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Minireview

AKAP79 and the evolution of the AKAP model

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Abstract A molecular explanation for the specificity of the cAMP-dependent protein kinase (PKA) can be provided by its compartmentalization through association with A-kinase-anchoring proteins (AKAPs). Structural and functional studies have led to the development of an anchoring model proposing that AKAPs contain a common PKA binding domain and a unique subcellular targeting domain. The discovery that AKAPs can bind other signaling enzymes led to the addition of a third property, that of scaffolding molecule. Recent research has now expanded the role of AKAPs to members of multiunit complexes containing both upstream activators and downstream targets.

1. Introduction

Some of the best-understood signal transduction pathways involve the generation of cAMP and the activation of the cAMP-dependent protein kinase (PKA). Work over the past 40 years has defined numerous transmembrane receptors, heterotrimeric G proteins and adenylyl cyclases [1–3]. These molecules combine to form molecular relay systems that convert signals transmitted by hormones into the generation of the intracellular second messenger cAMP. Once generated, cAMP activates PKA. A tremendous research emphasis to biochemically characterize the PKA holoenzyme has resulted in the understanding of its mechanism of catalysis [4], the identification of three catalytic subunit and four regulatory subunit genes [5–7], and the resolution of the crystal structures of certain R and C subunits [8,9].

Unfortunately, one aspect of PKA action that has remained elusive is an explanation of how an enzyme with such a broad range of substrate specificity is able to mediate specific phosphorylation events at particular sites within the cell. Subcellular targeting of PKA via the association with A-kinase-anchoring proteins (AKAPs) has emerged as an important mechanism by which PKA is maintained with distinct subcellular targets [10]. PKA anchoring not only maintains the kinase at its site of action, but also limits access to a subset of physiological substrates [11]. The discovery that some AKAPs not only bind PKA but also anchor protein phosphatases has broadened our view of these molecules. The identification of anchored kinase/phosphatase units suggests that some AKAPs serve as scaffolding proteins to localize enzymes that coordinate the phosphorylation status of certain cellular substrates [12]. In the past year, a new concept of AKAP signaling networks has emerged from findings that certain AKAPs may themselves be recruited into much larger multiunit signaling complexes comprising of both upstream activators and downstream targets [13,14]. Understanding these complex signaling networks will undoubtedly add to our knowledge of the specificity and regulation of PKA action.

There are 70 AKAP sequences currently known and this family of proteins represents a functionally related group of molecules that serve to localize kinases and phosphatases (reviewed by [10,15]). In order to highlight progress on this topic, we have chosen to focus on the AKAP79/150 family. This is a family consisting of three orthologs; bovine AKAP75, first identified as a contaminant of PKA holoenzyme preparations [16]; murine AKAP150, a fragment of which was isolated by interaction cloning using RII as a probe [17], and human AKAP79, the prototypic member of the family that was originally identified as a component of the postsynaptic densities in neurons [18]. All three proteins are highly related and differ only in their molecular weights, which is predominantly a consequence of repeat sequences present in the larger AKAP150. Since most of the work has been conducted with AKAP79, it will be used as an example to demonstrate the complexity and importance of AKAPs in the regulation of cellular events.

2. Development of the PKA anchoring model: anchoring and targeting

Studies in the 1970s utilized cAMP binding assays of various subcellular fractions to show that the PKA holoenzyme containing the type I regulatory subunit (RIα or RIβ) is predominantly cytoplasmic, whereas the majority of the type II PKA holoenzyme containing the type II regulatory subunit (RIIα or RIIβ) is associated with cellular structures and organelles [19,20]. Later reports described the microtubule-associated protein MAP2 and AKAP75 as the first AKAPs [16,21]. In both cases, these molecules were originally identified as contaminants of type II PKA holoenzyme preparations which associated with RII. By the 1990s, structure/function analysis of AKAPs had demonstrated that only the amino-terminal regions of the RII dimer were necessary for interaction with MAP2 or AKAP75 [22,23]. These findings were consolidated into the original AKAP model which proposed that each anchoring protein contained two motifs: a PKA targeting domain and a unique subcellular targeting domain [24]. The conserved PKA binding domain consists of an amphipathic helix which fits into a hydrophobic pocket formed by the extreme N-terminus of the regulatory subunit [24–26]. In fact, peptides mimicking this amphipathic helix serve as

