Physiol Rev
84: 137–167, 2004; 10.1152/physrev.00021.2003.

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

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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 dissambly 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 β-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 (RIα, RIβ, RIIα, RIIβ) and the catalytic (Cα, Cβ, Cγ) 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).

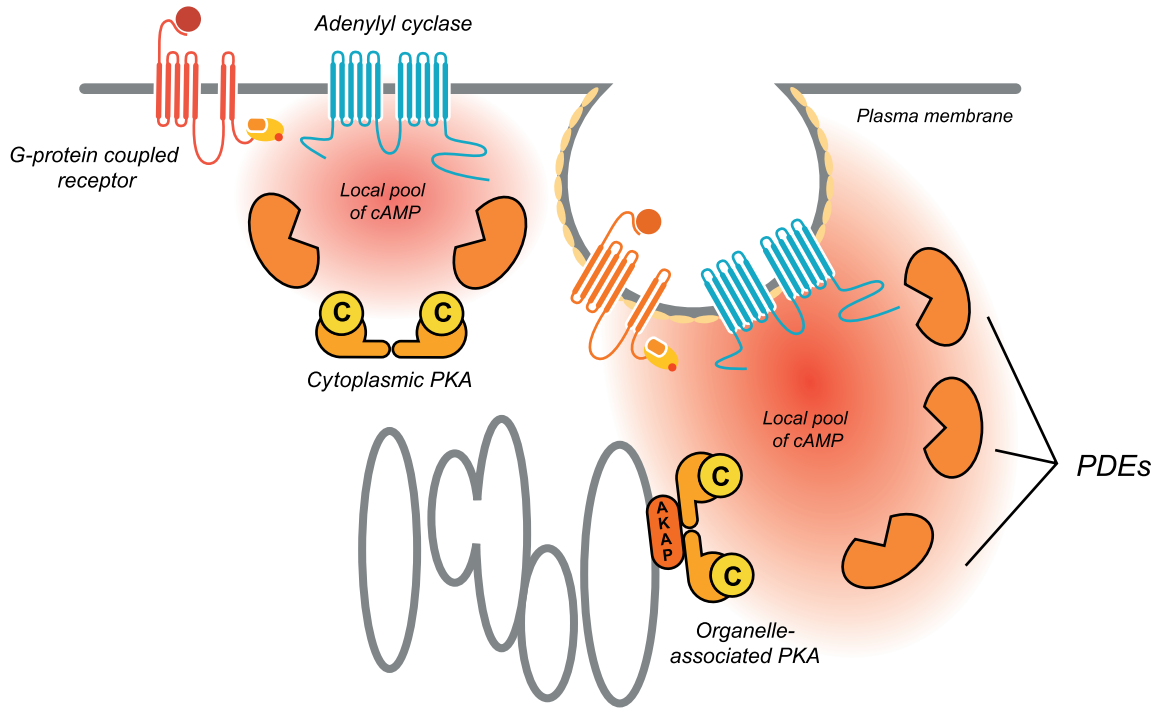

FIG. 1. Ligand binding to various G protein-coupled receptors activates adenylyl cyclases in their proximity and generates pools of cAMP. The local concentration and distribution of the cAMP gradient is limited by phosphodiesterases (PDEs). Particular G protein-coupled receptors are confined to specific domains of the cell membrane in association with intracellular organelles or cytoskeletal constituents. The subcellular structures may harbor specific isozymes 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 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 α-subunit ($G_s\alpha$) and reduced upon binding of the inhibitory G protein α-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 cerebellum. 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 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, β_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 NH_2 terminus and targeting domains which we are only beginning to discover (67, 110, 147). The substrate specificites 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 the endoplasmatic 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 diacyglycerol (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 β-adrenoreceptors through interaction with β-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 of PKA signaling by AKAP anchoring and temporal 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).

