AKAPs: from structure to function

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Compartmentalization of signalling molecules through association with anchoring proteins ensures specificity in signal transduction by placing enzymes close to their appropriate effectors and substrates. For example, 'A-kinase anchoring proteins' (AKAPs) bind to the regulatory subunit of cAMP-dependent protein kinase (PKA) to direct the kinase to discrete intracellular locations. Recently, functional studies aimed at disrupting AKAP–PKA complexes have demonstrated a role for anchored PKA in various cellular processes, including gene transcription, hormone-mediated insulin secretion and ion-channel modulation. By binding to additional signalling molecules, AKAPs might function to coordinate multiple components of signal-transduction pathways.

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The transduction of extracellular signals across the cell membrane to intracellular targets is a tightly regulated process that underlies diverse physiological functions. As many signalling enzymes are widely distributed and often have rather broad substrate selectivity, a crucial element in signal transduction is the control of specificity. How does an individual hormone or neurotransmitter direct the correct pool of second messengers to particular downstream enzymes, and, in turn, how does each enzyme find its appropriate set of protein substrates? In some cases, specificity is achieved by enzymes that have limited or precise substrate recognition. However, distinct mechanisms must be in place to enhance the specificity of signalling enzymes with broader actions. Subcellular targeting through association with anchoring proteins has emerged as an important mechanism by which cells localize signalling enzymes to sites where they can be accessed optimally by activators and, in turn, interact with particular substrates[1]. This review focuses on recent advances in our understanding of the molecular mechanisms that govern the cellular compartmentalization of protein kinase A (PKA) through the use of A-kinase anchoring proteins (AKAPs).

cAMP signalling pathways and AKAPs

One of the best-understood signal-transduction pathways involves the second-messenger cAMP[2]. cAMP is generated following receptor activation by many different hormones and neurotransmitters, leading to the activation of PKA. Many, if not all, of the components of this signalling cascade – receptors, heterotrimeric G proteins, adenylyl cyclases and kinase subunits – have been cloned and characterized. The PKA holoenzyme consists of two catalytic subunits (C) and a regulatory subunit (R) dimer. There are three C subunit isoforms (Cα, β, γ), which have virtually identical kinetic and physiochemical properties[3]. By contrast, the different R subunits (RI, RII) exhibit distinct cAMP binding affinities and are localized differentially inside cells. The type I PKA holoenzyme (RIα and RIβ) is predominantly cytoplasmic, whereas the majority of type II PKA (RIIα and RIIβ) associates with cellular structures and organelles[4]. This appears to be due, in large part, to the anchoring of RII subunits by AKAPs. RII subunits bind to AKAPs with nanomolar affinity[5], and many AKAP–RII complexes have been isolated from cell extracts. By contrast, RI subunits bind to AKAPs with only micromolar affinity[6], and it remains to be determined whether AKAP–RI complexes form *in vivo*. However, it is worth noting that a novel AKAP was identified recently that binds specifically to the RI-like regulatory subunit of *Caenorhabditis elegans* PKA[7].

The microtubule-associated protein MAP2 was the first protein shown to copurify and directly interact with RII[8], and, in the 15 years thereafter, numerous AKAPs have been identified by gel overlays and expression-library screening (Ref. 9; reviewed in Ref. 10). Today, multiple cDNAs encoding AKAPs have been isolated from diverse species, ranging from *C. elegans* and *Drosophila* to human (Table 1). Surprisingly, extensive biochemical analysis and searching of the yeast genome has not identified any AKAPs in *Saccharomyces cerevisiae*. Among the mammalian AKAPs, at least three are subject to alternative splicing: AKAP-KL, S-AKAP84/D-AKAP1 and yotiao/AKAP350[11–15]. In the latter two cases, genomic and biochemical analyses have identified multiple isoforms with differing tissue expression. Collectively, the existing data support the notion that AKAPs are a group of functionally, rather than structurally, related proteins, which each contain a common RII-binding site. We now propose that a more stringent criterion be used to classify a protein as an AKAP – namely, the ability to copurify C subunit activity from cell extracts by immunoprecipitation of the anchoring protein. This criterion is a functional test of the ability of the candidate anchoring protein to target the catalytic subunit of PKA inside cells; however, it assumes that the PKA–AKAP interaction is of sufficiently high affinity to permit copurification of the holoenzyme.

