Functional Anchoring of the cAMP-Dependent Protein Kinase

Brian J. Murphy* and John D. Scott

The precision of cAMP-responsive events is controlled in part through compartmentalization of the signal transduction machinery. Recent evidence suggests that the cAMP-dependent protein kinase (PKA) is localized to specific subcellular compartments through association with A Kinase Anchoring Proteins (AKAPs). The AKAPs now represent a functionally related family of regulatory proteins that contain a conserved PKA binding domain and unique targeting sequences that direct the PKA–AKAP complex to subcellular structures. In this review, the recent evidence suggesting that AKAPs facilitate PKA anchoring close to key membrane substrates, such as glutamate receptors, calcium-activated potassium channels, and skeletal or cardiac muscle calcium channels, is surveyed. (Trends Cardiovasc Med 1998;98:89–95).

Protein phosphorylation is an ubiquitous mechanism for the regulation of a myriad of intracellular processes including effects on metabolism, regulation of intracellular enzyme activity, membrane receptor function, ion channel activity, the modulation of gene expression, and cell cycle regulation. Protein activities that are regulated by phosphorylation are also counterbalanced by protein phosphatases that dephosphorylate the same substrates. The dynamic equilibrium between the phosphorylated and nonphosphorylated state of any given protein is governed by the activities of the responsible protein kinases and protein phosphatases.

Many protein kinases exhibit a broad substrate specificity such that upon activation they are capable of phosphorylating multiple protein substrates within a given cell. Likewise, protein phosphatases can also exhibit broad substrate specificities and are capable of dephosphorylating many of the kinase targets (Cohen 1989). An important question in cellular biology is how the phosphorylation state of limited substrate pools is regulated in a cell upon hormonal activation of individual protein kinases and phosphatases.

An emerging paradigm is that the specificity of phosphorylation and dephosphorylation events can be modulated by site-selective localization of kinases and phosphatases within the cell. Localizing or targeting these enzymes near their substrates can promote specific regulatable phosphorylation and dephosphorylation events within spatially distinct substrate pools (Hubbard and Cohen 1993, Faux and Scott 1996b).

Targeting of protein kinases and protein phosphatases can occur through direct attachment of the catalytic or regulatory subunits of kinases/phosphatases to their substrates, or nearby subcellular structures. Localization near substrate molecules can also occur via attachment of the kinases and/or phosphatases to specific targeting or anchoring proteins that bind to their substrates or nearby subcellular structures. Finally, the kinases and their substrates can be assembled into multifunctional complexes via "scoffolding" proteins to ensure the proximity of multiple components of a sequential signaling system (Faux and Scott 1996a).

This review describes the known proteins involved in the anchoring of cAMP-dependent protein kinase (PKA) within cells and reviews the evidence to date that suggests that anchoring or subcellular targeting of PKA has functional importance. This issue is particularly pertinent for the role of PKA in cardiac and skeletal muscle function, as some evidence now suggests that anchored kinase pools facilitate the modulation of Ca^{2+} channels and govern phosphorylation events at the plasma membrane and the sarcoplasmic reticulum.

• Protein Kinase A

Since its discovery, cAMP has been the focus of extensive research aimed at understanding its role as an effector of second messenger-mediated signaling events. The major intracellular receptor for cAMP is PKA. The PKA holoenzyme consists of two regulatory subunits and two catalytic subunits that form an inactive heterotetramer (Krebs 1985, Taylor 1989). Each regulatory subunit binds two cAMP molecules, causing the release of active soluble catalytic subunits. Two classes of regulatory subunits exist (RI and RII), giving rise to type I (containing RI) and type II (containing RII) holoenzymes. Although type I PKA is generally soluble and cytoplasmic, the type II isoform is associated with the cell particulate fraction to varying degrees in various tissues (Rubin et al. 1979).

Despite its broad substrate specificity, PKA activity can be highly selective in a physiological setting. Specific hormones, each capable of raising intracellular cAMP, can result in the preferential phosphorylation of different target substrates (Livesey et al. 1982, Harper et al. 1985). Recent work has demonstrated that cAMP accumulation can be compartmentalized within the cell (Adams et al. 1991, Hempel et al. 1996, Jurevicius and Fischmeister 1996) and that PKA is specifically localized within the cell (Joachim and Schwoch 1990, Salvatori et al. 1990). Together, these findings em-

Brian J. Murphy was at the Department of Pharmacology, University of Washington, Seattle, Washington, and John D. Scott is at the Howard Hughes Medical Institute, Vollum Institute, Oregon Health Sciences University, Portland, Oregon, USA.
*Address correspondence to: Dr. Brian J. Murphy, Neurocrine Biosciences Inc., 8050 Science Park Road, San Diego, CA 92121 (present affiliation).

phasize that PKA phosphorylation of various target substrates depends not only on whether cAMP levels are increased but also on where within the cell this increase occurs and whether PKA and its substrates are localized at the site.