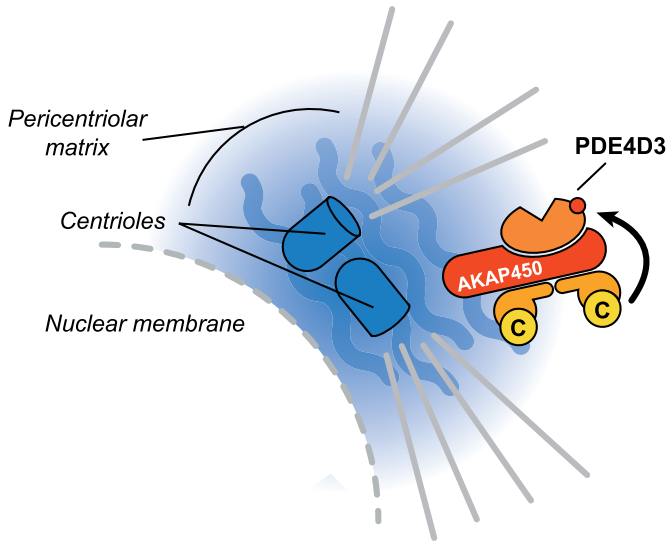

FIG. 2. AKAP450 targets PKA type II and PDE4D3 to the centrosomal region in Sertoli cells. A similar mechanism operates in cardiomyocytes, where mAKAP binds and targets both PDE4D3 and PKA type II to the perinuclear region. Colocalized PKA and PDE provides spatial control of PKA signaling via anchoring 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 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^+ 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 moities (79). The cAMP binding domains in both Epac1 and Epac2 function as inhibitors of the COOH-terminal GEF domains in the absence of cAMP, wheras 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 RIα, RIβ, RIIα, and RIIβ as well as four C subunits Cα, Cβ, Cγ, 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 (RIIα_2C$_2$, RIIβ_2C$_2$) typically activate with an activation constant (K_{act}) of 200–400 nM of cAMP, type I holoenzymes (RIα_2C$_2$, RIβ_2C$_2$) 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, thyrold, 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 epithellal cells	Multiple splice variants	88
AKAP110 (*AKAP3*)	Testis	Axoneme	Binds $G_{13}\alpha$	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, calclneurin (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 $\alpha,\beta,\gamma,\delta$ (*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^+ and L-type Ca^{2+} 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/centromsomes/ 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 $G_{12}\alpha$ to Rho	49, 85, 179
MAP2B	Ubiquitous	Microtubules	Binds tubulin; modulation of L-type Ca^{2+} channels	76, 208, 282, 332
Ezrin/AKAP78	Secretory epithelia	Actin cytoskeleton	Linked to CFTR via EBP50/NHERF	92, 318, 319
T-AKAP80	Testis	Fibrous sheath of sperm tail		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	Ubiquitous	Cytoskeleton		189
PAP7	Steroid-producing cells (adrenal gland and gonads)	Mitochondria	Hormonal regulation of cholesterol transport into mitochondria; binds RI in vivo	201
Neurobeachin	Brain	Golgi		348
AKAP28	Primary airway cells	Ciliary axonemes	Modulation of ciliary beat frequency	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 RIα/RIβ and RIIα/RIIβ 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
$AKAP_{CE}$	*Caenorhabditis elegans*		Binds to RI-like subunit; RING-finger protein with FYVE and TGF-β receptor binding domain	11, 12, 142
DAKAP550	*Drosophila*	Plasma membrane/cytoplasm	Contains two RII-binding sites	133
DAKAP200	*Drosophila*	Plasma membrane	Binds F-actin and Ca calmodulin	202, 276
AKAP97/radial spoke protein 3 (*RSP3*)	*Chlamydomonas*	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; β-AR, β-adrenergic receptor; PDE, phosphodiesterase; PKA, protein kinase A; CFTR, cystic fibrosis transmembrane conductance regulator; FSH, follicle stimulating hormone; TGF-β, transforming growth factor-β; 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_2 terminus in an antiparallel fashion forming an X-type, four-helix bundle that is necessary for both AKAP binding (NH_2-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_2-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_2 terminus and encompasses amino acids 12 to 61 (21, 22, 195). The extreme NH_2 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.

