REVIEW

Specificity and spatial dynamics of protein kinase A signaling organized by A-kinase-anchoring proteins

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Abstract

Protein phosphorylation is the most common post-translational modification observed in cell signaling and is controlled by the balance between protein kinase and phosphatase activities. The cAMP–protein kinase A (PKA) pathway is one of the most studied and well-known signal pathways. To maintain a high level of specificity, the cAMP–PKA pathway is tightly regulated in space and time. A-kinase-anchoring proteins (AKAPs) target PKA to specific substrates and distinct subcellular compartments providing spatial and temporal specificity in the mediation of biological effects controlled by the cAMP–PKA pathway. AKAPs also serve as scaffolding proteins that assemble PKA together with signal terminators such as phosphoprotein phosphatases and cAMP-specific phosphodiesterases as well as components of other signaling pathways into multiprotein-signaling complexes.

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Introduction

In multicellular organisms, cell signaling constitutes communication mechanisms between and inside cells to ensure coordinated behavior of the organism and to regulate various biological processes such as cell proliferation, development, metabolism, gene expression, and other more specialized functions of specific target cells and tissues. Intracellularly, the systems for cellular communication transmit information based on well-controlled post-translational modifications. Protein phosphorylation is one of the most common post-translational modifications and is controlled by the opposing actions of various specific protein kinases and phosphatases (PPs). The phosphorylation status of a protein may affect its enzymatic activity, cellular localization, and/or association with other proteins. Kinases and phosphatases have a central role in signal transduction pathways and differ in substrate specificity, acting on serine, threonine, or tyrosine residues. Since protein phosphorylation affects many cellular processes, its regulation must be specific and act on a defined subset of cellular targets to ensure signal fidelity. Specificity is achieved through assembly of distinct complexes of enzymes at subcellular localizations by anchoring and scaffolding proteins (for review, see Scott & Pawson (2009)). Thus, organization of anchoring provides means for spatiotemporal regulation of cellular signaling and constitutes a concept by which a common signaling pathway can perform many different functions. One of the best-characterized signal transduction pathways is the cAMP-signaling pathway where ligand binding to G protein-coupled receptors (GPCRs) via G proteins and adenyl cyclase (AC) leads to an intracellular increase in the levels of the second messenger cAMP. Next, this leads to activation of effector molecules such as cAMP-dependent protein kinase A (PKA; Tasken & Aandahl 2004, Beene & Scott 2007, Taylor et al. 2008; Fig. 1).

Transmembrane and intracellular signaling pathways require a high level of spatial and temporal regulation to convey the appropriate inputs. The temporal control of the cAMP-signaling pathway is not only due to signal generation by the AC, but also depends on the action of cAMP–phosphodiesterases (PDEs), and different cell types may have high or low constitutive turnover of cAMP (Baillie et al. 2005, Conti & Beavo 2007). Moreover, spatial control is achieved through association with anchoring proteins that ensure specificity in signal transduction by placing enzymes close to their cognate effectors and substrates. A-kinase-anchoring proteins (AKAPs) provide the means to achieve both spatial control of PKA signaling by regulating proximity as well as facilitating good temporal control by placing PKA optimally versus small pools of cAMP that are discretely temporally regulated. Indeed, AKAPs target
cAMP signaling and compartmentalization

Heterotrimeric G proteins are comprised of α, β, and γ subunits (Gα, Gβγ, and Gγ). In the resting state, the α subunit is bound to GDP and is associated with βγ subunits. The GDP-bound heterotramer is coupled to GPCRs, which upon binding of an extracellular ligand activates the trimeric G protein by increasing the rate of GDP–GTP exchange on the Gα subunit. The GTP-bound, active, Gα subunit dissociates from the Gβγ subunits; these subunits then activate their respective effectors. The ubiquitously expressed G protein Gαs mediate receptor-dependent activation of one of several isoforms of AC resulting in increase in intracellular cAMP synthesized from ATP. cAMP mediates its effects through several effector molecules including PKA (Walsh et al. 1968), exchange proteins activated by cAMP (Epac; De Rooij et al. 1998, Kawasaki et al. 1998), and the cyclic nucleotide-gated ion channels (Nakamura & Gold 1987). For review and references, see Tasken & Aandahl (2004), Bos (2006), Beene & Scott (2007), Taylor et al. (2008) and Biel (2009) and Fig. 1.

Live-cell imaging and fluorescence resonance energy transfer (FRET) have allowed for the visualization of microdomains with a high concentration cAMP, indicating that cAMP accumulation is restricted to specific locations within the cell (Zaccolo & Pozzan 2002). The compartmentalized pools of cAMP are controlled in space and time by ACs and PDEs inside the cell. While a family of nine membrane-bound AC isoforms is responsible for the production of cAMP (Hanoune & Defer 2001, Cooper 2003), the termination of cAMP signaling is conferred by a large superfamily of enzymes including over 40 different PDE isozyme variants that catalyze the degradation of cAMP into AMP (Baillie et al. 2005, Lugnier 2006). The intracellular concentrations of cAMP are therefore regulated by the

PKA to their specific substrates and assemble multi-protein signal complexes that also include phospho-protein phosphatases and PDEs, which control the temporal aspect of the regulation of the cAMP-signaling pathway (Michel & Scott 2002, Tasken & Aandahl 2004, Baillie et al. 2005; Fig. 2). The role of AKAPs in the specificity of cAMP signal transduction is discussed in this review.