• A Kinase-Anchoring Proteins

Biochemical experiments have shown that Type II PKA is predominantly associated with the particulate fraction from cells by virtue of its attachment to a variety of subcellular structures including the plasma membrane, endoplasmic reticulum, microtubules, Golgi, peroxisomes, mitochondria, and nucleus (Faux and Scott 1996b) (Table 1). Type II PKA is tethered to subcellular structures by virtue of the attachment of RII to targeting proteins termed *A kinase-anchoring proteins* (AKAPs). One valuable biochemical observation was that AKAPs retain their ability to bind RII in protein overlays following sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransfer to nitrocellulose (Lohmann et al. 1984). The use of purified RII protein to probe blots of cellular extracts has facilitated the detection of AKAPs from a variety of tissues ranging in size from 15 to 420 kD (Carr et al. 1992a: Table 1). Radiolabeled RII protein has also been used to screen cDNA expression libraries, enabling the determination of the primary structure of several AKAPs (Carr and Scott 1992, Hirsch et al. 1992; Table 1). Despite their amino acid sequence diversity, AKAPs share certain structural features. All known AKAPs bind to a common site at the N terminus of the RII protein and

Table 1. Identification, tissue distribution, and salient features of known A kinase anchoring proteins

AKAP	*Distribution*	*Features*	*Reference*
MAP-2	Complexed with microtubules in neurons	Calmodulin-binding protein	Lohmann et al. 1984
AKAP-75 (p75)	Bovine cerebral cortex, liver	Calmodulin-binding protein	Sarkar et al. 1984 Hirsch et al. 1992
AKAP-80 AKAP-120	Flagellar cytoskeleton	AKAP-80, and AKAP-120 in rat flagella. AKAP-120 in bovine flaggella	Horowitz et al. 1988
AKAP-150 (p150)	Rat brain and lung	Homologue of p75	Bregman et al. 1989
Ht-31		Cloned from human thyroid library	Carr et al. 1992a
AKAP-85	Golgi from human fibroblasts	Behaves as an integral membrane protein	Rios et al. 1992
AKAP-79	Postsynaptic density and periphery in HEK 293 cells	Human homologue of p75 and p150. Binds phosphatase-2B and PKC	Carr et al. 1992b Coghlan et al. 1995b Klauck et al. 1996
AKAP-350 AKAP-160	Centrosome in human lymphoblastic cell line	May be involved in microtube nucleation	Keryer et al. 1993
AKAP-80 AKAP140	Granulosa cells	Expression regulated by follicle stimulating hormone and estrogen	Carr et al. 1993
AKAP-95	Nuclear	Contains DNA-binding domain	Coghlan et al. 1994
AKAP-420	Aplysia neuronal tissue	Six other RII-binding proteins seen in overlay	Cheley et al. 1994
AKAP-72	Human sperm	Microtubule bound	Pariset and Weinman 1994
AKAP-82 pro-AKAP-82	Fibrous sheath of flagella	Developmentally regulated and tyrosine phosphorylated	Carrera et al. 1994 Carrera et al. 1996
sAKAP-84	Mitochondria of flagella	Developmentally regulated	Lin et al. 1995
AKAP-100	Sarcoplasmic reticulum of cardiac and skeletal muscle	Also found in brain to a limited extent	McCartney et al. 1995
AKAP-220	Testicular peroxisomes	Contains a PTS-1 motif	Lester et al. 1996
AKAP-149	Thymus, prostate, testis, ovary, gastrointestinal tract	Putative splice variant of sAKAP-84 containing an RNA-binding motif	Trendelenberg et al. 1996
AKAP-250 (Gravin)	Neurons, fibroblasts, endothelial cells	Autoantigen recognized by serum from myasthenia gravis patients. Binds PKC	Nauert et al. 1997
AKAP-78 (Ezrin)	Epithelial cells	Membrane-cytoskeletal linker protein/microvili biogenesis.	Dansfield et al. 1997
D-AKAP1	Most adult tissues	Binds both RI and RII subunits	Huang et al. 1997
AKAP-15	Skeletal muscle calcium channels	Involved in frequency/voltage-dependent potentiation	Gray et al. 1997

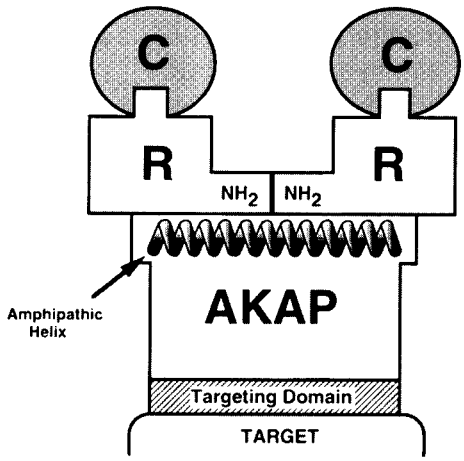

Figure 1. Schematic representation of a hypothetical PKA anchoring complex. The PKA holoenzyme consists of two catalytic subunits (C) bound to two regulatory subunits (R) that are dimerized at their amino terminus (NH_2). The amino terminal portion of dimerized RII binds to an amphipathic helix domain contained in each AKAP. The AKAP/PKA complex is localized to subcellular targets by an AKAP-specific targeting domains.