Structural determinants of AKAP–RII interaction

Early on, deletion-mapping studies identified a region of MAP2 that mediated association with RII[16,17]. Subsequent computer-aided secondary-structural analysis predicted that sequences of 14–18 residues in MAP2 and other AKAPs have a high probability of amphipathic helix formation,

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TABLE 1 – PROPERTIES OF AKAPS

AKAP	Tissue/compartment	Features	Refs
AKAP15/18	Plasma membrane	Targeted to plasma membrane via fatty acid modifications Modulation of L-type Ca^{2+} channels	27,28,51
Ht31	Isolated from thyroid library Cytoplasm	Peptide corresponding to RII binding site disrupts PKA anchoring in cells	18–21
AKAP75/79/150	Plasma membrane Postsynaptic density	Bovine, human and rat orthologues, respectively Also binds to PKC and calcineurin (PP2B) Polybasic domains target to plasma membrane and dendrites	9,29,31,32
Ezrin	Actin cytoskeleton	Might be linked to CFTR via EBP50/NHERF	36
TAKAP80	Fibrous sheath of sperm tail	Testis specific	52
AKAP82	Fibrous sheath of sperm tail	Potential role in sperm capacitation	53
AKAP95	Nuclear matrix	Zinc-finger motif	54
AKAP-KL	Kidney and lung Actin cytoskeleton Apical membrane of epithelial cells	Multiple splice variants	11
AKAP220	Vesicles	Also binds to PP1	35
Gravin	Actin cytoskeleton	Also binds to PKC	34
mAKAP	Cardiac and skeletal muscle and brain Nuclear membrane	Spectrin repeat domains involved in subcellular targeting Developmentally regulated	–[a]
Yotiao AKAP350	Postsynaptic density Neuromuscular junction Centrosomes	Multiple splice variants Yotiao targets PKA and PP1 to NMDA receptor	14,15
S-AKAP84/D-AKAP1 AKAP121/AKAP149	Outer mitochondrial membrane	Dual-specificity AKAP, binds to RI and RII Multiple splice variants	12,13,26, 55
D-AKAP2	Wide tissue distribution as assessed by northern blot	Dual-specificity AKAP, binds to RI and RII	56
$AKAP_{CE}$	Isolated from *Caenorhabditis elegans* expression library	Binds to RI-like subunit RING-finger protein	7
DAKAP550	Identified by RII overlay of *Drosophila* embryos	Contains two RII-binding sites	57

[a]M. S. Kapiloff and J. D. Scott, pers. commun.
Abbreviations: AKAP, A-kinase anchoring protein; CFTR, cystic fibrosis transmembrane conductance regulator; NMDA, *N*-methyl-D-aspartate; PKA, protein kinase A; PKC, protein kinase C.

with hydrophobic residues aligned along one face of the helix and charged residues along the other[18]. The human thyroid anchoring protein Ht31 served as a prototype to test this model. Indeed, introduction of amino acids predicted to disrupt helix formation in the AKAP abolished RII binding *in vitro* and *in vivo*[18,19]. Furthermore, peptides corresponding to this region of Ht31 bind to RII with nanomolar affinity, which is well within the intracellular concentrations of the AKAP and the enzyme[5]. These peptides can compete effectively for RII binding to AKAPs and thus have been used extensively as reagents to disrupt PKA anchoring inside cells[20–22].