**Figure 1** cAMP signal pathways. Ligand binding to various G protein-coupled receptors (GPCRs) activates adenylyl cyclase in their proximity and generates pools of cAMP. The local concentration and distribution of the cAMP gradient are limited by phosphodiesterases (PDEs). Particular GPCRs 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 PKA that, through anchoring via AKAPs, are localized in the vicinity of the receptor and the cyclase. PDEs are also anchored and serve to limit the extension and duration of cAMP gradients. These mechanisms serve to localize and limit the assembly and triggering of specific pathways to a defined area of the cell close to the substrate. cAMP (red filled circles) has effects on a range of effector molecules encompassing 1) PKA, 2) PDEs, 3) guanine nucleotide exchange factors (GEFs) known as exchange proteins activated by cAMP (Epacs), and 4) cyclic nucleotide-gated ion channels.

**Figure 2** Illustration of AKAP properties. (A) AKAPs share three common properties: 1) AKAPs bind to the regulatory subunit of PKA through a conserved anchoring domain; 2) a unique subcellular targeting domain directs AKAP-signaling complexes to discrete locations inside a cell; and 3) additional binding sites for other signaling proteins such as kinases, phosphatases, or potential substrates. (B) Ribbon diagram of the NMR structure of RII(1–43) dimer (yellow, blue, and red) and the AKAP amphipathic helix peptide (green; Newton et al. 2001) depicted using Accelrys Discovery Studio 2.5.1 based on the coordinates from PDB (http://www.rcsb.org/pdb/explore/explore.do?structureId=2DRN).
counterbalancing activities of ACs and PDEs that establish local pools of cAMP close to the effector molecules (Conti & Jin 1999, Houslay & Adams 2003). A local pool of cAMP can be generated by a distinct ligand and lead the signal to follow a specific route inside the cell by reaching and activating a subset of PKA–AKAP complex to mediate a biological effect (Zaccolo et al. 2002). PDEs contribute to the establishment of local gradients of cAMP by being localized to subcellular compartments and by being recruited into multiprotein-signaling complexes organized by AKAPs and other scaffold proteins (Dodge et al. 2001, Tasken et al. 2001).

cAMP-dependent protein kinase

The PKA holoenzyme is a heterotetramer composed of two catalytic (C) subunits held in an inactive state by association with a regulatory (R) subunit dimer (Corbin et al. 1973, Corbin & Keely 1977, Potter & Taylor 1979). Two classes of PKA holoenzymes, type I and II, have been identified, which differ in their R subunits (RI and RII; Reimann et al. 1971, Corbin et al. 1975). Both R subunits (RI and RII) and the C subunit exist as multiple isoforms (RIz, RIβ, RIIz, RIIβ, Cz, Cβ, Cγ, and PRKX). Although there are major differences in tissue distribution and biochemical and physical properties of the R subunit isoforms, the holoenzyme contains two C subunits bound to RI homo- or heterodimers or RII homodimers (Scott 1991, Tasken et al. 1993). 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 (Kopperud et al. 2002). The C subunits become catalytically active and phosphorylate nearby target substrates on serine or threonine residues presented in a sequence context of Arg-Arg-X-Ser/Thr, Arg-Lys-X-Ser/Thr, Lys-Arg-X-Ser/Thr, or Lys-Lys-X-Ser/Thr (reviewed in Shabb (2001) and Ruppelt et al. (2009)). Type I PKA is more sensitive to cAMP binding than type II PKA with an activation constant (K_{act}) of 50–100 nM of cAMP and was classically known to be mainly cytosolic; while about 75% of type II PKA is associated with organelles and specific cellular structures and it presents a K_{act} of 200–400 nM of cAMP (Cadd et al. 1990, Dostmann & Taylor 1991, Gamm et al. 1996). Each R subunit of PKA contains an N-terminal docking and dimerization (D/D) domain, a PKA inhibitor site, and two tandem cAMP-binding domains (Heller et al. 2004). The D/D domain is connected to cAMP-binding domain A by an extended, highly disordered linker that contains an autoinhibitory sequence and several putative phosphorylation sites. All PKA isoforms display high sequence homology in the D/D domain and the cAMP-binding domains (Canaves & Taylor 2002). In contrast, the linker regions are highly variable in both length and sequence (Vigil et al. 2004). In addition, the R subunits are differentially expressed in different cells. Whereas RIz and RIIz subunits are ubiquitously expressed (Lee et al. 1983, Scott et al. 1987), RIβ is primarily expressed in endocrine tissues, brain, fat, and reproductive tissues, and RIβ is mainly found in the brain (Jahnsen et al. 1986, Clegg et al. 1988, Cadd & McKnight 1989). These differences in cAMP affinities and localization between PKA isoforms are proposed to contribute to specificity in the cAMP-signaling pathway (Skalhegg & Tasken 2000). Subcellular localization of PKA is mainly due to anchoring of the R subunits by AKAPs.

