *Biol Reprod* 63: 607–611, 2000.
  268. Ren D, Navarro B, Perez G, Jackson AC, Hsu S, Shi Q, Tilly JL, and Clapham DE. A sperm ion channel required for sperm motility and male fertility. *Nature* 413: 603–609, 2001.
  269. Riabowol KT, Fink JS, Gilman MZ, Walsh DA, Goodman RH, and Feramisco JR. The catalytic subunit of cAMP-dependent protein kinase induces expression of genes containing cAMP-responsive enhancer elements. *Nature* 336: 83–86, 1988.
  270. Ridley AJ and Hall A. The small GTP binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 70: 389–399, 1992.
  271. Ridley AJ, Paterson HF, Johnston CL, Diekmann D, and Hall A. The small GTP binding protein rac regulates growth factor-induced membrane ruffling. *Cell* 70: 401–410, 1992.
  272. Rios E and Stern MD. Calcium in close quarters: microdomain feedback in excitation-contraction coupling and other cell biological phenomena. *Annu Rev Biophys Biomol Struct* 26: 47–82, 1997.
  273. Rios RM, Celati C, Lohmann SM, Bornens M, and Keryer G. Identification of a high affinity binding protein for the regulatory subunit RII beta of cAMP-dependent protein kinase in Golgi enriched membranes of human lymphoblasts. *EMBO J* 11: 1723–1731, 1992.
  274. Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, and Huganir RL. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16: 1179–1188, 1996.
  275. Rosenmund C, Carr DW, Bergeson SE, Nilaver G, Scott JD, and Westbrook GL. Anchoring of protein kinase A is required for

- modulation of AMPA/kainate receptors on hippocampal neurons. *Nature* 368: 853–856, 1994.
276. **Rossi EA, Li Z, Feng H, and Rubin CS.** Characterization of the targeting, binding, and phosphorylation site domains of an A kinase anchor protein and a myristoylated alanine-rich C kinase substrate-like analog that are encoded by a single gene. *J Biol Chem* 274: 27201–27210, 1999.
  277. **Ruiz ML and Karpén JW.** Single cyclic nucleotide-gated channels locked in different ligand-bound states. *Nature* 389: 389–392, 1997.
  278. **Saitoh N, Goldberg IG, Wood ER, and Earnshaw WC.** ScII: an abundant chromosome scaffold protein is a member of a family of putative ATPases with an unusual predicted tertiary structure. *J Cell Biol* 127: 303–318, 1994.
  279. **San Agustín JT, Leszyk JD, Nuwaysir LM, and Witman GB.** The catalytic subunit of the cAMP-dependent protein kinase of ovine sperm flagella has a unique amino-terminal sequence. *J Biol Chem* 273: 24874–24883, 1998.
  280. **San Agustín JT and Witman GB.** Differential expression of the C(s) and C(alph) isoforms of the catalytic subunit of cyclic 3',5'-adenosine monophosphate-dependent protein kinase testicular cells. *Biol Reprod* 65: 151–164, 2001.
  281. **Sanchez C, Diaz-Nido J, and Avila J.** Phosphorylation of microtubule-associated protein 2 (MAP2) and its relevance for the regulation of the neuronal cytoskeleton function. *Prog Neurobiol* 61: 133–168, 2000.
  282. **Sarkar D, Erlichman J, and Rubin CS.** Identification of a calmodulin-binding protein that co-purifies with the regulatory subunit of brain protein kinase II. *J Biol Chem* 259: 9840–9846, 1984.
  283. **Schillace RV, Andrews SF, Liberty GA, Davey MP, and Carr DW.** Identification and characterization of myeloid translocation gene 16b as a novel kinase anchoring protein in T lymphocytes. *J Immunol* 168: 1590–1599, 2002.
  284. **Schillace RV and Scott JD.** Association of the type 1 protein phosphatase PP1 with the A-kinase anchoring protein AKAP220. *Curr Biol* 9: 321–324, 1999.
  285. **Schillace RV, Voltz JW, Sim AT, Shenolikar S, and Scott JD.** Multiple interactions within the AKAP220 signaling complex contribute to protein phosphatase 1 regulation. *J Biol Chem* 276: 12128–12134, 2001.