RII is a modular protein, comprising an N-terminal dimerization domain, a region that binds to the catalytic subunit and two cAMP-binding sites[3] (Fig. 1a). The first 30 residues of RII are necessary for AKAP binding and also mediate dimerization of RII. Although dimer formation is a prerequisite for AKAP binding, the dimerization and AKAP-binding regions are separable – deletion of residues 1–5 in RII abolishes AKAP binding without disrupting dimer formation[23]. In particular, isoleucines 3 and 5 are essential determinants for interaction with AKAPs[23]. Interestingly, branched-chain hydrophobic amino acids are conserved at these positions in all RII subunits. Two recent studies have confirmed and extended these biochemical data. Neutron and x-ray scattering experiments have defined the quaternary structure of the PKA holoenzyme as an extended antiparallel dumbbell, where the bar is formed by the R subunit dimerization domains, and the bells represent the interaction between the R and C subunits[24]. Recently, the solution structure of an N-terminal fragment of RIIα (1–44) was solved by NMR[25]. The structure consists of an antiparallel dimer of two polypeptide chains in a helix–turn–helix motif, forming an X-type four-helix bundle. The N-terminal RII fragment can be divided into two regions: residues 1–23, which form the AKAP-binding surface and residues 24–44, which encompass the bulk of the dimer contacts. Furthermore, nuclear magnetic resonance data on a complex of RIIα (1–44)

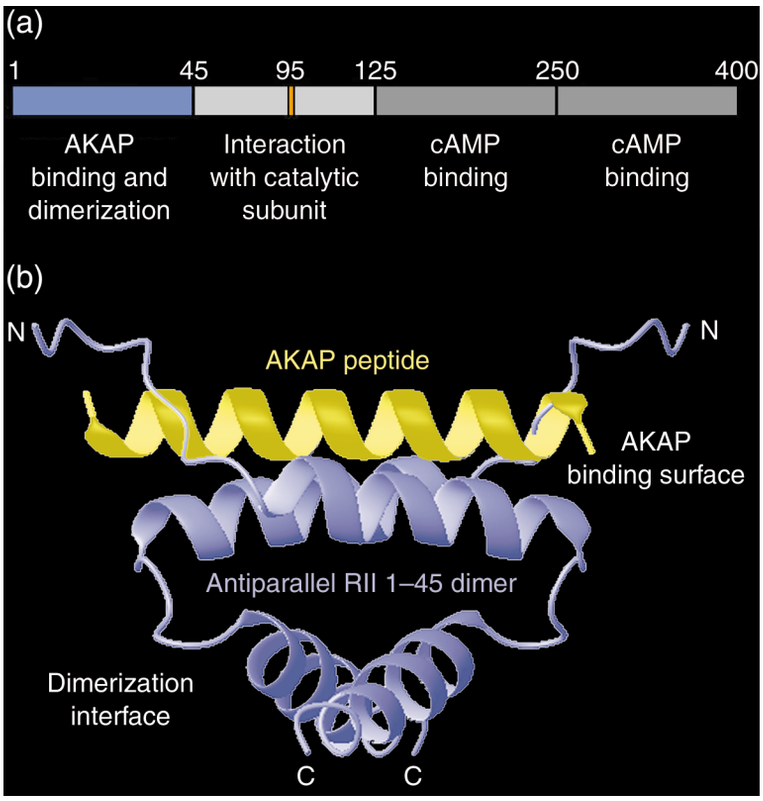

FIGURE 1

(a) Schematic of RII subunit of protein kinase A (PKA). The various binding regions of RII are indicated (orange box indicates autophosphorylation site). The three-dimensional (3-D) structure of the AKAP-binding region (indicated in blue) is shown in (b). (b) Structural model of the PKA–AKAP complex. The 3-D structure of the RIIα (1–45)–AKAP peptide complex is shown as a ribbon diagram. The four helices of the RIIα (1–45) dimer are indicated in blue. The upper two helices are predicted to form contacts with the AKAP; the lower two mediate dimerization. The backbone ribbon of the AKAP (Ht31) peptide, shown in yellow, has been modelled to fit into its putative binding site on RII. (Image kindly provided by Patricia Jennings, UCSD, USA.)

with the Ht31 peptide indicate that there is an entire surface of contacts between the two proteins, suggesting that the association is due probably to direct helix–helix contacts between hydrophobic residues on each protein[25]. In all, there are a total of 18 side-chain contacts between the two surfaces, which might explain the nanomolar binding affinity of the Ht31–RII complex (Fig. 1b).