bind with high affinity to dimerized RII protein only (Figure 1) (Luo et al. 1990, Scott et al. 1990). Recent studies have also identified the specific regions on AKAPs that bind to RII (Obar et al. 1989, Rubino et al. 1989). Although these regions share no significant primary sequence homology, they each consist of single amphipathic helix domains (Carr et al. 1991). In addition to an RII binding domain, each AKAP is proposed to have a unique targeting domain responsible for localizing the AKAP and therefore the type II PKA to specific subcellular structures within the cell. The targeting domains of the AKAPs MAP-2 (Joly et al. 1989), AKAP 75 (Glantz et al. 1993), AKAP 220 (Lester et al. 1996), and AKAP-250 (Nauert et al. 1997) have been identified, and the locations and structures of other AKAP targeting domains are currently under investigation.

Until recently, all AKAPs identified specifically and exclusively bound the type II regulatory subunit (RII) of PKA. Recently, a new class of AKAPs has been identified based on its ability to bind both RII and RI. The first member of this family designated D-AKAP1 (dual specificity AKAP1) was isolated from a yeast two hybrid screen using RI as bait (Huang et al. 1997). Although D-AKAP1 binds RI and RII it appears to bind RI with a 25-fold lower affinity than RII. The discovery of dual specificity AKAPs is intriguing and implies that both RI and RII may be anchored to specific subcellular locations within the cell.

• AKAP 100

Previous studies (Kawano et al. 1992) have proposed that the type II PKA is associated with native sarcoplasmic reticulum vesicles from cardiac muscle. This finding is supported by immunocytochemical data showing that AKAP100 and a significant proportion of the cellular RII pool appear to be localized at the sarcoplasmic reticulum in cardiac muscle (McCartney et al. 1995). In fact, double labeling immunofluorescence experiments (McCartney et al. 1995) suggest that a predominant proportion of RII is targeted to the sarcoplasmic reticulum in cardiac muscle. In light of both observations, it is plausible that AKAP100 functions to adapt PKA for a role in the phosphorylation of proteins in or surrounding the sarcoplasmic reticulum. Although the precise identity of these target substrates remains to be determined, anchoring of kinases close to ion channels is an attractive hypothesis. Of interest is the notion that anchored PKA may function to modulate the calcium release channels in the sarcoplasmic reticulum. The protein phospholamban is another potential target for PKA anchoring in the sarcoplasmic reticulum. Phospholamban plays an important role in controlling the rate of calcium uptake into the sarcoplasmic reticulum via the Ca^{2+}–ATPase and therefore the rate of myocardial relaxation. Phospholamban normally exerts an inhibitory effect on the Ca^{2+} pump. Upon phosphorylation by PKA, however, phospholamban's inhibitory effect on the pump is relieved, leading to an increased rate of Ca^{2+} reuptake.

• Functional Consequences of PKA–AKAP Interactions

A central question has been whether anchoring of PKA to certain subcellular structures facilitates the selective action of the kinase. So far, two experimental strategies have been used: overexpression of AKAPs or RII-binding fragments, and the introduction of peptides that disrupt anchoring. Overexpression of AKAP75 or its human homologue AKAP79 redirects the location of RII and other enzymes to the pheriphery of HEK 293 cells (Klauck et al. 1996, Li et al. 1996). In addition, expression of an untargeted RII-binding fragment called AKAP45 prevents membrane targeting of RIIβ in the thyroid-derived cell line FRTL-5 (Feliciello et al. 1996). The functional consequences of these studies are not yet clear, although there is some evidence that expression of the AKAP45 fragment prevents nuclear accumulation of the C subunit and decreases phosphorylation of the nuclear transcription factor CREB. The latter results suggest a role for anchored PKA in cAMP-mediated transcriptional regulation.

Peptides encompassing the amphipathic helix region of Ht31 (residues 493–515) effectively compete for RII-AKAP interaction in vitro and disrupt the subcellular location of PKA inside cells (Rosenmund et al. 1994, Johnson et al. 1994). As is discussed in detail later in this article, these "anchoring inhibitor peptides" have proven to be valuable in dissecting the actions of PKA in single cells. For example, perfusion of Ht31 peptides into neurons or muscle modifies the activity of two classes of ion channels. These studies suggest that the Ht31 peptide displaces PKA from anchored sites, thereby decreasing the probability of channel phosphorylation. More recently, cell-permeant anchoring inhibitor peptides have been used to demonstrate a role for PKA anchoring in the cAMP responsive attenuation of IL-2 transcription in Jurkat T cells (M. Howard personal communication), and the application of stearated Ht31 peptide analogues has been shown to arrest the motility of mammalian sperm (Vijayaraghavan et al. 1997). The mechanism of PKA action in regulation of these two biological systems is not fully understood, and the specific PKA substrates involved have not been identified.