  286. **Schmid A, Renaud JF, and Lazdunski M.** Short term and long term effects of beta-adrenergic effectors and cyclic AMP on nitrendipine-sensitive voltage-dependent Ca<sup>2+</sup> channels of skeletal muscle. *J Biol Chem* 260: 13041–13046, 1985.
  287. **Schmidt PH, Dransfield DT, Claudio JO, Hawley RG, Trotter KW, Milgram SL, and Goldenring JR.** Akap350, a multiply spliced protein kinase A-anchoring protein associated with centrosomes. *J Biol Chem* 274: 3055–3066, 1999.
  288. **Schneider A, Biernat J, Von Bergen M, Mandelkow E, and Mandelkow EM.** Phosphorylation that detaches tau protein from microtubules (Ser262, Ser214) also protects it against aggregation into Alzheimer paired helical filaments. *Biochemistry* 38: 3549–3558, 1999.
  289. **Schwencke C, Yamamoto M, Okumura S, Toya Y, Kim SJ, and Ishikawa Y.** Compartmentation of cyclic adenosine 3',5'-monophosphate signaling in caveolae. *Mol Endocrinol* 13: 1061–1070, 1999.
  290. **Schwinger RHG and Frank KF.** Calcium and the failing heart: phospholamban, good guy or bad guy? *Sci STKE* 2003: pe15, 2003.
  291. **Sculptoreanu A, Scheuer T, and Catterall WA.** Voltage-dependent potentiation of L-type Ca<sup>2+</sup> channels due to phosphorylation by cAMP-dependent protein kinase. *Nature* 364: 240–243, 1993.
  292. **Serrano L, Avila J, and Maccioni RB.** Controlled proteolysis of tubulin by subtilisin: localization of the site for MAP2 interaction. *Biochemistry* 23: 4675–4681, 1984.
  293. **Sewer MB and Waterman MR.** Insights into the transcriptional regulation of steroidogenic enzymes and StAR. *Rev Endocr Metab Disorders* 2: 269–274, 2001.
  294. **Shih M, Lin F, Scott JD, Wang HY, and Malbon CC.** Dynamic complexes of beta2-adrenergic receptors with protein kinases and phosphatases and the role of gravin. *J Biol Chem* 274: 1588–1595, 1999.
  295. **Shih M and Malbon CC.** Oligodeoxynucleotides antisense to mRNA encoding protein kinase A, protein kinase C, and beta-adrenergic receptor kinase reveal distinctive cell-type-specific roles in agonist-induced desensitization. *Proc Natl Acad Sci USA* 91: 12193–12197, 1994.
  296. **Shih M and Malbon CC.** Protein kinase C deficiency blocks recovery from agonist-induced desensitization. *J Biol Chem* 271: 21478–21483, 1996.
  297. **Sillibourne JE, Milne DM, Takahashi M, Ono Y, and Meek DW.** Centrosomal anchoring of the protein kinase CK1delta mediated by attachment to the large, coiled-coil scaffolding protein CG-NAP/AKAP450. *J Mol Biol* 322: 785–797, 2002.
  298. **Skalhegg BS, Huang Y, Su T, Idzerda RL, McKnight GS, and Burton KA.** Mutation of the C(alpha) subunit of PKA leads to growth retardation and sperm dysfunction. *Mol Endocrinol* 16: 630–639, 2002.
  299. **Skalhegg BS, Landmark BF, Doskeland SO, Hansson V, Lea T, and Jahnsen T.** Cyclic AMP dependent protein kinase type I mediates the inhibitory effects of 3',5'-cyclic adenosine monophosphate on cell replication in human T lymphocytes. *J Biol Chem* 267: 15707–15714, 1992.
  300. **Skalhegg BS and Tasken K.** Specificity in the cAMP/PKA signaling pathway. Differential expression, regulation, and subcellular localization of subunits of PKA. *Front Biosci* 5: D678–D693, 2000.