Targeting regions

A property unique to each AKAP is a targeting sequence that determines the location of the protein in the cell. This is demonstrated in Fig. 2 by a montage of some compartment-specific AKAPs. A combination of subcellular-fractionation and immunohistochemical studies have identified AKAPs in association with a variety of cellular compartments, including centrosomes, dendrites, endoplasmic reticulum, mitochondria, nuclear membrane, plasma membrane and vesicles (Table 1). Although the subcellular location of many AKAPs has been determined, the precise details of targeting have been determined for only a few molecules. For example, members of the S-AKAP84/D-AKAP1 family contain a consensus sequence for targeting to the outer mitochondrial membrane[13,26]. The low-molecular-weight anchoring protein AKAP15/18 is targeted to the plasma membrane through lipid modification – myristoylation of glycine 1 and palmitoylation of cysteines 4 and 5[27,28] (Fig. 2). Recently, mAKAP, a muscle-selective anchoring protein, was shown to be targeted to a perinuclear location through a region that contains spectrin-like repeats (M. S. Kapiloff and J. D. Scott, pers. commun.; Fig. 2). Undoubtedly, additional sequences will be identified as further studies examine the mechanism by which other AKAPs are targeted.

Recent evidence suggests that AKAP targeting is more sophisticated than believed originally. For example, AKAP79 and yotiao both localize to the neuronal postsynaptic membrane. Three polybasic targeting regions in AKAP79 participate in electrostatic interactions with membrane phospholipids, thereby directing PKA to substrates on the inner face of the postsynaptic membrane[29] (Fig. 2). A more precise mechanism of PKA targeting at the synapse might be mediated by yotiao, which was identified originally as an *N*-methyl-D-aspartate (NMDA) receptor-associated protein[30]. Heterologous co-expression of yotiao and NMDA receptor subunits enhances cAMP-dependent potentiation of channel currents[14]. Thus, yotiao maintains an anchored pool of PKA that is physically attached to its synaptic substrate, the NMDA receptor. The location of two anchoring proteins at the postsynaptic membrane raises the intriguing possibility that, within a single cellular compartment, different AKAPs function to target PKA to specific substrates.

AKAPs as multivalent anchoring proteins

Although AKAPs have been defined on the basis of their interaction with PKA, an additional feature of many of these molecules is their ability to bind to other signalling enzymes. By simultaneously binding enzymes with opposing actions, such as kinases and phosphatases, these multivalent anchoring proteins could target entire signalling complexes to specific substrates (Fig. 3). For example, AKAP79 binds to PKA, protein kinase C (PKC) and the protein phosphatase calcineurin (PP2B)[31,32] (Fig. 3a). Each enzyme is inhibited when bound to the anchoring protein, providing an additional level of regulation. As distinct activation signals are necessary to release and activate each enzyme, AKAP79 might provide a point of convergence for multiple second-messenger signals, such as cAMP, Ca^{2+} and phospholipids[33]. Likewise, gravin targets both PKA and PKC to the cell periphery and filopodia of macrophage-like cells[34]. AKAP220 recently has been shown to bind to protein phosphatase 1 (PP1) in addition to PKA[35]. When bound, PP1 is inhibited, suggesting that AKAP220 might function to regulate phosphatase activity. Similarly, recent work has shown that yotiao binds to PP1 as well as to PKA. In contrast to AKAP220, yotiao holds PP1 in an active state[14] (Fig. 3b). The functional consequence of this appears to be a limiting of NMDA channel activity. Activation of PKA by cAMP overcomes this inhibition, leading to an enhancement of the channel current. In this way,