A-kinase-anchoring proteins

The intracellular targeting and compartmentalization of PKA are controlled through association with AKAPs. AKAPs are a structurally diverse family of functionally related proteins that now include more than 50 members when splice variants are included (Table 1). They are defined on the basis of their ability to bind to PKA and co-precipitate catalytic activity. However, the functional importance further involves targeting the enzyme to specific subcellular compartments thereby providing spatial and temporal regulation of the PKA-signaling events. All anchoring proteins contain a PKA-binding domain and a unique targeting domain directing the PKA–AKAP complex to defined subcellular structures, membranes, or organelles (Fig. 2). In addition to these two domains, several AKAPs are also able to form multivalent signal transduction complexes by interaction with phosphoprotein phosphatases, other kinases, PDEs, and other proteins involved in signal transduction (Coghlan et al. 1995, Schillace & Scott 1999, Feliciello et al. 2001, Tasken et al. 2001). Through this essential 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. Type II PKA is typically particulate and confined to subcellular structures and compartments anchored by cell- and tissue-specific AKAPs, which explain why the majority of the AKAPs identified to date bind type II PKA (Colledge & Scott 1999, Dodge & Scott 2000, Diviani & Scott 2001). However, several RI-specific AKAPs have also been characterized, although the type I PKA, which is classically known to be biochemically soluble, has been assumed to be mainly cytoplasmic. In addition,
<table>
<thead>
<tr>
<th>AKAP (gene nomenclature committee name)</th>
<th>Tissue</th>
<th>Subcellular localization</th>
<th>Properties/function</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-AKAP84/D-AKAP1/AKAP121/AKAP149 (AKAP1)</td>
<td>Testis, thyroid, heart, lung, liver, skeletal muscle, and kidney</td>
<td>Outer mitochondrial membrane/endoplasmic reticulum/nuclear envelope/sperm midpiece</td>
<td>Dual-specific AKAP. Binds lamin B and PP1. Multiple splice variants. Binds PDE7A</td>
</tr>
<tr>
<td>AKAP-KL (AKAP2)</td>
<td>Kidney, lung, thymus, and cerebellum</td>
<td>Actin cytoskeleton/apical membrane of epithelial cells</td>
<td>Binds Gz13. Potential role in sperm motility and capacitation. Multiple splice variants. Binds both RI and RII</td>
</tr>
<tr>
<td>AKAP110 (AKAP3)</td>
<td>Testis</td>
<td>Axoneme</td>
<td></td>
</tr>
<tr>
<td>AKAP82/FSC1 (AKAP4)</td>
<td>Testis</td>
<td>Fibrous sheath of sperm tail</td>
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<tr>
<td>AKAP75/79/150 (AKAP5)</td>
<td>Bovine/human/rat orthologs Brain</td>
<td>Plasma membrane/post-synaptic density</td>
<td>Polybasic domains target to plasma membrane and dendrites. Binds PKC, calcineurin (PP2B), β-AR, SAP97, and PSD-95</td>
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<tr>
<td>mAKAP (AKAP6)</td>
<td>Heart (mAKAPβ, short), skeletal muscle, and brain (mAKAPα, long)</td>
<td>Nuclear membrane</td>
<td>Binds PDE4D3. Spercin repeat domains involved in subcellular targeting. Organizes signal complex with PKA, PDE4D3, Epac, and a MEKK/MEK5/ERK5 module. Longer mAKAPα also anchors PDK1</td>
</tr>
<tr>
<td>AKAP15/18 α, β, γ, δ (AKAP7)</td>
<td>Brain, skeletal muscle, pancreas, and heart</td>
<td>Basolateral (α) and apical (β) plasma membrane, cytoplasm (γ), secretory vesicles (δ)</td>
<td>Targeted to plasma membrane via fatty acid modifications. Modulation of Na+ and L-type Ca2+ channels. ADH-mediated translocation of AQP2 from vesicles to apical membrane in distal kidney tubules. Involved in the initiation of chromosome condensation. Binds Eg7/condensin Zinc-finger motif. Binds PDE7A</td>
</tr>
<tr>
<td>AKAP95 (AKAP8)</td>
<td>Heart, liver, skeletal muscle, kidney, and pancreas</td>
<td>Nuclear matrix</td>
<td></td>
</tr>
<tr>
<td>AKAP450/AKAP350/Yotiao/CN-NAP/Hyperion (AKAP9)</td>
<td>Brain, pancreas, kidney, heart, skeletal muscle, thymus, spleen, placenta, lung, and liver</td>
<td>Post-synaptic density/neuromuscular junction/centrosomes/Golgi</td>
<td>Targets PKA and PP1 to the NMDA receptor. Multiple splice variants. Dual-specific AKAP. Binds PP1. Dual-specific AKAP</td>
</tr>
<tr>
<td>D-AKAP2 (AKAP10)</td>
<td>Liver, lung, spleen, and brain</td>
<td>Vesicles/peroxisomes/centrosome</td>
<td>Binds PKC and β-AR. Xgravin-like (Xgl) is also a putative AKAP</td>
</tr>
<tr>
<td>AKAP220/hAKAP220 (AKAP11)</td>
<td>Testis and brain</td>
<td>Actin cytoskeleton/cytoplasm</td>
<td></td>
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<tr>
<td>Gravin (AKAP12)</td>
<td>Endothelium</td>
<td>Actin cytoskeleton/cytoplasm</td>
<td></td>
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<tr>
<td>AKAP-Lbc/Ht31/Rt31 (AKAP13)</td>
<td>Ubiquitous</td>
<td>Cytoplasm</td>