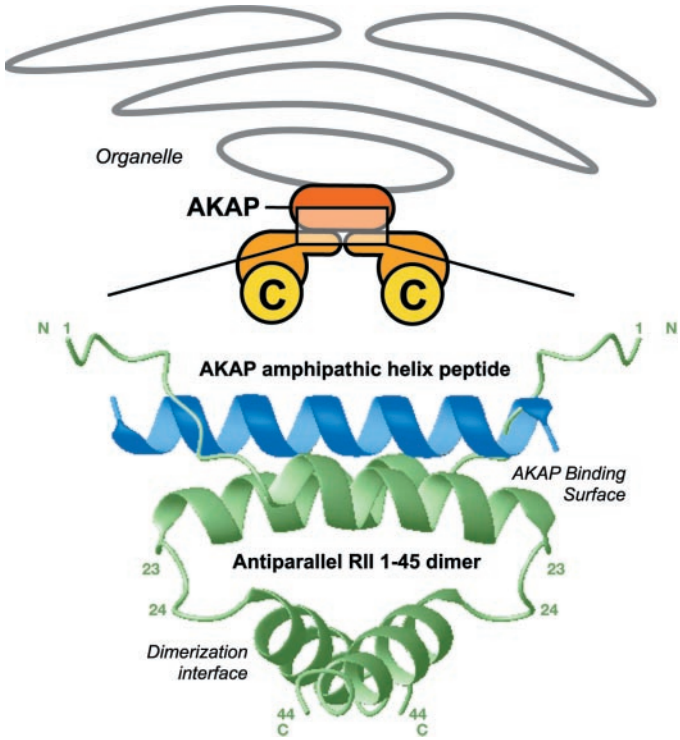

FIG. 3. The AKAP amphipathic helix binds to a hydrophobic groove in the dimerization domain of the PKA R subunit. Anchoring of PKA to AKAPs involves binding of the NH_2-terminal dimerization and docking domain of the PKA R subunit to an amphipathic helix that constitutes the PKA binding domain of the AKAP. Solution of the NMR structure (*bottom*) reveals that the antiparallel RII dimer forms an X-type, 4-helix bundle where the two NH_2-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_2 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.)

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-

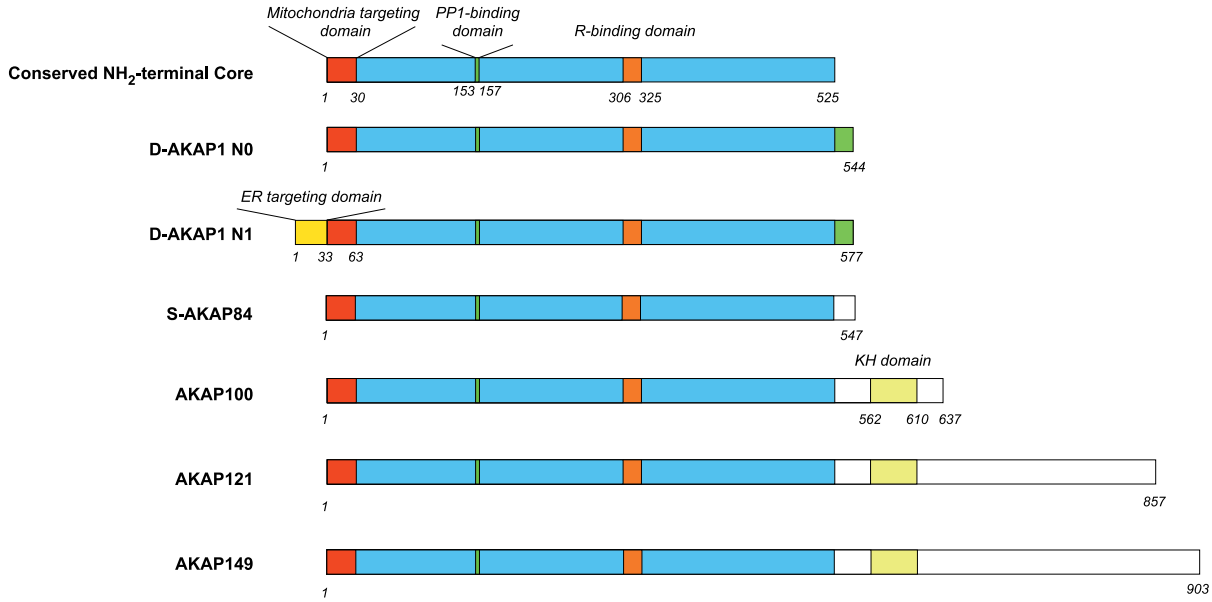

FIG. 4. Multiple splice variants originate from the AKAP1 gene. A total of 6 different splice products have been identified originating from the AKAP1 gene (151). D-AKAP1-N0 (150), S-AKAP84 (205), AKAP100 (151), AKAP121 (57), and AKAP149 (337) all have an NH_2-terminal mitochondrial targeting domain but differ in their COOH termini. D-AKAP1-N1 has an additional NH_2-terminally spliced endoplasmatic 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_2-terminally spliced ER signal as AKAP149 is found in the ER and nuclear envelope (313).

geting 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_2C_2) with high affinity (low nanomolar range). In contrast, their interaction with PKA type I (RI_2C_2) 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 $AKAP_{CE}$ 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_2 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_2 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_2-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 β_2-adrenergic receptors (β_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 β_2-AR also induces a switch in the G protein coupling from G_s to G_i (74). This promotes a mitogenic signaling cascade mediated by G_i, β-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, β-arrestin recruits PDE4 which control β_2-AR phosphorylation by PKA and hence the G_s to G_i switch (17, 252).