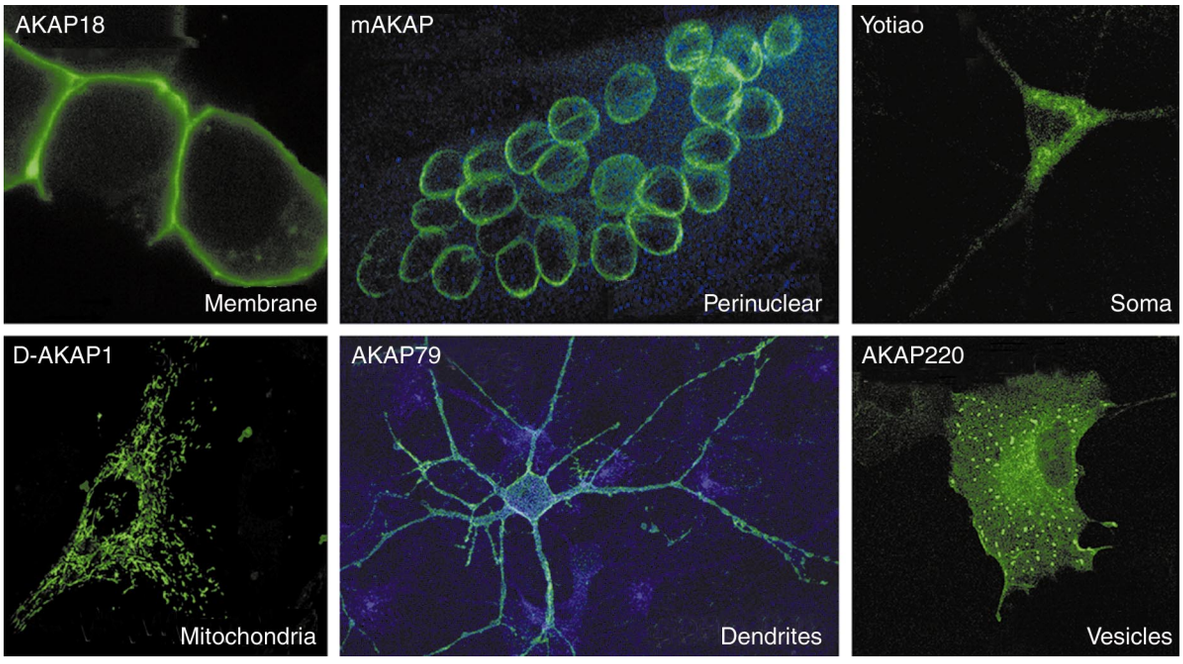

FIGURE 2

Compartmentalization of AKAPs. Targeting of various AKAPs to different cellular compartments is illustrated. In each panel, the location of the AKAP was visualized by immunofluorescent labelling using antibodies specific for the indicated AKAP. In all cases, the green label indicates the AKAP. (The D-AKAP1 image was kindly provided by Susan Taylor, UCSD, USA.)

yotiao directly targets a kinase and a phosphatase, with opposing functions, to their channel substrate.

AKAPs also have binding sites for other proteins. For example, the cytoskeletal component ezrin, an RII-binding protein[36], also binds to EBP50/NHERF, a protein involved in regulation of Na^+–H^+ transport in the apical membrane of epithelial cells. A pair of recent studies demonstrate that EBP50/NHERF binds to the cystic fibrosis transmembrane conductance regulator (CFTR)[37,38] (Fig. 3b). Since cAMP-mediated signalling is involved in regulation of CFTR, these data implicate a role for anchored PKA, via an ezrin–EBP50 complex.