• Voltage-Dependent Ca^{2+} Channels

Heart muscle contraction is regulated by the influx of Ca^{2+} ions into myocardial cells via L-type voltage-dependent Ca^{2+} channels located in the plasma membrane. Movement of Ca^{2+} ions through these channels is responsible for the plateau phase of the cardiac action potential. These channels are also the target

for the calcium channel blockers, a therapeutically important group of cardiovascular drugs.

Repetitive membrane depolarization of heart cells, as seen during rapid pacing of the heart, leads to a facilitation or potentiation of Ca^{2+} current termed the positive staircase effect, causing increased myocardial contraction. In skeletal muscle, transverse tubules L-type Ca^{2+} channels are thought to initiate muscle contraction by triggering the release of calcium from the sarcoplasmic reticulum through the ryanodine receptor (Catterall 1991). Calcium entering directly through L-type calcium channels, however, is thought to be important in regulating the force of skeletal muscle contraction, even though it is not required for single twitches of skeletal muscle. High-frequency stimulation of skeletal muscle fibers causes potentiation of calcium channel activity, an effect that may play a role in the increased force of muscle contraction during tetanus (Sculptoreanu et al. 1993a).

This frequency- and voltage-dependent potentiation of cardiac and skeletal muscle calcium channel activity has been shown to require phosphorylation by PKA, as a peptide inhibitor of PKA (PKI, 5-24 peptide) blocks experimentally induced potentiation (Sculptoreanu et al. 1993a and b). The role of PKA anchoring in the potentiation of skeletal muscle Ca^{2+} current was examined by Johnson et al. (1994). These authors showed that the introduction of the Ht31 anchoring inhibitor peptide into skeletal muscle myotubes (Johnson et al. 1994) or heterologous cells expressing skeletal muscle Ca^{2+} channel subunits (Johnson et al. 1997) eliminates Ca^{2+} current potentiation, presumably by displacing PKA from an endogenous AKAP. Potentiation could be restored in the presence of Ht31 peptide by adding an excess of purified exogenous catalytic subunit of PKA, indicating that high concentrations of free catalytic subunit of PKA obviate the requirement for anchoring. Furthermore, the potentiation was not inhibited by a proline substituted version of the Ht31 peptide that is devoid of RII-binding activity. These results show that the frequency-dependent potentiation of skeletal muscle Ca^{2+} currents requires the anchoring of PKA near the channel via an AKAP and provides an example demonstrating a possible physiological role for PKA anchoring. It remains to be confirmed that frequency-dependent potentiation of cardiac Ca^{2+} currents can be blocked by AKAP anchoring disruption; however, anchored PKA likely plays an important role in the increase in contractile force of skeletal muscle during tetanus and may contribute to the positive inotropy seen with the "positive staircase" effect in the heart.

Recent biochemical studies have complemented the aforementioned electrophysiological studies. An endogenous protein kinase activity has been shown to copurify with both the cardiac and skeletal muscle L-type Ca^{2+} channel through several chromatographic steps, suggesting that it may be physically linked to the channel (Murphy and Tuana 1989). Recent studies (Gray et al. 1997) have identified this kinase as PKA. Furthermore, Gray et al. (1997) have identified a 15-kD AKAP (AKAP-15) associated with skeletal muscle Ca^{2+} channels. AKAP-15 copurifies with the skeletal muscle Ca^{2+} channel through several chromatographic steps and coimmunoprecipitates with the channels. These results suggest that PKA is physically linked to the calcium channel complex via AKAP-15 (Gray et al. 1997). It remains to be determined whether AKAP-15 binds directly to one of the Ca^{2+} channel subunits or if it is associated with the channel via an intermediary protein.

A recent study by Goa et al. (1997) provides evidence suggesting a potential role of PKA anchoring in the cAMP-dependent modulation of cardiac L-type Ca^{2+} channels. These authors show that the anchoring inhibitory peptide HT-31 diminishes isoproterenol-stimulated Ca^{2+} currents measured in dissociated adult mouse cardiac myocytes. The control peptide HT-31P fails to diminish the stimulation of currents by isoproterenol. These results suggest that PKA anchoring plays a role in adrenergic receptor-mediated stimulation of L-type Ca^{2+} currents in the heart. The authors partially reconstituted this system in human HEK 293 cells by coexpressing the cardiac L-type α subunit and the cardiac β subunit. They were able to obtain forskolin-stimulated Ca^{2+} currents in cells coexpressing AKAP79 but not in cells coexpressing a PKA binding deficient mutant of AKAP79. These results again suggest that anchoring of PKA may play a role in cAMP-dependent modulation of cardiac L-type Ca^{2+} channel currents. AKAP79 is not found in the heart, so it remains to be determined whether a cardiac AKAP that modulates L-type Ca^{2+} channel activity exists. A small molecular mass AKAP similar to AKAP-15 also copurifies with the cardiac Ca^{2+} channel (B. Murphy unpublished data). AKAP-15 and its cardiac counterpart are excellent candidates for mediating the role of anchoring PKA to L-type Ca^{2+} channels in cardiac and skeletal muscle.