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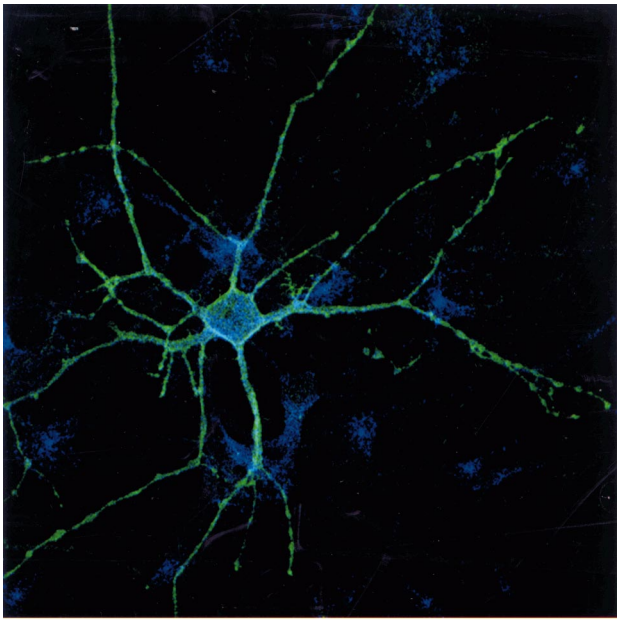

Fig. 1. Subcellular localization of AKAP79 in neurons. The subcellular distribution of AKAP79 at the plasma membrane in neurons is illustrated. AKAP79 was visualized by immunofluorescent labeling using antibodies specific for AKAP79 (green) and the RII subunit of PKA (blue).

efficient anchoring inhibitor reagents that compete for RII–AKAP interaction in vitro and inside cells [26,27]. These peptides, generically termed Ht31, have been used widely to demonstrate the functional implications of PKA anchoring as it relates to a growing number of PKA responsive events [10]. The first functional use of the peptides was by Rosenmund et al. who reported that intracellular infusion of Ht31 in cultured hippocampal neurons prevented the PKA-mediated regulation of AMPA/kainate currents [27]. These results highlighted a role for PKA anchoring in the regulation of synaptic signaling events. Although it was unclear at that time which PKA anchoring event was disrupted by the Ht31 peptides, the subcellular distribution of AKAP79 and its colocalization with these ion channels made it a prime candidate (Fig. 1).

As stated above, each anchoring protein contains a unique domain responsible for targeting of the AKAP–PKA complex [10]. Three N-terminal polybasic sequences in AKAP79 mediate binding to acidic phospholipids and, thus, attachment to membranes, including postsynaptic sites [28]. Analysis of these domains identified phosphorylation sites for PKA and PKC. Furthermore, activation of PKC was shown to release AKAP79 from membrane particulate fractions. These findings inferred that AKAP79 association with the plasma membrane may be regulated by phosphorylation, although this has not been fully established to date. Recent evidence found that AKAP79 is selectively retained at the post-synaptic densities in neurons, suggesting that the subcellular localization of AKAP79 is more complex than originally proposed [14]. Thus, additional targeting determinants that involve protein–protein interactions are likely to facilitate the precise subcellular location of AKAP79 in neurons.

3. The third property: kinase/phosphatase scaffolding units

Although AKAPs are defined by their ability to bind PKA, continuing studies suggest that an equally important function may be to bind other enzymes (Fig. 2). This provides a mechanism for the formation of multicomponent signaling complexes and forces an amendment of the original AKAP model to incorporate this property. Coghlan et al. initially demonstrated that the phosphatase calcineurin (PP2B) was an AKAP79 binding partner through a yeast-2-hybrid screen [29]. The PP2B binding site was mapped to the C-terminal region of AKAP79 to a fragment encompassing 108–427 of the anchoring protein [28]. Interestingly, the association of PP2B with AKAP79 was shown to inhibit phosphatase activity in vitro, suggesting that AKAP79 exhibits an additional level of regulation on the enzyme [29,30]. However, it is hypothesized that when bound to AKAP79, the phosphatase would be optimally positioned to dephosphorylate substrates, although the mechanism for activation is at present unknown.