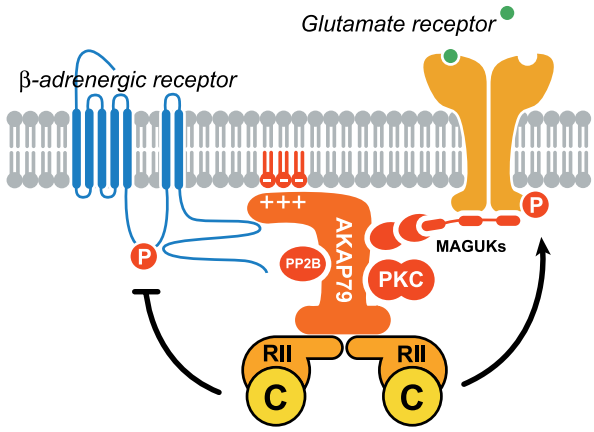

FIG. 5. AKAP79 is targeted to the plasma membrane by three NH_2-terminal basic domains and is recruited to the AMPA receptor by binding to the membrane-associated guanylate kinase (MAGUK) proteins. AKAP79 also associates with β_2-adrenergic receptors (β_2-AR). This enhances β_2-AR-induced cAMP-PKA signaling by recruiting PKA close to the receptor and the site of adenylyl cyclase activation. PKA phosphorylation of the β_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 β-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 α-isoform) is an 81-residue protein containing an amphipathic helix that binds PKA and NH_2-terminal myristoyl and palmitoyl lipid anchors that target the PKA-AKAP complex to the plasma

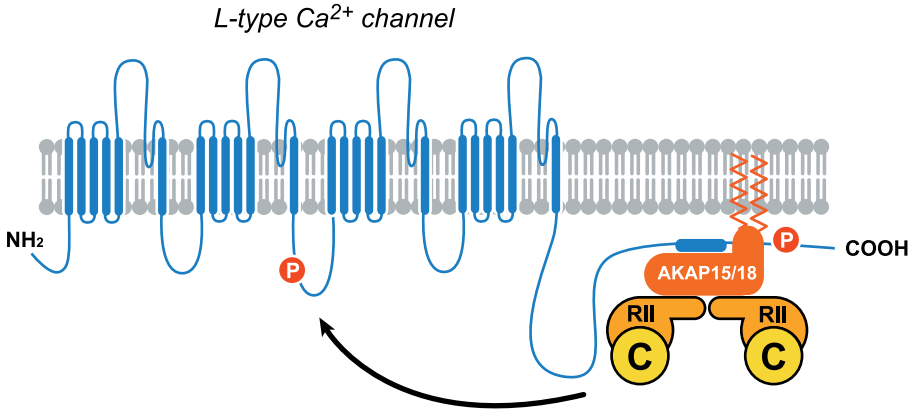

FIG. 6. AKAPs target PKA to ion channels to provide necessary proximity for phosphorylation to occur rapidly enough to regulate the channel in the short time frame of opening and closing of the channels. For example, the α-isoform of AKAP15/18 anchors PKA to the COOH terminus of L-type Ca^{2+} channels in skeletal muscle. PKA phosphorylation enhances the activity of the channel. 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 α_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 α_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 AKAP18α, and is localized to the basolateral membrane compartment in polarized cells. Furthermore, other splice variants from this gene have apical targeting (AKAP18β) and localization to cytoplasm (AKAP18γ) or to secretory granules (AKAP18δ) (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 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-radexin (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 Na^+/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 Na^+/H^+ exchanger in the renal brush border by the Na^+/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 gastic 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 consitutents 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 cognititive 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. Wheras 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 filpodia 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 β_2-AR (294) in a complex that also includes the G protein-linked receptor kinase 2 (GRK2) and transiently β-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 β_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 fascilitating 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_2 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 γ-tubulin lattice (33, 370). With its crucial role in nucleation and organization of microtubules, the centrosome is important in cellular procesesses 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 RIIα 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 RIIα 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 RIIα 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, inluding PKN, PP1, PP2A, and the immature nonphosphorylated form of PKCε (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