• Glutamate Receptor Ion Channels

A physiological role for PKA anchoring has also been suggested for the regulation of α-amino-3-hydroxy-5-methyl-4 isoxazolepropionic acid (AMPA)/kainate channels in hippocampal neurons (Rosenmund et al. 1994). Basal hippocampal (AMPA)/kainate channel currents diminish when the internal contents of cells are dialyzed away during electrophysiological recording from these cells in the whole cell voltage clamp configuration. This rundown of current is prevented by the addition of ATP to the cells, suggesting a role for protein phosphorylation in maintaining basal channel activity. Currents, however, continued to run down in the presence of ATP and PKI, suggesting that basal activity of (AMPA)/kainate channels requires PKA-dependent phosphorylation (Greengard et al. 1991, Wang et al. 1991, Rosenmund et al. 1994). Rosenmund et al. (1994) showed that when the anchoring inhibitor peptide HT-31 or a peptide derived from the RII binding region of AKAP79 was dialyzed into hippocampal cells, the rundown of current was equivalent to that seen when cells were incubated in the presence of PKI. Rundown was, however, prevented in the presence of a 16-amino acid control peptide derived from HT-31 that does not bind RII. These results suggest that anchoring of PKA is required for PKA-dependent maintenance of (AMPA)/kainate currents in hippocampal cells. The specific AKAP responsible for anchoring PKA near the (AMPA)/kainate channel has not been identified.

Recent evidence has been presented suggesting that anchored PKA may also play a role in the regulation of the activity of calcium activated potassium channels (K_{Ca}) measured in excised inside-out patches from tracheal smooth muscle

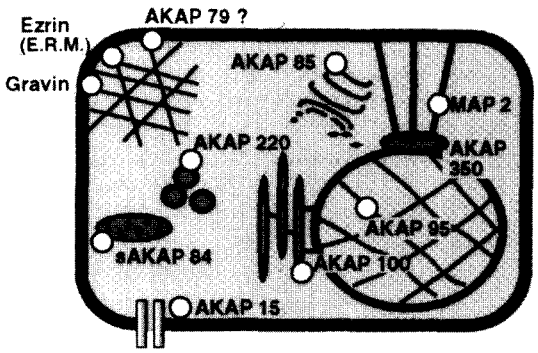

Figure 2. Schematic representation of proposed subcellular distribution of known AKAPs. PKA is targeted near the membrane by association with AKAP79 and ezrin, to the mitochondria through AKAP84, to peroxisomes through AKAP220, to the Golgi through AKAP85, to the cell periphery by gravin, to microtubules through MAP 2, to the endoplasmic reticulum through AKAP100, and to voltage-gated Ca^{2+} channels through AKAP-15.

cells (Wang and Kotlikoff 1996). In the absence of added PKA, the addition of ATP to the cytoplasmic face of the patch augmented K_{Ca} currents, suggesting that a protein kinase was associated with the excised patch. The protein kinase involved was PKA, as PKI blocked the increase in K_{Ca} currents seen the presence of ATP (Wang and Kotlikoff 1996). Application of the Ht31 anchoring inhibitor peptide blocked the stimulatory effect of ATP, suggesting that the PKA responsible for modulation of the smooth muscle K_{Ca} channel remains attached to the excised patches via an AKAP (Wang and Kotlikoff 1996).

• Conclusion

Increasing evidence suggests that the subcellular location of PKA is an important determinant in its specificity of action. The goal of this minireview has been to highlight some of the recent advances that suggest A-Kinase Anchoring Proteins (AKAPs) contribute to this process, targeting the kinase to specific subcellular compartments. The concept of PKA compartmentalization is a relatively new idea and most of the research emphasis to date has focused on a concerted effort to clone and characterize the AKAPs. This information has been incorporated into Table 1 and Figure 2, which list the known AKAPs. These proteins have been designated AKAPs on the basis of their ability to bind RII *in vitro*, although it has been surprising to find that there is no overall sequence similarity between these molecules. It would appear that the AKAPs represent a convergent group of proteins sharing a common RII-binding motif. The cloning of additional AKAPs should provide a more complete database of sequences and will establish whether gene families of anchoring proteins exist.

Information about the common RII-binding domain has enabled the development of peptides that disrupt the localization of PKA inside cells. Despite their limitations, the use of anchoring inhibitor peptides inside cells has provided the most compelling evidence to support a role for PKA anchoring in the modulation of cAMP responsive events. As the specificity of these anchoring inhibitor reagents increases, it is hoped that the mislocalization of individual AKAP signaling complexes will have site-selective effects on PKA phosphorylation events.

