A-Kinase Anchoring Proteins

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The precise transmission of information from a plasma membrane receptor to the downstream target inside the cell is essential for the control of dynamic cellular functions. It has been proposed that the coordination of signaling pathways inside cells is achieved, in part, by the localization of signaling enzymes such as kinases and phosphatases near their intended protein substrates. The anchoring of these enzymes into signaling scaffolds facilitates the phosphorylation state of specific proteins at appropriate time and place.

Protein Phosphorylation

Protein phosphorylation is a predominant form of covalent modification of proteins inside cells. This bidirectional process, catalysed by protein kinases and reversed by phosphoprotein phosphatases, provides a flexible means of influencing the proteins that control cellular metabolism, transcription, division, and movement. The utility of this regulatory mechanism is underscored by evidence that ~30% of intracellular proteins are phosphoproteins.

Protein Kinase A

One well-studied "protein phosphorylation pathway" is regulated by the second messenger cAMP. When extracellular messengers bind to heptahelical receptors on the surface of the cell and recruit heterotrimeric G proteins to activate adenylyl cyclases on the inner face of the plasma membrane cAMP synthesis is triggered. This newly synthesized "second messenger" then diffuses to its sites of action. Although cAMP can modulate a few classes of signaling molecules, the predominant intracellular receptors are cAMP dependent protein kinases (PKA). The PKA holoenzyme is composed of two catalytic (C) subunits that are held in an inactive state by association with a regulatory (R) subunit dimer. The C subunits are expressed from three genes; Cα, Cβ, and Cγ, whereas the R subunits are transcribed from four genes; RIα, RIβ, RIIα, and RIIβ. The type I PKA (composed of RI dimers) is predominantly cytoplasmic and is most highly expressed in the immune system, whereas type II PKA (composed of RII dimers) associates with cellular structures and organelles and is abundant in the heart and brain.

Phosphorylation Specificity

One unresolved issue in cAMP signaling is the question of how this commonly used pathway is able to selectively regulate so many different cellular processes. For that reason, the mechanism by which PKA discriminates among its substrates is a topic of considerable interest. One hypothesis proposes that specific pools of the kinase are compartmentalized within the cell and are activated in close proximity to particular substrates. This can only occur if there is a means to both (1) selectively control the level of subcellular pools of cAMP and (2) maintain PKA in these environments. It has been proposed that a balance between adenylyl cyclase and phosphodiesterase activities leads to the establishment of intracellular gradients of cAMP. An equally important component of this model requires "scaffolding" proteins called "A-kinase anchoring proteins" that keep the kinase in close proximity to its substrates. This article will discuss the compartmentalization of the protein kinase A and other enzymes through their association with A-kinase anchoring proteins (AKAPs).

The PKA Anchoring Hypothesis

The first AKAPs that were identified remained tightly associated with the type II R subunits during purification from tissues and were therefore designated "RII-binding proteins". Over 30 AKAPs have now been identified and are recognized as a family of diverse proteins that are classified on the basis of their interaction with the PKA inside cells. It has also become apparent that most AKAPs share some other common properties (Figure 1). These include a common R subunit binding sequence (Figure 1A), localization regions that target the PKA/AKAP complex to precise intracellular environments (Figure 1B) and binding sites for other enzymes to form signaling complexes (Figure 1C). Each property is discussed here.