<td>Ht31 RII-binding site used in peptides to disrupt PKA anchoring. Rho-GEF that couples Gz12 to Rho. Binds tubulin. Modulation of L-type Ca2+ channels. Connected to CFTR via EB200/ASGER. Dual specific AKAP. Pieters RII due to RISS domain. Scaffolds PAK-Csk immunomodulating pathway. ERM homolog Radixon also probably AKAP</td>
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<tr>
<td>MAP2B</td>
<td>Ubiquitous</td>
<td>Microtubules</td>
<td></td>
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<tr>
<td>Ezrin/AKAP78</td>
<td>Secretory epithelia</td>
<td>Actin cytoskeleton</td>
<td>Linked to CFTR via EB50/NHERF. Dual specific AKAP. Pieters RII due to RISS domain. Scaffolds PAK-Csk immunomodulating pathway. ERM homolog Radixon also probably AKAP</td>
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<tr>
<td>T-AKAP80</td>
<td>Testis</td>
<td>Fibrous sheath of sperm tail</td>
<td>(continued)</td>
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<tr>
<td><strong>Table 1 Continued</strong></td>
<td><strong>Tissue</strong></td>
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<td><strong>Properties/function</strong></td>
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<tr>
<td>AKAP80/MAP2D</td>
<td>Ovarian granulosa cells</td>
<td></td>
<td>FSH-regulated protein, identified as MAP2D</td>
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<tr>
<td>SSeCKS (Src-suppressed C kinase substrate)</td>
<td>Testis and elongating spermatids, Ubiquitous</td>
<td>Actin remodelling, Centrosome</td>
<td>Gravin-like, Binds dynein and γ-γ-tubulin, Unique RII-binding domain</td>
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<tr>
<td>Pericentrin</td>
<td></td>
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<td>Binds Abl and Wrp. Involved in sensorimotor and cognitive function</td>
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<tr>
<td>WAVE-1/Scar</td>
<td>Brain</td>
<td>Actin cytoskeleton</td>
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<tr>
<td>Myosin VIIA</td>
<td>Ubiquitous</td>
<td>Cytoskeleton</td>
<td>Binds RI</td>
</tr>
<tr>
<td>PAP7</td>
<td>Steroid-producing cells (adrenal gland and gonads)</td>
<td>Mitochondria</td>
<td>Hormonal regulation of cholesterol transport into mitochondria. Binds RI <em>in vivo</em>, contains RISR</td>
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<tr>
<td>Neurobeachin</td>
<td>Brain</td>
<td>Golgi</td>
<td></td>
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<tr>
<td>AKAP28</td>
<td>Primary airway cells</td>
<td>Ciliary axonemes</td>
<td>Modulation of ciliary beat frequency</td>
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<tr>
<td>Myeloid translocation gene (MTG) 8 and 16b</td>
<td>Lymphocytes</td>
<td>Golgi</td>
<td>Binds PDE7A</td>
</tr>
<tr>
<td>AKAP140</td>
<td>Granulosa cells and meiotic oocytes</td>
<td></td>
<td>Upreregulated by FSH in granulosa cells. Phosphorylated by CDK1 in oocytes. Not cloned</td>
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<tr>
<td>AKAP85</td>
<td>Lymphocytes</td>
<td>Golgi</td>
<td>Not cloned</td>
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<tr>
<td>BIG2 (Brefeldin A-inhibited guanine nucleotide-exchange protein 2)</td>
<td></td>
<td></td>
<td>GEF for ADP-ribosylation factor GTPases. Binds RII/RII and RII/RII through three separate PKA-binding domains. cAMP regulated translocation of BIG from cytosol to Golgi</td>
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<tr>
<td>Rab32</td>
<td></td>
<td>Mitochondria</td>
<td>Regulation of mitochondrial dynamics and fission</td>
</tr>
<tr>
<td>AKAP CE</td>
<td><em>Caenorhabditis elegans</em></td>
<td></td>
<td>Binds to RII-like subunit INF-1/FYVE and TGF- receptor-binding domain</td>
</tr>
<tr>
<td>DAKAP550</td>
<td><em>Drosophila</em></td>
<td>Plasma membrane/cytoplasm</td>
<td>Contains two RII-binding sites</td>
</tr>
<tr>
<td>DAKAP200</td>
<td><em>Drosophila</em></td>
<td>Plasma membrane</td>
<td>Binds F-actin and Ca–calmodulin</td>
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<tr>
<td>Nervy</td>
<td><em>Drosophila</em></td>
<td>Axons</td>
<td>MTG family member. Regulates repulsive axon guidance by clustering PKA and Semaphorin 1a (Sema–1a) receptor Plexin A (PlexA)</td>
</tr>
<tr>
<td>AKAP97/radial spoke protein 3 (RSP3)</td>
<td><em>Chlamydomonas</em></td>
<td>Flagellar axonemes</td>
<td>Located near inner arm dyneins and possibly regulates flagellar motility</td>
</tr>
<tr>
<td>Synemin</td>
<td>Heart</td>
<td>Intercalated discs, sarcolemma, IFs, and Z-lines</td>
<td>PKA–RII-binding AKAP. Serves dual functions in adult and neonatal cardiac myocytes as both a cytoskeletal protein and as an AKAP</td>
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(continued)
dual-specificity AKAPs (D-AKAPs) are capable of anchoring both types of R subunits (Huang et al. 1997a, b).