Although the principal function of the AKAPs is undoubtedly to target PKA, a fascinating new aspect has been the discovery of anchoring proteins such as AKAP79 and AKAP250/gravin, which simultaneously bind more than one signaling enzyme (Coghlan et al. 1995b, Klauck et al. 1996, Nauert et al. 1997). It is thought that these multivalent AKAPs serve as scaffolds for the assembly of signaling complexes consisting of several kinases and phosphatases. This is an appealing variation on the anchoring theme, as it provides a model for reversible phosphorylation in which the opposing effects of kinase and phosphatase action are colocalized in a multiprotein transduction complex (Coghlan et al. 1995a). Future studies will no doubt focus on the potential roles of these AKAP-mediated scaffolds in the modulation of compartmentalized signaling events.

• Acknowledgments

The authors' work is supported by an American Heart Association Research Grant (B.J.M.) and by DK44239 (J.D.S.).

The authors thank Peter Gray for helpful discussion and Victoria Tibbs for assistance in compiling Table 1 and for design of Figure 1.

References

Adams SR, Harootunian AT, Buechler YJ, Taylor SS, Tsien RY: 1991. Fluorescence ratio imaging of cyclic AMP in single cells. Nature 349:694–697.

Bregman DB, Bhattacharyya N, Rubin CS: 1989. High affinity binding protein for the regulatory subunit of cAMP-dependent protein kinase II-B. J Biol Chem 264:4648–4656.

Carr DW, Scott JD: 1992. Blotting and band-shifting: techniques for studying protein-protein interactions. Trends Biochem Sci 17:246–249.

Carr DW, Stofko-Hahn RE, Fraser ID, et al.: 1991. Interaction of the regulatory subunit (RII) of cAMP-dependent protein kinase with RII-anchoring proteins occurs through an amphipathic helix binding motif. J Biol Chem 266:14,188–14,192.

Carr DW, Hausken ZE, Fraser ID, Stofko-Hahn RE, Scott JD: 1992a. Association of the type II cAMP-dependent protein kinase with a human thyroid RII-anchoring protein: cloning and characterization of the RII-binding domain. J Biol Chem 267:13, 376–13,382.

Carr DW, Stofko-Hahn RE, Fraser ID, Cone RD, Scott JD: 1992b. Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins: characterization of AKAP79. J Biol Chem 267:16,816–16,823.

Carr DW, DeManno DA, Atwood A, Hunzicker-Dunn M, Scott JD: 1993. Follicle-stimulating hormone regulation of A-kinase anchoring proteins in granulosa cells. J Biol Chem 268:20,729–20,732.

Carrera A, Gerton GL, Moss SB: 1994. The major fibrous sheath polypeptide of mouse sperm: structural and functional similarities to the A-kinase anchoring proteins. Dev Biol 165:272–284.

Carrera A, Moos J, Ning XP, et al.: 1996. Regulation of protein tyrosine phosphorylation in human sperm by a calcium/calmodulin-dependent mechanism: identification of A kinase anchor proteins as major substrates for tyrosine phosphorylation. Dev Biol 180:284–296.

Catterall WA: 1991. Excitation–contraction coupling in vertebrate skeletal muscle: a tale of two calcium channels. Cell 64:871–874.

Cheley S, Panchal RG, Carr DW, Scott JD, Bayley H: 1994. Type II regulatory subunits of cAMP-dependent protein kinase and their binding proteins in the nervous system of *Aplysia californica*. J Biol Chem 269:2911–2920.

Coghlan VM, Langeberg LK, Fernandez A, Lamb NJ, Scott JD: 1994. Cloning and characterization of AKAP 95, a nuclear protein that associates with the regulatory subunit of type II cAMP-dependent protein kinase. J Biol Chem 269:7658–7665.

Coghlan VM, Lester L, Scott JD: 1995a. A targeting model for reversible phosphorylation. Adv Prot Phosphatases 6:51–61.

Coghlan VM, Perrino BA, Howard M, et al.: 1995b. Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. Science 267:108–111.

Cohen P: 1989. The structure and regulation of protein phosphatases. Annu Rev Biochem 58:453–508.

Dransfield DT, Bradford AJ, Smith J, et al.: 1997. Ezrin is a cyclic AMP-dependent protein kinase anchoring protein. EMBO 16: 35–43.

Faux MC, Scott JD: 1996a. Molecular glue: kinase anchoring and scaffold proteins. Cell 85:9–12.

Faux MC, Scott JD: 1996b. More on target with protein phosphorylation: conferring specificity by location. Trends Biochem Sci 21:312–315.

Feliciello A, Giuliano P, Porcellini A, et al.: 1996. The v-Ki-Ras oncogene alters cAMP nuclear signaling by regulating the location and the expression of cAMP-dependent protein kinase II beta. J Biol Chem. 271:26,350–25,359.

Gao T, Yatani A, Dell'Acqua ML, et al.: 1997. cAMP-dependent regulation of cardiac L-type Ca^{2+} channels requires membrane targeting of PKA and phosphorylation of channel subunits. Neuron 19:185–196.