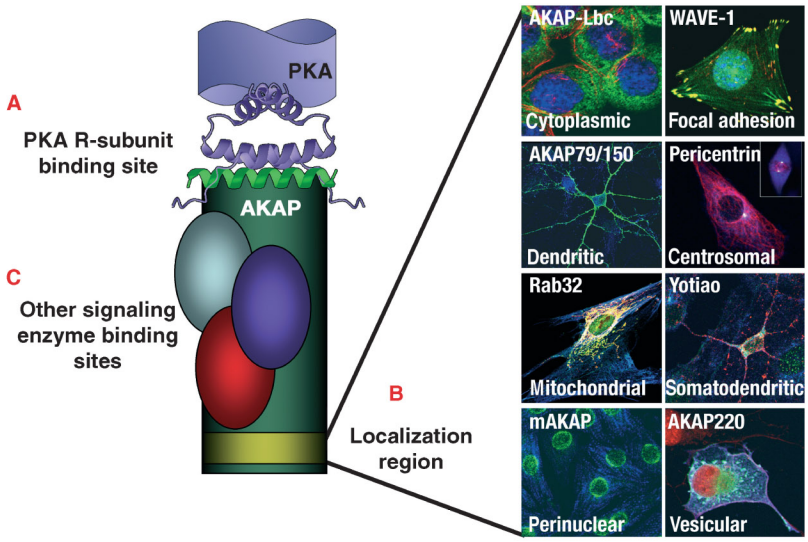

FIGURE 1 Properties of A-kinase anchoring proteins (A) A common identifying characteristic of AKAPs is a protein–protein interaction site that binds to protein kinase A. The amphipathic helix of the AKAP (green) tightly associates with the regulatory subunit amino terminal dimer of PKA (purple). (B) The localization region of the AKAP (yellow) is responsible for the precise subcellular targeting of the scaffold. Here we show the subcellular staining pattern for eight different AKAPs (in green, or yellow and white when colocalized with other stained proteins) found at distinct locations within the cell. (C) Multiple signaling enzymes associate with an AKAP via protein–protein interactions, nucleating a unique signaling complex for efficient signal transmission.

Protein Kinase A Binding

RII Subunit Binding Sequences

Most AKAPs contain a short sequence that forms a binding site for the R subunit dimmer and was first recognized in the human thyroid anchoring protein, AKAP-lbc. The region likely forms an amphipathic helix that slots into a binding pocket formed by the amino terminal regions of RII. This view is supported by evidence that a 24-amino acid peptide encompassing this region, called Ht31, binds RII with low nanomolar affinity. Cellular delivery of this peptide antagonizes PKA anchoring and has become a standard means to delineate a role for AKAPs in the coordination of cAMP-responsive events. Recently, a new and improved anchoring inhibitor peptide has been developed from a comprehensive analysis of 10 AKAP sequences. This 17-amino acid peptide, called "AKAP-is", selectively binds RII with subnanomolar affinity, efficiently disrupts PKA anchoring inside cells, and functions to block cAMP signaling to glutamate receptor ion channels in cells.

RI Subunit Binding

Although most AKAPs associate with the type II PKA, it is now clear that some anchoring proteins also target the type I kinase. Yeast two-hybrid screening and affinity purification techniques have identified anchoring proteins that can interact with either RI or RII (designated dual function AKAPs) and, in a few instances, RI selective AKAPs have been reported. Apparently, RI anchoring also proceeds through an amphipathic helix although other determinants may contribute to the compartmentalization of the type I PKA holoenzyme. Recently, a single nucleotide polymorphism (SNP) that causes a valine to isoleucine mutation in the anchoring helix of D-AKAP-2 has been shown to increase RI-binding affinity threefold. Although the functional ramifications of this valine to isoleucine change are unclear it is more prevalent in the aging population. Detailed structural analyses will be necessary to define the differences between type I and type II PKA anchoring.