One of the classical characteristics of an AKAP is that it binds to the R subunit of PKA through a stretch of 14–18 amino acids forming an amphipathic helix. This helix is found in almost all AKAPs characterized to date, and consists of hydrophobic residues aligned along one face of the helix and charged residues along the other (Carr et al. 1991). The residues form five turns of an α-helix, and this helical shape is important to maintain binding to PKA. Proline substitutions of one or more residues within the helix of numerous known AKAPs disrupt their PKA-binding capability (Carr et al. 1991). The RII subunits normally bind to AKAPs with affinity in the low nanomolar range (Herberg et al. 2000), while the affinity of RI subunits is usually high nanomolar to submicromolar. Nuclear magnetic resonance (NMR) data (Newlon et al. 1999, 2001) and crystal structures (Gold et al. 2006, Kinderman et al. 2006) of the RII–AKAP complex have demonstrated that the RII subunits dimerize at their N-terminus, which is necessary for AKAP binding. The D/D domain consists of an antiparallel dimer of two polypeptide chains in a helix-turn-helix motif, forming an X-type four-helix bundle that provides the docking surface for the AKAP helix (Fig. 3). The N-terminal RII fragment can be divided into two regions: residues 1–23 forming the AKAP-binding surface and residues 24–44, which cover the majority of the contacts between the two parts of dimer. Deletion of residues 1–5 in RII abolishes binding, without interfering with dimerization, since the branched side chains at residues Ile3 and Ile5 are needed for the interaction with the AKAPs in the hydrophobic groove formed on top of the N-terminal helices (Hausken et al. 1994, 1996). The RI subunits dimerize in a similar way by making a helix-turn-helix motif, although the dimerization domain is shifted further from the N-terminus and include amino acids 12–61 (Leon et al. 1997, Banky et al. 1998, 2000). The structural basis has recently been modelled using an NMR structure of the RI D/D domain (Banky et al. 2003; Fig. 3). The difference in AKAP-binding specificity between RI and RII may be explained by the fact that the extreme N-terminus in the RI subunit is believed to be helical and that this additional helix folds back on to the four-helix bundle. Furthermore, the RIβ homodimer is rather compact having a Y-like shape with a maximum diameter of 14 nm (Heller et al. 2004), while the RIIα and RIIβ dimers seem to be completely extended with a maximum diameter of nearly 20 nm (Vigil et al. 2004). In addition, the RIα subunit undergoes a major conformational change when it associates with the catalytic subunit (Vigil et al. 2005),

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<tr>
<td>AKAP (gene nomenclature committee name)</td>
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<tr>
<td>Myospryn</td>
<td>Muscle-specific</td>
<td>The costamere of striated muscle</td>
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<td>Alpha4 integrins</td>
<td></td>
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<tr>
<td>Sphingosine kinase type 1-interacting protein (SKIP)</td>
<td>Heart</td>
<td>Ventricular tissue</td>
</tr>
<tr>
<td>MyRIP (myosin Va–Rab27a-interacting protein)</td>
<td>Perinuclear region</td>
<td>Nucleus and sliceosome</td>
</tr>
<tr>
<td>SFRS17A (splicing factor, arginine–serine-rich 17A)</td>
<td>CNS, hippocampal neurons</td>
<td>Soma, dentrites</td>
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while the RIIβ subunits remain fully extended (Zhao et al. 1998). In contrast, the RIIβ subunits round up to form a globular protein upon complex formation with the catalytic subunits (Vigil et al. 2006).