Functional consequences of PKA anchoring

The biological relevance of anchoring is underscored by recent functional studies that use AKAPs as reagents to manipulate the subcellular distribution of PKA. To date, two approaches have been exploited: cellular disruption of PKA anchoring using inhibitor peptides and protein fragments derived from Ht31, and expression of compartment-specific AKAPs to redistribute the kinase to defined subcellular sites. Many of these studies have focused on rapid cAMP-responsive events, such as modulation of ion channels. Initial studies demonstrated that perfusion of cultured hippocampal neurons with Ht31 peptides, to displace anchored PKA, caused a time-dependent rundown in AMPA-type glutamate receptor currents[20]. Similarly, use of Ht31 peptides has demonstrated a role for anchored PKA in the regulation of cardiac and skeletal muscle L-type Ca^{2+} channels and Ca^{2+}-activated K^+ channels in smooth muscle[39–41]. Heterologous expression of AKAP79 targets PKA to the periphery of cells in proximity to transmembrane proteins. This approach has been used successfully to demonstrate increases in cAMP-dependent modulation of L-type Ca^{2+} channels and ROMK-type K^+ channels[39,42]. Likewise, overexpression of AKAP15/18 stimulates cAMP-dependent L-type Ca^{2+}-channel activity[27]. Isolation of complexes containing AKAP15/18 and L-type Ca^{2+} channels from cells suggests that AKAP15/18 is responsible for mediating channel regulation *in vivo* [28] (Fig. 3a). In a similar manner, it will be important to identify the particular AKAPs involved in the modulation of specific ion channels in native tissues.

AKAPs also orchestrate the role of PKA in more complicated physiological systems, such as hormone-mediated insulin secretion from pancreatic islet β cells[22]. Peptide-mediated disruption of the AKAP–RII interaction inside intact cells reduces insulin secretion, whereas membrane targeting of the kinase, through expression of AKAP15/18, stimulates this process. In part, this effect might result from enhanced Ca^{2+} entry through L-type Ca^{2+} channels[27]. AKAPs also might participate in cAMP-mediated signalling to the nucleus. Membrane localization of PKA by AKAPs appears to be important to this process. Translocation of PKA to the cytoplasm by overexpression of an untargeted RII-binding fragment of AKAP75 (the bovine orthologue of AKAP79) prevents nuclear accumulation of the C subunit and subsequent cAMP-dependent gene transcription[43]. Similarly, expression of full-length AKAP75, which presumably targets RII to the membrane, enhances cAMP-responsive transcription[44]. Finally, cell-soluble Ht31 inhibitor peptides have been shown to arrest bovine sperm motility. Interestingly, inhibition of the C subunit with the PKA inhibitor peptide PKI (5-24) does not mimic

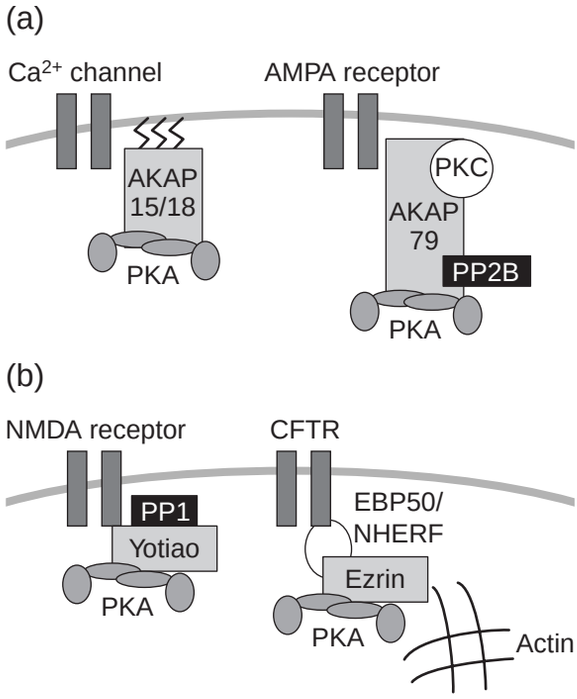

FIGURE 3

Targeting of AKAPs to ion channels. (a) By localizing signalling enzymes near ion channels, AKAPs function to modulate channel function. In the case of Ca^{2+} channels, channel regulation appears to result from association with AKAP15/18. AKAP79 might play a role in regulation of AMPA receptor function. (b) An additional level of specificity might be achieved by AKAPs that directly target signalling enzymes to their substrates. Ezrin might target protein kinase A (PKA) to the cystic fibrosis transmembrane conductance regulator (CFTR) through EBP50/NHERF. In neurons, yotiao targets PKA and protein phosphatase 1 (PP1), enzymes with opposing actions, to their substrate, the *N*-methyl-D-aspartate (NMDA) receptor.

the anchoring inhibitor effect, leading to the proposition that regulation of sperm motility is an independent function of the R subunit[45].