  301. **Skalhegg BS, Tasken K, Hansson V, Huitfeldt HS, Jahnsen T, and Lea T.** Location of cAMP-dependent protein kinase type I with the TCR-CD3 complex. *Science* 263: 84–87, 1994.
  302. **Smith CM, Radzio-Andzelm E, Madhusudan Akamine P, and Taylor SS.** The catalytic subunit of cAMP-dependent protein kinase: prototype for an extended network of communication. *Prog Biophys Mol Biol* 71: 313–341, 1999.
  303. **Smith FD and Scott JD.** Signaling complexes: junctions on the intracellular information super highway. *Curr Biol* 12: R42–R40, 2002.
  304. **Snyder GL, Fienberg AA, Haganir RL, and Greengard PA.** Dopamine/D1 receptor/protein kinase A/dopamine- and cAMP-regulated phosphoprotein (M<sub>r</sub> 32 kDa)/protein phosphatase-1 pathway regulates dephosphorylation of the NMDA receptor. *J Neurosci* 18: 10297–10303, 1998.
  305. **Soderling SH and Beavo JA.** Regulation of cAMP and cGMP signaling: new phosphodiesterases and new functions. *Curr Opin Cell Biol* 12: 174–179, 2000.
  306. **Soderling SH, Binns KL, Wayman GA, Davee SM, Ong SH, Pawson T, and Scott JD.** The WRP component of the WAVE-1 complex attenuates Rac-mediated signalling. *Nat Cell Biol* 4: 970–975, 2002.
  307. **Soderling SH, Langeberg LK, Soderling JA, Davee SM, Simerly R, Raber J, and Scott JD.** Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proc Natl Acad Sci USA* 100: 1723–1728, 2003.
  308. **Solberg R, Tasken K, Wen W, Coghlan VM, Meinkoth JL, Scott JD, Jahnsen T, and Taylor SS.** Human regulatory subunit RI beta of cAMP-dependent protein kinases: expression, holoenzyme formation and microinjection into living cells. *Exp Cell Res* 214: 595–605, 1994.
  309. **Spann TP, Moir RD, Goldman AE, Stick R, and Goldman RD.** Disruption of nuclear lamin organization alters the distribution of replication factors and inhibits DNA synthesis. *J Cell Biol* 136: 1201–1212, 1997.
  310. **Spina D.** Theophylline and PDE4 inhibitors in asthma. *Curr Opin Pulm Med* 9: 57–64, 2003.
  311. **Steen RL and Collas P.** Mistargeting of B type lamins at the end of mitosis: implications on cell survival and regulation of lamins A/C expression. *J Cell Biol* 153: 621–626, 2001.
  312. **Steen RL, Cubizolles F, Le Guellec K, and Collas P.** A kinase-anchoring protein (AKAP)95 recruits human chromosome-associated protein (hCAP)-D2/Eg7 for chromosome condensation in mitotic extract. *J Cell Biol* 149: 531–536, 2000.
  313. **Steen RL, Martins SB, Tasken K, and Collas P.** Recruitment of protein phosphatase 1 to the nuclear envelope by A-kinase anchoring protein AKAP149 is a prerequisite for nuclear lamina assembly. *J Cell Biol* 150: 1251–1262, 2000.
  314. **Steinberg SF and Brunton LL.** Compartmentation of G protein-coupled signaling pathways in cardiac myocytes. *Annu Rev Pharmacol Toxicol* 41: 751–773, 2001.

315. **Stocco DM.** StAR protein and the regulation of steroid hormone biosynthesis. *Annu Rev Physiol* 63: 193–213, 2001.
316. **Strada SJ, Martin MW, and Thompson WJ.** General properties of multiple molecular forms of cyclic nucleotide phosphodiesterase in the nervous system. *Adv Cyclic Nucleotide Protein Phosphorylation Res* 16: 13–29, 1984.
317. **Suetsugu S, Miki H, and Takenawa T.** Identification of two human WAVE/SCAR homologues as general actin regulatory molecules which associate with the Arp2/3 complex. *Biochem Biophys Res Commun* 260: 296–302, 1999.