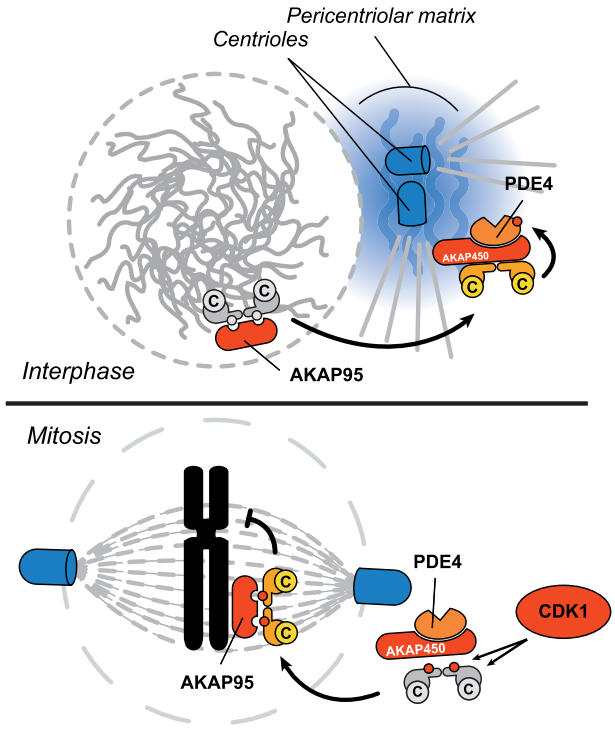

FIG. 7. PKA-AKAP associations may be dynamic. *Top*: during interphase, AKAP450 targets PKA to the pericentriolar matrix where PKA maintains the interphase microtubule network. AKAP450 anchors both PKA and PDE4, and PKA-mediated phosphorylation of PDE4 leads to increased phosphodiesterase activity, establishing 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 RIIα 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 RIIα binds to chromosome-associated AKAP95, and PKA prevents premature decondensation of chromatin during mitosis. At mitosis exit, an unknown phosphatase dephosphorylates RIIα; 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_2-terminal regulatory region (322). Interaction between AKAP450 and the nonphosphorylated form of PKCϵ is required for the phosphorylation-dependent maturation of PKCϵ. Recently, AKAP450 was reported to anchor protein kinase CK1δ, 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 kendrin) is an integral component of the pericentriolar matrix (90) and forms a centrosomal macromolecular complex with γ-tubulin (82) and dynein (258) important in the dynamic organization of centrosomes and spindles (90). Binding to γ-tubulin is required for microtubule nucleation during mitosis and meiosis, and the association with dynein is necessary for the transport of pericentrin-γ-tubulin complexes along microtubules to the centrosome. Pericentrin/kendrin shares a high degree of linear homology with AKAP450. This indicates a common origin, and pericentrin/kendrin 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 redundance 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 RIα in vivo (201), discussed in section IX*B*.

S-AKAP84 (205), AKAP121 (57), D-AKAP1 (150), and AKAP149 (337) share a 525-amino NH_2-terminal core but differ in the COOH-terminal domain as well as in their extreme NH_2 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 mitochondiral membrane, whereas alternate splicing of the NH_2 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 RIα is lower than for RIIα (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 α_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 RIIα (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 RIIα onto, or in the vicinity of, the metaphase plate. Recruitment of RIIα from a centrosome-Golgi localization during interphase to chromatin-bound AKAP95 at mitosis requires

phosphorylation of RIIα on threonine-54 by CDK1 (192) (Fig. 7). Conversely, release of RIIα from AKAP95 upon chromosome decondenseation 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 RIIα 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 dissambly and reassembly, and numerous intracellular transport mechansims. 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 β-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 Ca^{2+} is essential in cardiac electrical activity and is the direct activator of the myofilaments, which cause contraction (27). Myocyte mishandling of Ca^{2+} is a central cause of both contractile dysfunction and arrhythmias in pathophysiological conditions (256).