Glantz SB, Li Y, Rubin CS: 1993. Characterization of distinct tethering and intracellular targeting domains in AKAP75, a protein that links cAMP-dependent protein kinase II beta to the cytoskeleton. J Biol Chem 268:12,796–12,804.

Gray PC, Tibbs VC, Catterall WA, Murphy BJ: 1997. Identification of a 15-kD cAMP-dependent protein kinase-anchoring protein associated with skeletal muscle L-type calcium channels. J Biol Chem 272:6297–6302.

Greengard P, Jen J, Nairn AC, Stevens CF: 1991. Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. Science 253: 1135–1138.

Harper JF, Haddox MK, Johanson RA, Hanley RM, Steiner AL: 1985. Compartmentation of second messenger action: immunocytochemical and biochemical evidence. Vitam Horm 42:197–252.

Hempel CM, Vincent P, Adams SR, Tsien RY, Selverston AI: 1996. Spatio-temporal dynamics of cyclis AMP signals in an intact neural circuitm [see comments]. Nature 384:166–169.

Hirsch AH, Glantz SB, Li Y, You Y, Rubin CS: 1992. Cloning and expression of an intronless gene for AKAP 75, an anchor protein for the regulatory subunit of cAMP-dependent protein kinase II beta. J Biol Chem 267:2131–2134.

Horowitz JA, Wasco W, Leiser M, Orr GA: 1988. Interaction of the regulatory subunit of a type II cAMP-dependent protein kinase with mammalian sperm flagellum. J Biol Chem 263:2098–2104.

Huang LJ-S, Durick K, Weiner JA, Chun J, Taylor SS: 1997. Identification of a novel protein kinase A anchoring protein that binds both type I and type II regulatory subunits. J Biol Chem 272:8057–8064.

Hubbard MJ, Cohen P: 1993. On target with a new mechanism for the regulation of protein phosphorylation. Trends Biochem Sci 18(5): 172–177.

Joachim S, Schwoch G: 1990. Localization of cAMP-dependent protein kinase subunits along the secretory pathway in pancreatic and parotid acinar cells and accumulation of the catalytic subunit in parotid secretory granules following beta-adrenergic stimulation. Eur J Cell Biol 51:76–84.

Johnson BD, Scheuer T, Catterall WA: 1994. Voltage-dependent potentiation of L-type Ca^{2+} channels in skeletal muscle cells requires anchored cAMP-dependent protein kinase. Proc Natl Acad Sci USA 91:11,492–11,496.

Johnson BD, Brousal JP, Peterson BZ, et al.: 1997. Modulation of the cloned skeletal muscle L-type Calcium channel by anchored cAMP-dependent protein kinase. J Neurosci 17:1243–1255.

Joly JC, Flynn G, Purich DL: 1989. The microtubule-binding fragment of microtubule-associated protein-2: location of the protease-accessible site and identification of an assembly-promoting peptide. J Cell Biol 109:2289–2294.

Jurevicius J, Fischmeister R: 1996. cAMP compartmentation is responsible for a local activation of cardiac Ca^{2+} channels by beta-adrenergic agonists. Proc Natl Acad Sci USA 93:295–299.

Kawano S, Nakamura F, Tanaka T, Hiraoka M: 1992. Cardiac sarcoplasmic reticulum chloride channels regulated by protein kinase A. Circ Res 71:585–589.

Keryer G, Rios RM, Landmark BF, Skalhegg B, Lohmann SM, Bornens M: 1993. A high-affinity binding protein for the regulatory subunit of cAMP-dependent protein kinase II in the centrosome of human cells. Exp Cell Res 204:230–240.

Klauck TM, Faux MC, Labudda LK, Langeberg LK, Jaken S, Scott JD: 1996. Coordination of three signaling enzymes by AKAP79, a mammalian scaffold protein. Science 271: 1589–1592.

Krebs EG: 1985. The phosphorylation of proteins: a major mechanism for biological regulation: fourteenth Sir Frederick Gowland Hopkins memorial lecture. Biochem Soc Trans 13:813–820.

Lester LB, Coghlan VM, Nauert B, Scott JD: 1996. Cloning and characterization of a novel A-kinase anchoring protein. AKAP 220, association with testicular peroxisomes. J Biol Chem 271:9460–9465.

Li Y, Ndubuka C, Rubin CS: 1996. A kinase anchor protein 75 targets regulatory (RII) subunits of cAMP-dependent protein kinase II to the cortical actin cytoskeleton in non-neuronal cells. J Biol Chem 271:16, 862–16,869.

Lin RY, Moss SB, Rubin CS: 1995. Characterization of S-AKAP84, a novel developmentally regulated A kinase anchor protein of male germ cells. J Biol Chem 270:27,804–27,811.

Livesey SA, Kemp BE, Re CA, Partridge NC, Martin TJ: 1982. Selective hormonal activation of cyclic AMP-dependent protein kinase isoenzymes in normal and malignant osteoblasts. J Biol Chem 257:14,983–14,987.