318. **Sun F, Hug MJ, Bradbury NA, and Frizzell RA.** Protein kinase A associates with cystic fibrosis transmembrane conductance regulator via an interaction with ezrin. *J Biol Chem* 275: 14360–14366, 2000.
319. **Sun F, Hug MJ, Lewarchik CM, Yun CH, Bradbury NA, and Frizzell RA.** E3karp mediates the association of ezrin and protein kinase A with the cystic fibrosis transmembrane conductance regulator in airway cells. *J Biol Chem* 275: 29539–29546, 2000.
320. **Sunahara RK, Dessauer CW, and Gilman AG.** Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* 36: 461–480, 1996.
321. **Takahashi M, Mukai H, Oishi K, Isagawa T, and Ono Y.** Association of immature hypophosphorylated protein kinase Cepsilon with an anchoring protein CG-NAP. *J Biol Chem* 275: 34592–34596, 2000.
322. **Takahashi M, Mukai H, Toshimori M, Miyamoto M, and Ono Y.** Proteolytic activation of PKN by caspase-3 or related protease during apoptosis. *Proc Natl Acad Sci USA* 95: 11566–11571, 1998.
323. **Takahashi M, Shibata H, Shimakawa M, Miyamoto M, Mukai H, and Ono Y.** Characterization of a novel giant scaffolding protein, CG-NAP, that anchors multiple signaling enzymes to centrosome and the Golgi apparatus. *J Biol Chem* 274: 17267–17274, 1999.
324. **Takenawa T and Miki H.** Wasp and Wave family proteins: key molecules for rapid rearrangement of cortical actin filaments and cell movement. *J Cell Sci* 114: 1801–1809, 2001.
325. **Takeuchi S, Takayama Y, Ogawa A, Tamura K, and Okada M.** Transmembrane phosphoprotein Cbp positively regulates the activity of the carboxyl-terminal Src kinase, Csk. *J Biol Chem* 275: 29183–29186, 2000.
326. **Tansey JT, Huml AM, Vogt R, Davis KE, Jones JM, Fraser KA, Brasaemle DL, Kimmel AR, and Londos C.** Functional studies on native and mutated forms of perilipins a role in protein kinase A-mediated lipolysis of triacylglycerols in Chinese hamster ovary cells. *J Biol Chem* 278: 8401–8406, 2003.
327. **Tash JS and Means AR.** Regulation of protein phosphorylation and motility of sperm by cyclic adenosine monophosphate and calcium. *Biol Reprod* 26: 745–763, 1982.
328. **Tasken K, Skalhegg BS, Tasken KA, Solberg R, Knutsen HK, Levy FO, Sandberg M, Orstavik S, Larsen T, Johansen AK, Vang T, Schrader HP, Reinton NT, Torgersen KM, Hansson V, and Jahnsen T.** Structure, function and regulation of human cAMP-dependent protein kinases. *Adv Second Messenger Phosphoprotein Res* 31: 191–204, 1997.
329. **Tasken KA, Collas P, Kemmer WA, Witczak O, Conti M, and Tasken K.** Phosphodiesterase 4D and protein kinase A type II constitute a signaling unit in the centrosomal area. *J Biol Chem* 276: 21999–22002, 2001.
330. **Taylor SS, Buechler JA, and Yonemoto W.** cAMP-dependent protein kinase: framework for a diverse family of regulatory enzymes. *Annu Rev Biochem* 59: 971–1005, 1990.
331. **Taylor SS, Radzio-Andzelm E, Madhusudan Cheng X, Ten Eyck L, and Narayana N.** Catalytic subunit of cyclic AMP-dependent protein kinase: structure and dynamics of the active site cleft. *Pharmacol Ther* 82: 133–141, 1999.
332. **Theurkauf WE and Vallee RB.** Molecular characterization of the cAMP-dependent protein kinase bound to microtubule-associated protein 2. *J Biol Chem* 257: 3284–3290, 1982.
333. **Thorn P.** Spatial domains of Ca<sup>2+</sup> signaling in secretory epithelial cells. *Cell Calcium* 20: 203–214, 1996.
334. **Torgersen KM, Vaage JT, Levy FO, Hansson V, Rolstad B, and Tasken K.** Selective activation of cAMP-dependent protein kinase type I inhibits rat natural killer cell cytotoxicity. *J Biol Chem* 272: 5495–5500, 1997.