Another enzyme component of the AKAP79 scaffolding complex is the calcium/phospholipid-dependent protein kinase (PKC) [31,32]. Various PKC isoforms associate with AKAP79 through interactions with the conserved catalytic core of the kinase at a site distinct from both the PP2B and RII binding domains [33]. Interestingly, binding of the calcium/calmodulin complex to AKAP79 releases PKC from the anchoring protein, liberating the active kinase [33,34]. Thus, signals that proceed through calcium/calmodulin can lead to the liberation and activation of an anchored pool of PKC at the postsynaptic densities [35]. This mechanism demonstrates another feature of the AKAP79 signaling scaffold as a site for the integration of various second messenger signals. This type of regulation may be particularly relevant at postsynaptic sites where there is a plethora of signal-generating machinery including adenylyl cyclases that produce cAMP, several enzymes that generate phospholipids and diacylglycerol and calcium entry through ion channels including NMDA receptors [35].

The physiological importance of the AKAP79 signaling complex has also been demonstrated in the rat uterus, where PKA is a known uterine relaxant which acts via the inhibition of phospholipase C (PLC) activity [36,37]. Dodge et al. found that PKA associated with the plasma membrane via binding to an AKAP, presumably AKAP79, is responsible for this PKA-mediated inhibition of PLC activity [37]. During pregnancy in the rat, PKA-mediated inhibition of PLC activity is

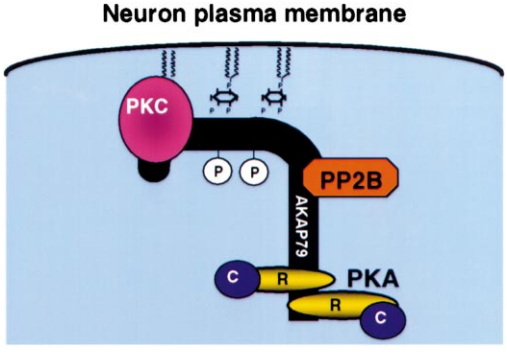

Fig. 2. The AKAP79 signaling complex. An additional property of AKAPs is their ability to bind not only PKA, but other signaling enzymes. AKAP79 maintains a signaling scaffold of PKA, PKC and PP2B. The signaling complex is attached to the inner face of membranes via association with negatively-charged phospholipids. It is proposed that these targeting interactions facilitate close association of these enzymes with target substrates.

lost on the day of parturition and this loss correlates with a dramatic decrease in PKA associated with AKAP79 [36]. Furthermore, on the same day of pregnancy, PP2B-associated AKAP79 is increased. The demonstration that PP2B could dephosphorylate PLC implicated PP2B in the regulation of uterine contraction by opposing the uterine relaxation pathways. These data suggest the significance of AKAP79 in regulating the pathways that control uterine contraction and in the timing of parturition.

4. The fourth property: recruitment into macromolecular complexes

Recent advances in our understanding of AKAP79 have highlighted a previously unappreciated property of the anchoring protein, namely the ability to be recruited into a larger transduction unit where the signaling complex is physically associated with upstream activation elements and downstream substrates (Fig. 3). This represents the fourth postulate of our anchoring hypothesis. A classical upstream element in PKA signaling is the β2-adrenergic receptor (β2-AR) [3]. Interestingly, the β2-AR is also a known target for PKA phosphorylation, which is consistent with our idea that many targets of PKA phosphorylation may be associated with an AKAP signaling complex. Precedence for the association of receptors and kinases has been defined by studies showing the association of activated receptors with G-protein receptor kinases [38]. However analogous mechanisms for receptor association with other second messenger kinases have not been elucidated to date. The recent work of Fraser et al. demonstrates that AKAP79 coprecipitates with the β2-AR from both cell and tissue extract, allowing for an extended complex composed of PKA, PKC, PP2B and the β2-AR [13]. The association of PKA with the β2-AR was shown to enhance phosphorylation of the receptor independent of agonist. This action helps to facilitate a switch in receptor/G-protein coupling from Gαs to Gαi, and subsequently stimulates the MAP kinase cascade [39]. These findings suggest that β2-AR/AKAP79 association not only regulates β2-AR signaling pathways, but also the activation of PKA by switching the β2-AR signaling cascade from one that stimulates PKA to one that inhibits.