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

Sympathetic stimulation of the heart through β-adrenergic receptors increases both contraction force (inotropy) and heart rate (chronotropy). In order for the heart rate to increase, relaxation and Ca^{2+} decline must occur faster. β-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 Ca^{2+} channels, ryanodine receptor (RyR), troponin I, and myosin binding protein C], thus regulating the Ca^{2+} flux from L-type Ca^{2+} channel and SR (Fig. 8). Furthermore, PKA phosphorylates phospholamban that regulates the activity of SERCA2 and leads to increased reuptake of Ca^{2+} into SR, a process which is affected in failing hearts (111, 234, 290). Localized signaling is clearly important in the regulation of Ca^{2+} in the heart. Considerable amounts of evidence exist showing that the cAMP increase in response to β-adrenergic stimuli is local (for review and references, see Refs. 28, 314) as well as controlled temporally as recently illustrated by use of fluorecense 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 β-AR and the L-type Ca^{2+} channel have known AKAPs associated that are present in heart (AKAP79, AKAP18α, 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 RIα and is associated with changes in electrocardiogram recordings and cardiac dysfunction, implicating D-AKAP2 in targeting of PKA, possibly to an ion

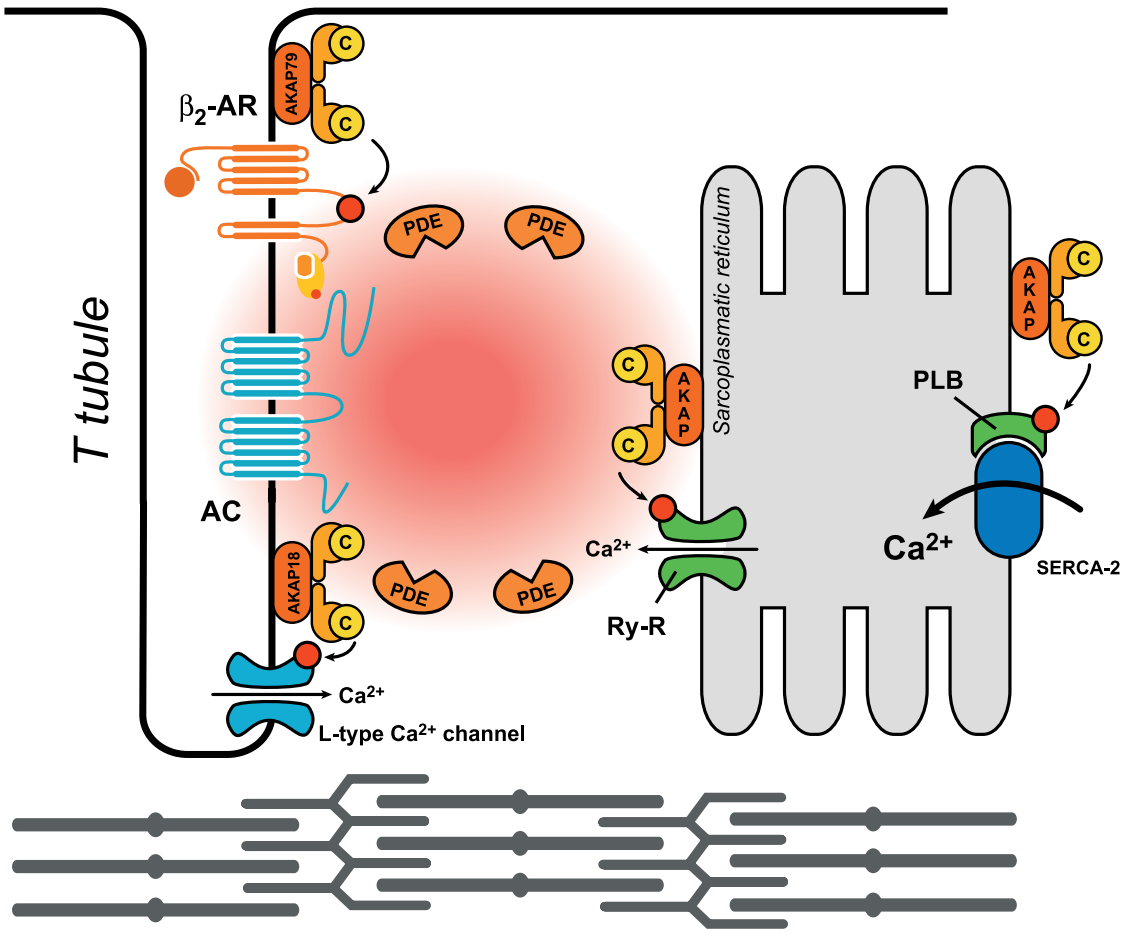

FIG. 8. Local gradients of cAMP and anchored pools of PKA mediate β-adrenergic regulation of cardiac function. Signaling through the β_2-adrenergic receptor localized in microdomains of the sarcolemma formed by t tubules generates a local gradient of cAMP that is also shaped by phosphodiesterases. cAMP activates PKA localized via AKAPs in the vicinity of its substrates such as the β_2-adrenergic receptor (β_2-AR), the L-type Ca^{2+} channel, and the ryanodine receptor (RyR) that regulates Ca^{2+} outflux from the sarcoplasmic reticulum (SR) and phospholamban (PLB) that through sarco(endo)plasmic reticulum (SERCA2) regulates 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 overy and Leydig cells of the tesis, 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, $p450^{SCC}$ (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 RIα and RIIα (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-