335. **Torgersen KM, Vang T, Abrahamsen H, Yaqub S, Horejsi V, Schraven B, Rolstad B, Mustelin T, and Tasken K.** Release from tonic inhibition of T cell activation through transient displacement of C-terminal Src kinase (Csk) from lipid rafts. *J Biol Chem* 276: 29313–29318, 2001.
336. **Torgersen KM, Vang T, Abrahamsen H, Yaqub S, and Tasken K.** Molecular mechanisms for protein kinase A-mediated modulation of immune function. *Cell Signal* 14: 1–9, 2002.
337. **Trendelenburg G, Hummel M, Riecken EO, and Hanski C.** Molecular characterization of AKAP149, a novel A kinase anchor protein with a KH domain. *Biochem Biophys Res Commun* 225: 313–319, 1996.
338. **Trotter KW, Fraser ID, Scott GK, Stutts MJ, Scott JD, and Milgram SL.** Alternative splicing regulates the subcellular localization of A-kinase anchoring protein 18 isoforms. *J Cell Biol* 147: 1481–1492, 1999.
339. **Vang T, Abrahamsen H, and Tasken K.** Protein kinase A intersects Src signaling in membrane microdomains. *J Biol Chem* 278: 17170–17177, 2003.
340. **Vang T, Torgersen KM, Sundvold V, Saxena M, Levy FO, Skalhegg BS, Hansson V, Mustelin T, and Tasken K.** Activation of the COOH-terminal Src kinase (Csk) by cAMP-dependent protein kinase inhibits signaling through the T cell receptor. *J Exp Med* 193: 497–507, 2001.
341. **Veillette A, Latour S, and Davidson D.** Negative regulation of immunoreceptor signaling. *Annu Rev Immunol* 20: 669–707, 2002.
342. **Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, Smith HO, Yandell M, Evans CA, Holt RA, Gocayne JD, Amanatides P, Ballew RM, Huson DH, Wortman JR, Zhang Q, Kodira CD, Zheng XH, Chen L, Skupski M, Subramanian G, Thomas PD, Zhang J, Gabor Miklos GL, Nelson C, Broder S, Clark AG, Nadeau J, McKusick VA, Zinder N, Levine AJ, Roberts RJ, Simon M, Slayman C, Hunkapiller M, Bolanos R, Delcher A, Dew I, Fasulo D, Flanigan M, Florea L, Halpern A, Hannenhalli S, Kravitz S, Levy S, Mobarry C, Reinert K, Remington K, Abu-Threideh J, Beasley E, Biddick K, Bonazzi V, Brandon R, Cargill M, Chandramouliswaran I, Charlab R, Chaturvedi K, Deng Z, Di FV, Dunn P, Eilbeck K, Evangelista C, Gabrielian AE, Gan W, Ge W, Gong F, Gu Z, Guan P, Heiman TJ, Higgins ME, Ji RR, Ke Z, Ketchum KA, Lai Z, Lei Y, Li Z, Li J, Liang Y, Lin X, Lu F, Merkulov GV, Milshina N, Moore HM, Naik AK, Narayan VA, Neelam B, Nusskern D, Rusch DB, Salzberg S, Shao W, Shue B, Sun J, Wang Z, Wang A, Wang X, Wang J, Wei M, Wides R, Xiao C, Yan C, Yao A, Ye J, Zhan M, Zhang W, Zhang H, Zhao Q, Zheng L, Zhong F, Zhong W, Zhu S, Zhao S, Gilbert D, Baumhueter S, Spier G, Carter C, Cravchik A, Woodage T, Ali F, An H, Awe A, Baldwin D, Baden H, Barnstead M, Barrow I, Beeson K, Busam D, Carver A, Center A, Cheng ML, Curry L, Danaher S, Davenport L, Desilets R, Dietz S, Dodson K, Doup L, Ferreira S, Garg N, Gluecksmann A, Hart B, Haynes J, Haynes C, Heiner C, Hladun S, Hostin D, Houck J, Howland T, Ibegwam C, Johnson J, Kalush F, Kline L, Koduru S, Love A, Mann F, May D, McCawley