ANALYSIS OF A NOVEL A-KINASE ANCHORING PROTEIN 100, (AKAP 100).

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Considerable research has focused on determining the role of cAMP in intracellular signal transduction, (1). Krebs and colleagues in 1968 (2) made a key discovery, which ultimately linked the action of cAMP to the then novel cAMP-dependent protein kinase, which through phosphorylation of target substrates potentiates the hormonal response by subtly altering the biological activity of key enzymes and structural proteins. It has been well documented that PKA is a multifunctional kinase with broad substrate specificity able to trigger discrete physiological responses, even in the same cell (2-3). For example, phosphorylation of nuclear transcription factors (e.g. CREB) alters the activity of certain genes (4), while in heart cells PKA regulates calcium channel, (5-6) potassium channel, (7-8) and chloride channel, (9-10) ion current, by phosphorylation of the channels directly or by phosphorylation of closely associated regulatory proteins. Differential localization of the type II cAMP-dependent protein kinase (PKA) holoenzyme via interaction of the regulatory subunit (RII) with A-Kinase Anchor Proteins (AKAPs), (11-13) is a means which can facilitate protein specific cellular phosphorylation events. The biochemical properties of the newly identified PKA binding protein, AKAP 100 which has been colocalised with RIIα to the sarcoplasmic reticulumn of the cardiac and skeletal muscle cell lines, H9c2 and L6P respectively, are discussed in this article.

Utilising an interaction cloning strategy with radiolabeled RIIα as the initial probe and a human foetal brain expression library, full length cDNA (1965 base pairs) encoding a protein of 655 amino acids, (AKAP 100) was isolated, (14). Northern analysis showed that AKAP 100 was expressed predominately, with an 8kb message, in human cardiac and skeletal muscle. Recombinant AKAP 100 was able to bind RIIα. A peptide specifically designed to disrupt RIIα binding to the amphipathic helix of AKAPs was able to displace all binding of RIIα to AKAP 100. Anti-AKAP-100 antibodies revealed expression of AKAP-100 in the rat cardiac and skeletal muscle cell lines H9c2 and L6P respectively. Immunohistochemical analysis with RIIα and AKAP 100 protein antibodies revealed colocalisation of RIIα and AKAP 100 to the sarcoplasmic reticulumn of H9c2 and L6P cells. In addition AKAP-100 copurified with RIIα, by cAMP agarose from an H9c2 cell extract, (Figure 1). Slot blot analysis comparing the affinity of AKAP 100 and Ht31 for RIIα revealed AKAP 100 and Ht31 to have IC_{50} values of 1μmol/100μl and 0.25μmol/100μl respectively for RIIα, a difference of four fold, (Figure 2). This four fold difference in affinity is emphasised in the copurification experiment shown in Figure 1, where it can clearly be seen (lane 4), that only a small fraction of AKAP 100 is copurified with RIIα and indeed a significant proportion of the protein remains unbound to RIIα, (Figure 1 lane 2). These results suggest that AKAP-100 is a novel protein which has been demonstrated *in vitro* to bind RIIα, with a four fold lower affinity than Ht31, show selective expression in human tissue and is colocalised with RIIα to the sarcoplasmic reticulumn in the muscle cell lines examined.

Figure 1.
AKAP 100 copurifies with RII on cAMP agarose. Separate filters were probed for either RIIα (A) or AKAP 100 (B) using purified Abs. A solubilised extract of L6P cells (lane 1) was incubated with cAMP agarose. Unbound protein (lane 2), final wash (lane 3) and elution with 75mM cAMP (lane 4) are indicated. Molecular weight markers are also indicated.

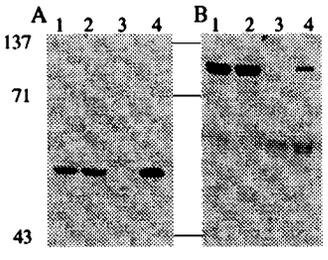
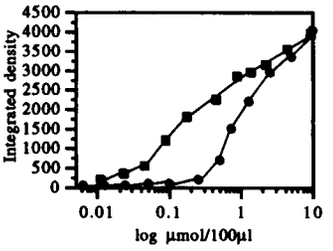


Figure 2.
Slot Blot Analysis of AKAP 100 and Ht31. A concentration range of μmol/100μl of AKAP 100 (●) and Ht 31 (●) were directly bound to Immobilon P. The samples were washed three times with tris buffered saline (pH 7.4), under vacuum. Blots were subsequently incubated over night with 32P-RIIα, 500,000cpm total.



The signal transduction pathways of numerous mammalian cells utilise multifunctional kinases and phosphatases which often share substrate specificity. To account for this it has been proposed that key regulatory proteins/enzymes function with dual specificity localizing both kinase and phosphatase cellular activities. Recently, (15) it has been demonstrated that bovine AKAP 79 located at the postsynaptic densities of rat hippocampal neurons, not only binds the regulatory subunit (RII) of PKA but also binds the protein phosphatase calcineurin. Additionally, it has recently been shown (16) that disruption of PKA localization, by blocking the binding of RII subunits to AKAPs, including AKAP 79, using Ht31 peptide, prevented modulation of the AMPA/Kainate receptors in hippocampal neurons. It is an attractive hypothesis that AKAP 100 is able to form a multimeric complex with RII and other as yet unidentified proteins, within the SR of cardiac cells exerting control on (1) chloride and/or potassium conductance and (2) the release or uptake of intracellular Ca^{2+}. Recently (17), it has been shown that the 565,000-dalton ryanodine receptor (Ryr), four of which form intracellular Ca^{2+} release channels of the SR and ER and are subject to phosphorylation by PKA, form a complex with the FK506-binding protein (FKBP12). It has also previously been reported that protein kinase activity is closely associated with a reconstituted calcium-activated potassium channel (7) and it has been speculated that the chloride channel of the SR may be intrinsically associated with PKA (9). In conclusion, there is a growing field of evidence which supports the notion that many functional membrane ion channels in addition to being targets for phosphorylation by PKA can also complex with PKA and/or other membrane proteins which may themselves be subject to phosphorylation and regulation and ultimately can have subtle or indeed dramatic effects on the regulation of ion channel activity. This association may be via AKAPs or may indeed be a direct association of PKA with these channels. Ultimately, however the ability of AKAPs to interact with other intracellular proteins in addition to PKA adds an exciting and dramatic level of control in the intracellular signal transduction pathway.

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