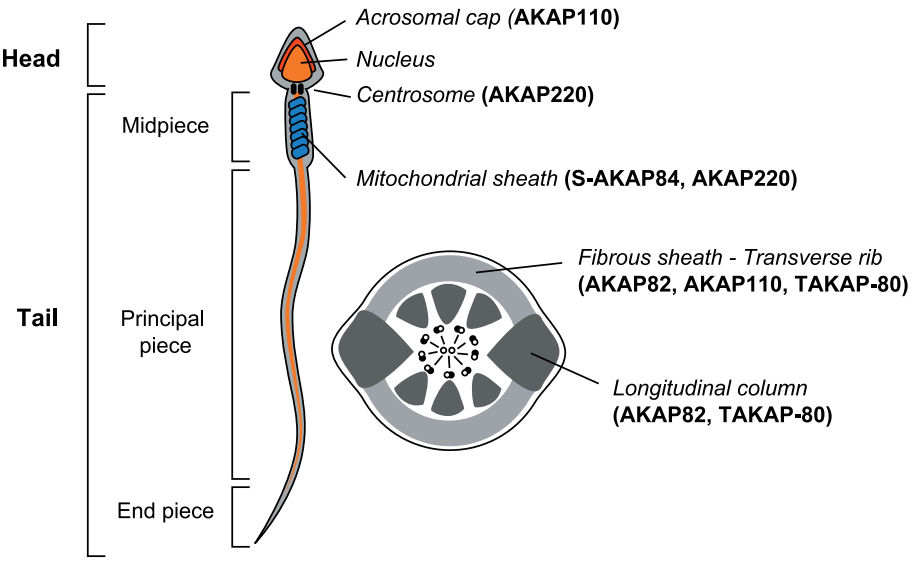

FIG. 9. Several AKAPs have been identified and localized to the different subcellular compartments of polarized mammalian sperm. The enlarged cross-section shows the flagellar structure and the localization of the various AKAPs in the midsection of the principal piece. [Adapted from Moss and Gerton (233).]

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 RIIα-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, RIα appears to rescue the phenotype in the RIIα null-mutant mice (45). Furthermore, knockout of the Cα-catalytic subunit of PKA in exon 2, which ablates both the constitutively expressed and the sperm-specific Cα-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. Cathecholamines stimulate lipolysis and thermogenesis in adipocytes, and this process is controlled largely by the β-ARs. Insulin is the most important physiological inhibitor of cathecholamine-mediated lipolysis. β-ARs are members of the large family of G protein-coupled receptors. Three β-ARs subtypes (β_1-AR, β_2-AR, β_3-AR) exist. While β_1-AR and β_2-AR are broadly expressed throughout all tissues of the body, β_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 β_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 β-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 RIIβ 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 RIα in the BAT. The RIα holoenzyme is more cAMP-responsive than the PKAIIβ isozyme (the regulatory RIα subunit of PKAIα 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 RIα, thermogenesis in BAT is increased (209). Furthermore, in WAT, the compensatory increase in RIα in RII$\beta^{-/-}$ animals is associated with increased basal kinase activity and increased basal rate of lipolysis. β-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 β-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 β-adrenergic receptors together with increased levels of the RIα subunit of PKA that lowers the threshold for activation by cAMP. This enhances signaling through the β-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 β-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 know about AKAPs in adipocytes. However, the observed differences in β-adrenergic regulation of metabolism in RIIβ-containing adipocytes from wild-type mice compared with adipocytes from RIIβ knock-out mice expressing RIα 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 β-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 β-cells is regulated by reversible phosphorylation of β-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 principial 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 excocytosis has not yet been precisely identified, AKAP18δ 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 ζ and CD3 ϵ, δ and γ 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-