S, McIntosh T, McMullen I, Moy M, Moy L, Murphy B, Nelson K, Pfannkoch C, Pratts E, Puri V, Qureshi H, Reardon M, Rodriguez R, Rogers YH, Romblad D, Ruhfel B, Scott R, Sitter C, Smallwood M, Stewart E, Strong R, Suh E, Thomas R, Tint NN, Tse S, Vech C, Wang G, Wetter J, Williams S, Williams M, Windsor S, Winn-Deen E, Wolfe K, Zaveri J, Zaveri K, Abril JF, Guigo R, Campbell MJ, Sjolander KV, Karlak B, Kejariwal A, Mi H, Lazareva B, Hatton T, Narechania A, Diemer K, Muruganujan A, Guo N, Sato S, Bafna V, Istrail S, Lippert R, Schwartz R, Walenz B, Yooseph S, Allen D, Basu A, Baxendale J, Blick L, Caminha M, Carnes-Stine J, Caulk P, Chiang YH, Coyne M, Dahlke C, Mays A, Dombroski M, Donnelly M, Ely D, Esparham S, Fosler C, Gire H, Glanowski S, Glasser K, Glodek A, Gorokhov M, Graham K, Gropman B, Harris M, Heil J, Henderson S, Hoover J, Jennings D, Jordan C, Jordan J, Kasha J, Kagan L, Kraft C, Levitsky A, Lewis M, Liu X, Lopez J, Ma D, Majoros W, McDaniel J, Murphy S, Newman M, Nguyen T, Nguyen N, and Nodell M.** The sequence of the human genome. *Science* 291: 1304–1351, 2001.

343. Verde I, Pahlke G, Salanova M, Zhang G, Wang S, Coletti D, Onuffer J, Jin SL, and Conti M. Myomegalin is a novel protein of the Golgi/centrosome that interacts with a cyclic nucleotide phosphodiesterase. *J Biol Chem* 276: 11189–11198, 2001.
344. Vijayaraghavan S, Goueli SA, Davey MP, and Carr DW. Protein kinase A-anchoring inhibitor peptides arrest mammalian sperm motility. *J Biol Chem* 272: 4747–4752, 1997.
345. Vijayaraghavan S, Liberty GA, Mohan J, Winfrey VP, Olson GE, and Carr DW. Isolation and molecular characterization of AKAP110, a novel, sperm-specific protein kinase A-anchoring protein. *Mol Endocrinol* 13: 705–717, 1999.
346. Wang LY, Orser BA, Brautigam DL, and MacDonald JF. Regulation of NMDA receptors in cultured hippocampal neurons by protein phosphatases 1 and 2A. *Nature* 369: 230–232, 1994.
347. Wang LY, Salter MW, and MacDonald JF. Regulation of kainate receptors by cAMP-dependent protein kinase and phosphatases. *Science* 253: 1132–1135, 1991.
348. Wang X, Herberg FW, Laue MM, Wullner C, Hu B, Petrasch-Parwez E, and Kilimann MW. Neurobeachin: a protein kinase A-anchoring, beige/Chediak-higashi protein homolog implicated in neuronal membrane traffic. *J Neurosci* 20: 8551–8565, 2000.
349. Watanabe G, Saito Y, Madaule P, Ishizaki T, Fujisawa K, Morii N, Mukai H, Ono Y, Kakizuka A, and Narumiya S. Protein kinase N (PKN) and PKN related protein raphilin as targets of small GTPase Rho. *Science* 271: 645–648, 1996.
350. Wei JY, Roy DS, Leconte L, and Barnstable CJ. Molecular and pharmacological analysis of cyclic nucleotide-gated channel function in the central nervous system. *Prog Neurobiol* 56: 37–64, 1998.
351. Weinman EJ, Steplock D, Donowitz M, and Shenolikar S. Nherf associations with sodium-hydrogen exchanger isoform 3 (NHE3) and ezrin are essential for cAMP-mediated phosphorylation and inhibition of NHE3. *Biochemistry* 39: 6123–6129, 2000.