Anchoring Protein Targeting Regions

While protein–protein interactions are responsible for the precise orientation of the kinase toward its substrates, it appears that protein–lipid interactions target the AKAP–PKA complex to the correct intracellular membranes and organelles. In the brain for example, repeat sequences that bind negatively charged phospholipids tether an AKAP called AKAP79/150 to the inner surface of synaptic membranes. In addition, protein–protein interactions with SAP97, an adapter protein that binds to the cytoplasmic tail of the AMPA receptor ion channel, places the AKAP79/150-PKA

complex in the vicinity of substrates. This elaborate molecular bridging facilitates the PKA phosphorylation of serine 845 in the cytoplasmic tail of GluR1. Serine 845 is an important regulatory site on the channel that is modified during chemically induced long term potentiation (LTP). The AKAP79/150 complex also includes the phosphatase PP2B which functions to dephosphorylate serine 845 leading to attenuation of GluR1 channels. In fact, peptide disruption of the PP2B-AKAP79/150 interaction prevents efficient dephosphorylation of the channel and suggests that targeting of the phosphatase with its substrate is necessary for modulation of channel activity. In a similar manner myristoylation and palmitoylation signals facilitate the protein–lipid tethering of another AKAP, AKAP15/18 in close proximity to PKA substrates such as calcium channels and sodium channels. AKAP15/18 may also be cross-linked to the α_1-subunit of the L type Ca^{2+} channel via a modified leucine zipper motif.

There are instances where multiple AKAPs mediate PKA targeting to the same organelle. Three anchoring proteins (D-AKAP-1/AKAP149, D-AKAP-2, and Rab32) anchor PKA at mitochondria, two AKAPs (AKAP350-450/CG-NAP and pericentrin) tether the kinase to centrosomes whereas Ezrin, WAVE-1 and AKAP-lbc tether PKA to distinct areas of the actin cytoskeleton. One explanation for these apparent redundancies may be the need to always maintain an anchored pool of PKA at certain sites. Alternatively, each compartment specific AKAP may direct the kinase to different microenvironments where specific substrates reside. Thus, compartmentalization of PKA is likely to be a more finely organized process than was initially appreciated.

Scaffolding Complexes

Multiple Enzyme Pathways

Perhaps the most important feature of AKAPs is their ability to simultaneously interact with several signaling proteins (Figure 1C). By localizing PKA with enzymes such as protein phosphatases, phosphodiesterases, G proteins, and other protein kinases, AKAPs provide focal points for the integration and processing of distinct intracellular signals. The notion was first proposed for the AKAP79/150 family, which maintains PKA, protein kinase C (PKC) and the phosphatase PP2B at the synaptic membrane. Subsequently, it has been shown that most, if not all, AKAPs nucleate signaling protein networks. For example, Yotiao, AKAP220, and AKAP149, tether protein phosphatase 1 to oppose the action of anchored kinases. This creates an environment where protein phosphorylation is only favored when kinase activity is sufficiently stimulated to overcome these basal dephosphorylation events. One variation on this theme occurs when signal termination enzymes that act upstream of protein kinases are recruited to AKAP signaling complexes. For example, AKAP450 and mAKAP co-localize a cAMP-metabolizing enzyme, the phosphodiesterase PDE4D3, with PKA. This creates a local environment where PDE activity reduces cAMP levels in the vicinity of the kinase. Presumably, these signaling complexes not only contribute to the formation of intracellular gradients of cAMP but also confer temporal control on PKA activation by generating pulses of kinase activity.

Parallel Signaling Pathways

Other AKAP complexes are known to participate in the parallel processing of distinct intracellular signals. In the brain WAVE-1 is a scaffolding protein that principally functions to relay signals from the plasma membrane via the small molecular weight GTPase Rac to the Arp2/3 complex, a group of seven related proteins that nucleate actin polymerization and branching to facilitate remodeling events in the cytoskeleton. However, WAVE-1 also anchors PKA and binds to the Abelson tyrosine kinase (Abl). Proteomic approaches have identified additional binding partners that are positive and negative regulators of WAVE-1 function and substrates for either kinase. Thus WAVE-1 is capable of recruiting different combinations of signaling enzymes to the neuronal cytoskeleton for control of distinct protein phosphorylation and actin-remodeling events. This multifaceted role is reflected in the complex phenotype of WAVE-1 knockout mice which exhibit abnormalities in their brain morphology as well as behavioral defects that may be linked to altered signaling in the cerebellum or hippocampus.