One of the more important downstream actions of PKA is the regulation of ion channel activity. The AMPA responsive glutamate receptor in the post-synaptic density is one such channel, but how PKA is targeted to the ion channel was unknown [40]. Recently, AKAP79 has been shown to interact directly with members of the MAGUK family of scaffolding molecules, which associate with many proteins in the post-synaptic densities, including glutamate receptors [14,41]. This interaction was important for PKA phosphorylation of the ion channel [14]. Thus, the two-fold contact of the MAGUK with glutamate receptors and with AKAP79 creates a protein bridge that links anchored kinases and phosphatases with their substrates.

5. Conclusions and perspectives

In the past few years, new evidence has deepened our understanding of AKAPs and the complexity of their signaling networks. Studies on AKAP79 have been instrumental in these discoveries, and the AKAP model has evolved to encompass the growing facets of AKAP79 signaling. The original model proposed suggested only two properties of AKAP function: a PKA binding domain and a targeting domain. However, the discovery that AKAP79 associated with other signaling enzymes forced a reevaluation of the function of AKAPs to that of scaffolding protein [12]. Now, new research on AKAP79 will again change the thinking on these multifaceted proteins to include the formation of multiunit signaling complexes comprising of both upstream activators and downstream targets [13,14].

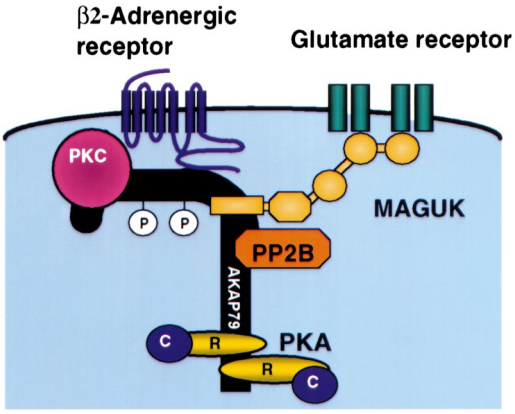

Fig. 3. Schematic diagram of a proposed synaptic signaling unit. AKAP79 would act as a bridge to link the β2-AR to the glutamate receptors that associate with MAGUKs. A variety of protein–protein interactions would act together to maintain the formation of the multiunit complex that brings together the AKAP79/150 scaffold with upstream activators such as the β2-AR and particular substrates for the anchored kinases and phosphatases such as the AMPA type glutamate receptor ion channels. Each component of the complex is named.

The regulation of AKAP targeting could potentially influence the actions of the anchoring protein. The membrane targeting of AKAP79 appears to be regulated at least in part by PKC phosphorylation [28]. However, other factors undoubtedly contribute to this process. For example, the association of AKAP79 with MAGUK may contribute to its localization at the post-synaptic density [14].

Perhaps the most intriguing property of AKAPs is their potential to assemble signaling complexes through association with multiple enzymes and binding partners. This idea is illustrated by AKAP79, which associates with both the β2-AR and MAGUK [13,14]. β-Adrenergic stimulation of PKA has been shown to regulate synaptic ion channels which associate with MAGUK [42,43]. Therefore, it is conceivable that a multiunit complex comprising of the β2-AR, AKAP79, MAGUK and glutamate receptors may exist at the synapse, which may be responsible for the adrenergic regulation of the ion channel (Fig. 3). No doubt future studies will focus on this possibility, as well as the possibility of similar complexes formed in other tissues, since AKAP79 displays a broad range of expression.

The crucial key in the understanding of the physiological importance of AKAPs will be in the development of knockout mice. Ablation of the genes encoding the C and RII subunit of the PKA holoenzyme have demonstrated the importance of PKA in the regulation of memory formation [44–46]. Since AKAPs bind the RII subunit of PKA and have been implicated in memory formation, it is conceivable that these animals would demonstrate deficiencies in brain function. Furthermore, these animals would not only demonstrate the

importance of PKA anchoring, but also provide a model to study the roles of the multitude of signaling molecules associated with AKAPs.

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