352. Weinman EJ, Steplock D, Wang Y, and Shenolikar S. Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane  $\text{Na}^+$ - $\text{H}^+$  exchanger. *J Clin Invest* 95: 2143–2149, 1995.
353. Wen W, Harootunian AT, Adams SR, Feramisco J, Tsien RY, Meinkoth JL, and Taylor SS. Heat-stable inhibitors of cAMP-dependent protein kinase carry a nuclear export signal. *J Biol Chem* 269: 32214–32220, 1994.
354. Wen W, Meinkoth JL, Tsien RY, and Taylor SS. Identification of a signal for rapid export of proteins from the nucleus. *Cell* 82: 463–473, 1995.
355. Wen W, Taylor SS, and Meinkoth JL. The expression and intracellular distribution of the heat-stable protein kinase inhibitor is cell cycle regulated. *J Biol Chem* 270: 2041–2046, 1995.
356. Westphal RS, Soderling SH, Alto NM, Langeberg LK, and Scott JD. Scar/WAVE-1, a Wiskott-Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J* 19: 4589–4600, 2000.
357. Westphal RS, Tavalin SJ, Lin JW, Alto NM, Fraser ID, Langeberg LK, Sheng M, and Scott JD. Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* 285: 93–96, 1999.
358. Winder DG and Sweatt JD. Roles of serine/threonine phosphatases in hippocampal synaptic plasticity. *Nat Rev Neurosci* 2: 461–474, 2001.
359. Witczak O, Skalhogg BS, Keryer G, Bornens M, Tasken K, Jahnsen T, and Orstavik S. Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450. *EMBO J* 18: 1858–1868, 1999.
360. Xavier R, Brennan T, Li Q, McCormack C, and Seed B. Membrane compartmentation is required for efficient T cell activation. *Immunity* 8: 723–732, 1998.
361. Yang J, Drazba JA, Ferguson DG, and Bond M. A kinase anchoring protein 100 (AKAP100) is localized in multiple subcellular compartments in the adult rat heart. *J Cell Biol* 142: 511–522, 1998.
362. Yao X, Karam SM, Ramilo M, Rong Q, Thibodeau A, and Forte JG. Stimulation of gastric acid secretion by cAMP in a novel alpha-toxin-permeabilized gland model. *Am J Physiol Cell Physiol* 271: C61–C73, 1996.
363. Yarwood SJ, Steele MR, Scotland G, Houslay MD, and Bolger GB. The RACK1 signaling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. *J Biol Chem* 274: 14909–14917, 1999.
364. Yau KW. Cyclic nucleotide-gated channels: an expanding new family of ion channels. *Proc Natl Acad Sci USA* 91: 3481–3483, 1994.
365. Yau KW and Nakatani K. Light-suppressible cyclic GMP sensitive conductance in the plasma membrane of a truncated rod outer segment. *Nature* 317: 252–255, 1985.
366. Zaccolo M, Magalhaes P, and Pozzan T. Compartmentalisation of cAMP and  $\text{Ca}^{2+}$  signals. *Curr Opin Cell Biol* 14: 160–166, 2002.
367. Zaccolo M and Pozzan T. Discrete microdomains with high concentration of cAMP in stimulated rat neonatal cardiac myocytes. *Science* 295: 1711–1715, 2002.
368. Zagotta WN and Siegelbaum SA. Structure and function of cyclic nucleotide-gated channels. *Annu Rev Neurosci* 19: 235–263, 1996.
369. Zimmer HG. Catecholamine-induced cardiac hypertrophy: significance of proto-oncogene expression. *J Mol Med* 75: 849–859, 1997.
370. Zimmerman W, Sparks CA, and Doxsey SJ. Amorphous no longer: the centrosome comes into focus. *Curr Opin Cell Biol* 11: 122–128, 1999.
371. Zimmermann B, Chiorini JA, Ma YL, Kotin RM, and Herberg FW. PrKX is a novel catalytic subunit of the cAMP-dependent protein kinase regulated by the regulatory subunit type I. *J Biol Chem* 274: 5370–5378, 1999.