Conclusions and perspectives

In the past few years, multiple lines of evidence have suggested that AKAPs are important in the organization of cAMP-mediated signalling events. The anchoring protein serves at least two functions: to place the PKA holoenzyme at locations where it can respond rapidly to the ebb and flow of cAMP production and to favour certain PKA phosphorylation events by placing the enzyme close to a particular subset of substrates. This latter point has been highlighted by a series of recent functional studies, suggesting that several ion channels might be served by their own membrane-targeted AKAPs, such as AKAP15/18 and yotiao[14,46]. Anchoring of PKA complexes with channels might be particularly relevant for the extremely rapid (millisecond) transduction of cAMP signals involved in the regulation of ion-channel currents. Nevertheless, slower PKA-mediated cellular events, such as transcriptional regulation, also appear to require AKAPs.

Undoubtedly, further analysis of AKAP function will be forthcoming from genetic approaches involving transgenic and knockout mice. It is anticipated that these studies will complement a series of elegant experiments that have systematically ablated the genes encoding the C and R subunits of the PKA holoenzyme[47]. Genetic analysis of anchoring proteins should permit a more precise dissection of particular AKAP–PKA complexes *in vivo*. Likewise, information from the RIIα–AKAP peptide structure could be useful in generating small-molecule inhibitors of PKA anchoring inside cells. Cell-soluble anchoring inhibitors of this type could supersede the use of Ht31 peptides by allowing greater flexibility in the analysis of AKAP-mediated functions. These reagents should be helpful in assessing which cAMP-dependent events involve anchored pools of PKA as it is not presently known what proportion of PKA is bound by AKAPs. In light of recent biochemical evidence that RI also binds to AKAPs, it will be important to determine what proportion of type I kinase is associated with anchoring proteins.

Recent structural data indicate that there is a single region of multiple contact sites between the RII subunit dimer and the AKAP, which presumably accounts for the high-affinity interaction. Functionally, this suggests that the AKAP–PKA complex is likely to be a constitutive interaction in cells and not subject to regulation. However, experimental evidence indicates that the distribution of the PKA holoenzyme can be altered upon induction of AKAP expression. Thus, regulation of PKA localization might be a function of AKAP targeting. In support of this idea, AKAP79 membrane targeting appears to be regulated at least in part by PKC phosphorylation and Ca^{2+}/calmodulin[29]. No doubt, future studies will focus on additional mechanisms that control AKAP expression and targeting.

Perhaps the most intriguing property of the AKAPs is their potential to assemble signalling complexes through association with multiple enzymes and potential substrates. To date, gravin, AKAP79, yotiao, AKAP220 and ezrin have been shown to act as multivalent anchoring proteins. The NMDA-receptor–yotiao complex is particularly sophisticated in its level of organization; yotiao directly localizes signalling enzymes with the potential substrate. In a similar manner, ezrin might target PKA to CFTR through association with EBP50/NHERF. By physically binding signalling enzymes, substrates and potential effectors, AKAPs might coordinate the formation of macromolecular signalling complexes in a manner analogous to the Sterile 5 mitogen-activated protein (MAP) kinase scaffold in yeast and the INAD phototransduction signalling complex in *Drosophila*[48–50]. It is becoming apparent that these AKAP signalling complexes might mediate a more precise level of organization than originally was appreciated. Conceivably, AKAP signalling complexes might not only organize the location of the cAMP signalling machinery with a particular substrate but also might segregate separate pathways within the same cellular compartment. This form of nanocompartmentalization might be the molecular key to specificity in signal-transduction pathways.

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