Delineation of PKA–AKAP interaction with disruptor peptides

To establish a functional role for various AKAPs, peptides have been developed to disrupt the interaction between PKA and AKAPs and serve as important tools to study the relevance of PKA in cellular processes. All peptides developed have attempted to mimic the PKA-binding domains of AKAPs and are made to be small stretches of amino acids forming amphipathic helices. The protein originally called Ht31, now known as AKAP-Lbc, was first characterized with respect to its PKA-binding domain and shown to contain an 18-amino acid amphipathic helix that binds PKA (Carr et al. 1991, 1992). Peptides encompassing this sequence display low nanomolar affinity for RII (K_D = 2.2 ± 0.3 nM; Newlon et al. 2001), were shown to disrupt R–AKAP interactions inside cells, and have been used extensively as the archetypical PKA-anchoring disruptor (RIAD) and the SuperAKAP-IS were developed with three-order magnitude selectivity for RI and RII binding. Upon substitutions selecting for either RI or RII specificity, the RI-anchoring disruptor (RIAD) and the SuperAKAP-IS were developed with three-order magnitude selectivity for RI and RII respectively (Carlson et al. 2006, Gold et al. 2006). From these studies, it became apparent that type I PKA binds more N-terminally in the A-kinase-binding region and tolerates more acidic residues on the hydrophilic face of the amphipathic helix (Carlson et al. 2006). Furthermore, the recent discovery of a region in D-AKAPs that enhance anchoring of type I PKA, the RISR specifier region (RISR), has led to more insight into how type I PKA can bind to AKAPs, although the affinity for the amphipathic helix is comparably lower (Jarnaess et al. 2008). In addition, a 13-mer peptide derived from RISR was developed and shown to disrupt the ezrin–type I PKA interaction independently of peptides that mimic the amphipathic helix, and this released cAMP-mediated inhibition of T-cell immune function (Jarnaess et al. 2008). These third generation anchoring disruptors serve as tools to delineate biological effects specifically mediated by the type I and the type II PKA, see Fig. 4 for illustration of effect (Carlson et al. 2006, Torheim et al. 2009).

AKAPs as unique targeting vehicles

In addition to the PKA-binding domain, AKAPs contain specialized targeting domains responsible for tethering the complex to different subcellular localizations through protein–lipid or protein–protein interaction.
AKAPs have been identified in association with a variety of cellular compartments, including centrosomes, endoplasmic reticulum, the Golgi apparatus, mitochondria, microtubules, cell membranes, nuclear matrices, secretory granules, and the cytoskeleton (Wong & Scott 2004). Several studies suggest that protein–lipid interactions target most AKAP–PKA complexes to the correct subcellular environment, while protein–protein interactions precisely orient the kinase toward its substrate (Carnegie & Scott 2003). For example, AKAP15/18α is targeted to the membrane by myristoylation and palmitoylation signals in close proximity to PKA substrates such as Ca^{2+} and Na^{+} channels (Fraser et al. 1998, Gray et al. 1998). In addition, AKAP15/18α interacts with L-type Ca^{2+} channel via a modified leucine zipper motif (Hulme et al. 2003). Furthermore, AKAP79 is localized to the post-synaptic membranes in neurons through repeated sequences that bind negatively charged phospholipids, whereas this anchoring protein is selectively coupled to substrates such as α-amin-3-hydroxy-5-methyl-4isoxazolepropionic acid-type glutamate receptor ion channels and Ca^{2+} or K^{+} channel through specific protein–protein interactions (Dodge & Scott 2000).

Different AKAPs can also be targeted to the same subcellular organelle, indicating that within a single cellular compartment, various AKAPs function to target PKA to distinct subdomains and to specific substrates. Also, splice variants and isoforms of an AKAP can be diversely targeted. Four anchoring proteins (D-AKAP1, D-AKAP2, PAP7, and Rab32) anchor PKA to the mitochondria, while ezrin, WAVE-1 (Wiskott-Aldrich syndrome protein family verprolin homologous protein), and AKAP-Lbc all tether PKA to distinct parts of the actin cytoskeleton (Tasken & Aandahl 2004). In addition, the AKAPs pericentrin and AKAP450 both secure precise PKA phosphorylation at the centrosomes (Tasken & Aandahl 2004). While AKAP450 is targeted to the centrosomes, its splice variant, Yotiao, is targeted to post-synaptic densities. Similarly, the different isoforms of AKAP18 have distinct subcellular localization. The α-isofrom is located at the lateral membrane of polarized epithelial cells, the β-isofrom is located at the apical membrane of polarized epithelial cells, while the γ-isofrom is mainly cytoplasmic (Trotter et al. 1999).

**Figure 4** Schematic illustration of the effect of specific anchoring disruptor peptides. (A) Model of type I PKA and type II PKA signal complexes organized by specific AKAPs and mediating biological effects 1 and 2 respectively. (B and C) Models for effect of RIAD or SuperAKAP-/S on biological effects 1 and 2. (D and E) Effect of RIAD and SuperAKAP-/S together or Ht31 on biological effects 1 and 2. cAMP, red-filled circles; phosphate, blue-filled circles.
AKAPs as multienzyme scaffolding complexes