Lohmann SM, DeCamilli P, Einig I, Walter U: 1984. High-affinity binding of the regulatory subunit (RII) of cAMP-dependent protein kinase to microtubule-associated and other cellular proteins. Proc Natl Acad Sci USA 81:6723–6727.

Luo Z, Shafit Zagardo B, Erlichman J: 1990. Identification of the MAP2- and P75-binding domain in the regulatory subunit (RII beta) of type II cAMP-dependent protein kinase: cloning and expression of the cDNA for bovine brain RII beta. J Biol Chem 265:21,804–21,810.

McCartney S, Little BM, Langeberg LK, Scott JD: 1995. Cloning and characterization of A-kinase anchor protein 100 (AKAP100): a protein that targets A-kinase to the sarcoplasmic reticulum. J Biol Chem 270:9327–9333.

Murphy BJ, Tuana BS: 1989. Phosphorylation and the identification of a protein kinase activity associated with the dihydropyridine receptor isolated from rabbit heart and skeletal muscle. Ann Rev NY Acad Sci 560:391–394.

Nauert JB, Klauck TM, Langeberg LK, Scott JD: 1997. Gravin, an autoantigen recognized by serum from myasthenia gravis patients, is a kinase scaffold protein. Curr Biol 7:52–62.

Obar RA, Dingus J, Bayley H, Vallee RB: 1989. The RII subunit of cAMP-dependent protein kinase binds to a common amino-terminal domain in microtubule-associated proteins 2A, 2B, and 2C. Neuron 3:639–645.

Pariset C, Weinman S: 1994. Differential localization of two isoforms of the regulatory subunit RII alpha of cAMP-dependent protein kinase in human sperm: biochemical and cytochemical study. Mol Reprod Dev 39:415–422.

Rios RM, Celati C, Lohmann SM, Bornens M, Keryer G: 1992. Identification of a high affinity binding protein for the regulatory subunit RII beta of cAMP-dependent protein kinase in Golgi enriched membranes of human lymphoblasts. EMBO J 11:1723–1731.

Rosenmund C, Carr DW, Bergeson SE, Nilaver G, Scott JD, Westbrook GL: 1994. Anchoring of protein kinase A is required for modulation of AMPA/kainate receptors on hippocampal neurons. Nature 368:853–856.

Rubin CS, Rangel Aldao R, Sarkar D, Erlichman J, Fleischer N: 1979. Characterization and comparison of membrane-associated and cytosolic cAMP-dependent protein kinases: physicochemical and immunological studies on bovine cerebral cortex protein kinases. J Biol Chem 254: 3797–3805.

Rubino HM, Dammerman M, Shafit-Zagardo B, Erlichman-J: 1989. Localization and characterization of the binding site for the regulatory subunit of type II cAMP-dependent protein kinase on MAP2. Neuron 3:631–638.

Salvatori S, Damiani E, Barhanin J, Furlan S, Salviati G, Margreth A: 1990. Co-localization of the dihydropyridine receptor and the cyclic AMP-binding subunit of an intrinsic protein kinase to the junctional membrane of the transverse tubules of skeletal muscle. Biochem J 267:679–687.

Sarkar D, Erlichman J, Rubin CS: 1984. Identification of a calmodulin-binding protein that co-purifies with the regulatory subunit of brain protein kinase II. J Biol Chem 259:9840–9846.

Scott JD, Stofko RE, McDonald JR, Comer JD, Vitalis EA, Mangili JA: 1990. Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. J Biol Chem 265:21,561–21,566.

Sculptoreanu A, Rotman E, Takahashi M, Scheuer T, Catterall WA: 1993a. Voltage-dependent potentiation of the activity of cardiac L-type calcium channel alpha 1 subunits due to phosphorylation by cAMP-dependent protein kinase. Proc Natl Acad Sci USA 90:10,135–10,139.

Sculptoreanu A, Scheuer T, Catterall WA: 1993b. Voltage-dependent potentiation of L-type Ca^{2+} channels due to phosphorylation by cAMP-dependent protein kinase. Nature 364:240–243.

Taylor SS: 1989. cAMP-dependent protein kinase: model for an enzyme family. J Biol Chem 264:8443–8446.

Trendelenburg G, Hummel M, Riecken EO, Hanski C: 1996. Molecular characterization of AKAP149, a novel A kinase anchor protein with a KH domain. Biochem Biophys Res Commun 225:313–319.

Vijayaraghavan S, Goueli SA, Davey MP, Carr DW: 1997. PKA anchoring inhibitor peptides arrest mammalian sperm motility. J Biol Chem 272:4747–4752.

Wang LY, Salter MW, MacDonald JF: 1991. Regulation of kainate receptors by cAMP-dependent protein kinase and phosphatases. Science 253:1132–1135.

Wang ZW, Kotlikoff MI: 1996. Activation of KCa channels in airway smooth muscle cells by endogenous protein kinase A. Am J Physiol 271:L100–105.

PII S1050-1738(97)00131-X **TCM**