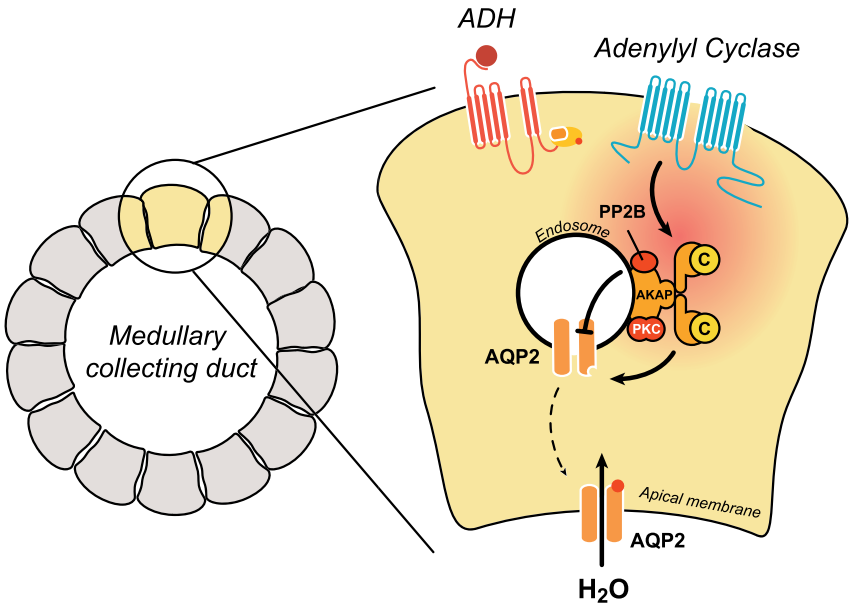

FIG. 10. Anchored PKA controls exocytotic processes. Antidiuretic hormone (ADH) elicits cAMP production by binding to the vasopressin V_2 receptors on the basolateral membrane surface of the principal cells in the renal collecting ducts. cAMP/PKA signaling is required for the translocation of aquaporin-2 (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 AKAP18δ (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_2 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 ($RI\alpha_2C_2$) 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 ζ-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 RIα (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_2-terminal myristyl group into rafts, or via interactions with a caveolin-like protein in T-cell rafts, similar to the PKA Cα 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 ζ-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-

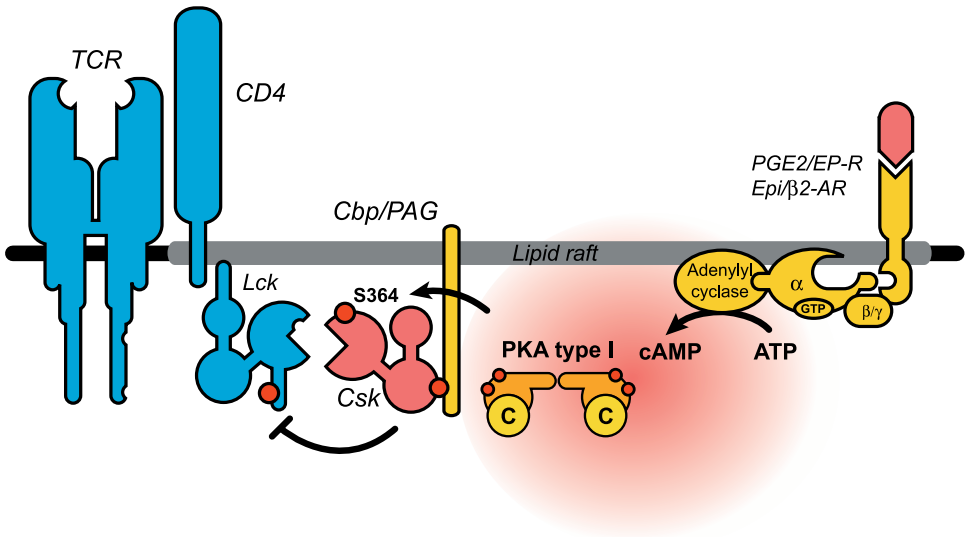

FIG. 11. cAMP inhibits T-cell activation through a PKA type I-Csk-Lck inhibitory pathway in lipid rafts. Proteins involved in proximal TCR signaling events are localized in lipid rafts, representing small regions of detergent-resistant lipid domains of the membrane. Both the cAMP-generating machinery (adenylyl cyclase) and the effectors (PKA type I and Csk) are localized in the lipid rafts. The mechanism involved in targeting RIα 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.

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