AKAPs mediate high levels of specificity and complexity in the cAMP–PKA signaling pathway through the interaction with various signaling enzymes and by assembly of multiprotein complexes. Several AKAPs with this property have been identified that provide precise spatiotemporal regulation of the cAMP–PKA pathway and integration with other signaling pathways in one signal complex. Recent examples are AKAP79, AKAP450, AKAP220, AKAP-Lbc, ezrin, gravin, mAKAP, and WAVE that all have been shown to form multienzyme complexes, and it is likely that we are still at the very beginning of understanding the roles AKAPs play in the orchestration of intracellular signaling events in health and disease. The potential for multivalency in AKAPs was first demonstrated for AKAP79, which in addition to PKA interacts with protein kinase C (PKC) and the calcium/calmodulin-dependent phosphatase, PP2B (Coghlan et al. 1995, Klauck et al. 1996). More recent work on AKAP79 using meticulously designed FRET reporters and mutant versions of the protein has shown that distinct combinations of enzymes targeted by AKAP79 in different contexts give functional diversity with respect to what signal pathways the AKAP-targeted complex participates in (Hoshi et al. 2003, 2005). Furthermore, the same authors recently demonstrated that interaction with AKAP79 alters the regulation and pharmacological profile of the bound PKC (Hoshi et al. 2010). Subsequently, other AKAPs have been shown to work as multi-scaffolding proteins as well. AKAP220 interacts with protein phosphatases 1 (PP1; Schillace & Scott 1999), AKAP450 anchors PKC, polynucleotide kinase, PP2A, PPI, and PDE4D3 to the centrosomes (for review, see Tasken & Aandahl (2004)), and AKAP149, when located at the nuclear membrane, binds PP1 and PKC (Steen et al. 2000, Kuntziger et al. 2006). An interesting and more complex example is the muscle-specific AKAP, mAKAP, that coordinates two integrated cAMP effector pathways. Both PKA and PDE4D3 are present in the mAKAP-signaling complex (Dodge et al. 2001), creating a negative feedback loop where PKA phosphorylation of PDE4D3 increases its activity and facilitates more rapid signal termination. Moreover, PDE4D3 serves as an adaptor protein for Epac and the extracellular signal-regulated kinase (ERK), ERK5 (Dodge-Kafka et al. 2005). ERK5 phosphorylation of PDE4D3 decreases the PDE activity (Hoffmann et al. 1999), thereby favoring a local increase of cAMP and successive PKA and Epac activation. Epac can then activate the small Ras-like GTPase Rap1, which inhibits the MAPK/ERK kinase kinase (MEKK) thereby releasing inhibition of PDE4D3 by ERK5 (Fig. 5). Thus, two coupled cAMP-dependent feedback loops are synchronized within the same AKAP complex, indicating that local control of cAMP signaling by AKAP proteins is more intricate than previously anticipated.

Figure 5 Signal complex consisting of mAKAP, PKA, PDE4D3, Epac, and a MEKK/MEK5/ERK5 module. mAKAP anchors PKA and PDE4D3, whereas PDE4D3 scaffolds an Epac–Rap1 pathway that coordinates a MEKK/MEK5/ERK5 module. cAMP, red-filled circle; phosphate, blue-filled circle.

Functional consequences of PKA anchoring

The biological relevance of anchoring is extensively studied using inhibitory peptides that disrupt PKA anchoring and expression of compartment-specific AKAPs to redistribute PKA to defined subcellular sites. Later studies have also used siRNA-mediated knockdown and reconstitution experiments expressing a mutant AKAP that does not bind PKA or employed engineering of mutant mice to study the function of AKAPs.

Early studies focused mainly on rapid cAMP-responsive events, such as modulation of ion channels. Use of the prototypical disruptor peptide Ht31 showed that anchored PKA is involved in the regulation of cardiac and skeletal muscle L-type Ca2+ channels and Ca2+-activated K+ channels in smooth muscle (Gao et al. 1997). AKAP79, AKAP15/18, and AKAP-KL have all been shown to be involved in the modulation of ion channels through directing pools of kinases and phosphatases near particular channel subunits (Fraser & Scott 1999). Expression of AKAP79 has been used to demonstran an increase in cAMP-dependant modulation of L-type Ca2+ channels (Gao et al. 1997) as well
as in the native K⁺ secretory channels (ROMK) in the kidney (Ali et al. 1998). Likewise, expression of AKAP15/18 stimulates the process of insulin secretion from intact cells by targeting PKA to the membrane, while peptide-mediated disruption of PKA–RII interaction leads to a decrease. This might be a result of enhanced Ca²⁺ channels (Fraser et al. 1998).

PKA was identified as the kinase involved in the recycling of β1-adrenergic receptor with the use of the peptide Ht31. However, it was through the use of siRNA interference that the AKAP responsible for anchoring PKA, AKAP79, was identified (Gardner et al. 2006). Moreover, while the use of Ht31 demonstrated the involvement of an AKAP in cAMP-mediated immunomodulation, siRNA-mediated knockdown of the AKAP ezrin eliminated type I PKA from T-cell lipid rafts and disrupted cAMP-mediated inhibition of T-cell immune function (Ruppelt et al. 2007), whereas reconstitution experiments reversed the effects seen with siRNA alone (Jarnaess et al. 2008).

The AKAP18δ is involved in the recruitment of PKA to a supramolecular complex containing phospholamban (PLB) and sarcoplasmic reticulum Ca²⁺-ATPase 2 (SERCA2), and is important to discretely regulate PKA phosphorylation of PLB and thereby the PLB-inhibitory effect on SERCA2 and Ca²⁺ reuptake into heart sarcoplasmic reticulum. Displacement of PLB from AKAP18δ and siRNA knockdown experiments revealed that scaffolding of the complex by AKAP18δ to allow precise spatiotemporal control of PKA phosphorylation of PLB is necessary for adrenergic effect on Ca²⁺ reuptake (Lygren et al. 2007; Fig. 6).