Alternatively Spliced Scaffolds

The AKAP350/450/CG-NAP/Yotiao family arise from alternative splicing of a single gene on chromosome 7q21. At least four anchoring proteins that are targeted to three distinct subcellular locations are transcribed from this gene. Initially a cDNA was isolated that encodes a 350 kDa protein believed to be a high molecular weight AKAP previously identified in centrosomal fractions. Around the same time variants encoding AKAP450 and CG-NAP were identified. The latter protein was named CG-NAP on the basis of its detection in centrosomal and Golgi fractions. Detailed analysis of CG-NAP has identified additional binding partners that include protein phosphatase 2A, the Rho-dependent-protein kinase PKN and the protein kinase C epsilon isoform. Functional studies propose that enzymes in this signaling complex may participate in membrane trafficking, microtubule nucleation and/or cell cycle progression.

Yotiao, the shortest splice variant in this family is targeted to synaptic sites where it anchors PKA and the type 1 protein phosphatase PP1 to regulate the phosphorylation state of NMDA receptor ion channels. Tonic PP1 activity negatively regulates NMDA receptors by favoring the dephosphorylated state. However, upon PKA activation the PP1 activity is overcome and the channel is phosphorylated resulting in increased NMDA receptor currents. More recently a requirement for yotiao targeting of PKA and PP1 to GABA(A) receptors at inhibitory synapses has been demonstrated in the dopaminergic regulation of cognitive processes. Thus, AKAP350/450, CG-NAP and yotiao organize PKA and a plethora of signaling enzymes in a variety of subcellular locations. Transcriptional regulation is undoubtedly a critical determinant for location and composition of each signaling complex maintained by these AKAP gene products.

Understanding the Function of Scaffolds

An emerging area of investigation is the genetic manipulation of AKAPs. Although several anchoring proteins have been identified in genetically tractable organisms including *C. elegans*, *D. melanogaster*, and *D. rerio* (zebrafish) the most significant advances have come from the characterization of genetically modified mice. Genetic disruption of the MAP2 gene causes a redistribution of the PKA holoenzyme in neurons that limits certain cAMP responsive phosphorylation events and causes reduction in dendrite length. In a similar manner disruption of the WAVE-1 gene has apparent effects on brain morphology and effects complex neuronal behaviors including coordination, balance, learning, and memory. These observations complement previous evidence that ablation of PKA subunit genes alters hippocampal-based forms of learning and memory.

Traditionally, these anchoring molecules were thought to exclusively control cAMP responsive events. However AKAP-mediated compartmentalization of other signaling enzymes may be an equally important function. Recent reports have implicated AKAP350/CG-NAP, AKAP220 and WAVE-1 networks in the control of Rho kinase signaling, glycogen synthase kinase 3 action and Rac mediated actin remodeling respectively. As the detailed dissection of these AKAP signaling complexes progresses it seems probable that their role in the coordination of both cAMP dependent and independent signaling events will become more evident.

Glossary

cytoskeleton The complex network of actin microtubules and microfilaments in the cytoplasm that provide structure to the cytoplasm of the cell and plays an important role in cell movement and maintaining the characteristic shape of the cells.

phosphorylation/dephosphorylation The activation of an enzyme by the addition of a phosphate group to the enzyme or the inactivation of an enzyme by the removal of a phosphate group from the enzyme.

protein kinase The enzyme that catalyzes the transfer of phosphate from ATP to the hydroxyl side chains of a protein causing changes in the function of the protein.

protein phosphatase An enzyme that catalyzes the removal of phosphate from a phosphorylated protein, thereby causing changes in the function of the protein.

receptor Proteins located either on the cell surface or within the cytoplasm that bind ligand, initiating signal transduction and cellular activity.

scaffold A protein that serves as a platform to bring together a unique assortment of signaling enzymes for the efficient transmission of intracellular messages.

Further Reading

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