A recent work has demonstrated that the RISR is found in D-AKAPs, and this sequence was utilized in a bioinformatic search to identify a novel anchoring protein, the splicing factor arginine–serine-rich 17A (SFRS17A). SFRS17A was found to bind both type I and type II PKA by several protein interaction methodologies, and was found to be co-localized with the catalytic (C) subunit of PKA in splicing factor compartments in the nucleus. In addition, SFRS17A regulated E1A alternative splicing as shown in E1A minigene experiments, indicating that SFRS17A targets PKA to splicing factor compartments to confer PKA-mediated regulation of pre-mRNA splicing (Jarnaess et al. 2009).

To study the function of the WAVE-1 signal complex in vivo, targeted disruption was used to knock out the WAVE-1 gene showing that loss of this AKAP causes sensorimotor retardation and reduced learning and memory in mice, phenotypes analogous to patients with 3p-syndrome mental retardation (Soderling et al. 2003). Later, null mutant mice have been generated for AKAP149 (Newhall et al. 2006), AKAP79 (Hall et al. 2007, Navedo et al. 2008, Tunquist et al. 2008), AKAP95 (Yang et al. 2006) and ezrin (Saotome et al. 2004) demonstrating distinct roles on differentiation, development and hormonal regulation of physiological function.

**AKAPs in disease**

A genetic polymorphism in D-AKAP2 (Ile646Val), which decreases the affinity for type I PKA R subunit, has been shown to be associated with familial breast cancer. Likewise, another study has shown that the AKAP-Lbc Lys526Gln polymorphism is associated with familial breast cancer. This amino acid substitution alters the secondary structure of AKAP-Lbc and affects the function of the protein. Carriers of both mutations, D-AKAP2 Ile646Val and AKAP-Lbc Lys526Gln, in combination have an even greater risk of developing breast cancer (Wirtenberger et al. 2007). Furthermore, the Ile646Val substitution in D-AKAP2 has been

![Figure 6](https://example.com/figure6.png)

**Figure 6** PKA–AKAP18δ–PLB–SERCA2 complex. (A) Resting situation, no adrenergic drive, low heart rate, SERCA2 inhibited by PLB with low ATP and energy consumption. (B) Adrenergic stimulation paces the heart and increased heart rate. SERCA2 released from PLB inhibition by PKA phosphorylation leading to fast Ca²⁺ reabsorption and high ATP and energy consumption.
associated with increased P–R interval in electrocardiography, whereas a Ser1570Lys substitution in Yotiao, which delocalizes the AKAP itself, has been linked to long QT syndrome and cardiac arrhythmias (Kammerer et al. 2003, Chen et al. 2007).

**Concluding remarks**

Although a number of early studies indicated possibilities of compartmentalization of cAMP, the predominating view just over a decade ago was still that cAMP would be elevated 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 have 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 AKAPs were starting to be identified 20 years ago, the prevailing view was still for a long time 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 (more than 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 in order to regulate specific substrates as well as to mediate a number of physiological effects has been extensively studied over the past decade. With few exceptions, it has been shown that most cAMP–PKA-regulated physiological processes require an anchored kinase. It has been made clear from studies discussed here that the localized cAMP microdomains require carefully compartmentalized and anchored pools of PDE enzymes. The concept described in this review that has emerged over the past 10–15 years and which is now well established is that cAMP–PKA-regulated physiological processes has been extensively studied over the past 10–15 years and which is now well established is that cAMP–PKA-regulated physiological processes have increased specificity and spatial dynamics of PKA signaling.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**References**


Cadd GG, Uhler MD & McKnight GS 1990 Holoenzymes of cAMP-dependent protein kinase containing the neural form of type I regulatory subunit have an increased sensitivity to cyclic nucleotides. *Journal of Biological Chemistry* 265 19502–19506.


Colledge M & Scott JD 1999 AKAPs: from structure to function. Trends in Cell Biology 9 216–221.


Dostmann WR & Taylor SS 1991 Identifying the molecular switches that determine whether (Rp)-cAMPS functions as an antagonist or an agonist in the activation of cAMP-dependent protein kinase I. Biochemistry 30 8710–8716.


Gao T, Yatani A, Dell’Acqua ML, Sako H, Green SA, Dascal N, Scott JD & Hossy MM 1997 cAMP-dependent regulation of cardiac L-type Ca2+ channels requires membrane targeting of PKA and phosphorylation of channel subunits. Neuron 1 185–196.


Kuntziger T, Rogné M, Folstad RL & Collas P 2006 Association of PP1 with its regulatory subunit AKAP149 is regulated by serine phosphorylation flanking the RVXF motif of AKAP149. *Biochemistry* **45** 5868–5877.


Schillace RV & Scott JD 1999 Association of the type I protein phosphatase PP1 with the Akinase anchoring protein AKAP220. *Current Biology* **9** 321–324.


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