Critical Review

A-kinase Anchoring Proteins: From Protein Complexes to Physiology and Disease

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Summary

Protein scaffold complexes are a key mechanism by which a common signaling pathway can serve many different functions. Sequestering a signaling enzyme to a specific subcellular environment not only ensures that the enzyme is near its relevant targets, but also segregates this activity to prevent indiscriminate phosphorylation of other substrates. One family of diverse, well-studied scaffolding proteins are the A-kinase anchoring proteins (AKAPs). These anchoring proteins form multi-protein complexes that integrate cAMP signaling with other pathways and signaling events. In this review, we focus on recent advances in the elucidation of AKAP function. © 2009 IUBMB
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INTRODUCTION

Protein phosphorylation regulated by protein kinases and protein phosphatases is a primary mechanism for the regulation of enzymatic activities (*1*) and inducing protein-protein interactions (*2*). This bi-directional process is a flexible means of influencing cellular activity. Not surprisingly, a breakdown in signal transduction may lead to pathophysiological outcomes and the development of disease (*3*).

The molecular organization of these signaling pathways is intriguing as most transduction cascades are composed of common elements. The initial signal is transduced through a receptor at the plasma membrane (such as a G-protein coupled receptor, or a receptor tyrosine kinase or phosphatase), which results in activation of the receptor or the mobilization of receptor-associated proteins to generate some form of intracellular message.

There has been a concerted research effort focused to understand how the subcellular location of protein kinases and phosphatases contributes to the regulation of phosphorylation events. Sequestering a signaling enzyme to a specific subcellular environment not only ensures that the enzyme is near to its relevant targets, but also segregates this activity to prevent indiscriminate phosphorylation of other substrates. Thus protein scaffold complexes are a key mechanism, by which a common signaling pathway can serve many different functions.

One family of diverse, well-studied scaffolding proteins are the A-kinase anchoring proteins (AKAPs) (*4*). To date over 50 AKAPs have been identified in mammals and lower organisms (*5*). AKAPs contribute to the precision of intracellular signaling events by directing anchored enzyme pools to a subset of their physiological substrates at specific subcellular locations (Fig. 1). AKAPs have little primary sequence similarity and, thus, were originally classified based on their ability to bind the cAMP-dependent protein kinase (protein kinase A; PKA) (*6*). As a result, evidence to support their function has primarily been gleaned from analysis of PKA phosphorylation events. However, it is now well recognized that an equally important role of AKAPs is their capacity to form multi-protein complexes that integrate cAMP signaling with other pathways and signaling events (*7,8*). In this review, we focus on recent advances in the elucidation of AKAP function.

PROTEIN KINASE A

PKA is a broad specificity Ser/Thr kinase that can phosphorylate a range of proteins. Many functions have been ascribed to this protein kinase, including metabolism (*9*), learning and memory (*10*), and exocytosis (*11*). Several of these processes contribute to various disease states. For example, PKA is implicated in pancreatic β-cell function and may provide a novel

means for combating diabetes (*12*). PKA is also important in the molecular mechanisms of learning and memory, making it an attractive target for therapies seeking to inhibit cognitive decline resulting from neurodegenerative diseases (*13*). The activity of PKA is regulated by its two regulatory subunits, which form a dimer that binds to the two catalytic subunits. Activation of PKA occurs through the binding of cAMP to the regulatory dimer, causing dissociation of the catalytic subunits (*14–16*). The two main PKA subtypes are defined by the identity of their regulatory subunits, RI and RII (*17*). Anchoring of PKA by AKAPs confines PKA activity to a relevant subset of potential substrates. The majority of known AKAPs bind specifically to the RII holoenzyme. However, several dual specificity AKAPs, which bind to both PKA subtypes, have been identified. These include the dual-function anchoring proteins D-AKAP1 (*18*) and

D-AKAP2 (*19*). Although the intracellular regulation of PKA activity has been well described in terms of anchoring proteins, specific biological functions for AKAPs have been harder to identify. At present, proteomic and biochemical techniques have been exhaustively used to identify the majority of AKAPs and to characterize the components of their signaling complexes (*20*). Only recently, however, are we beginning to gain an understanding of the role that AKAPs play in cellular physiology.
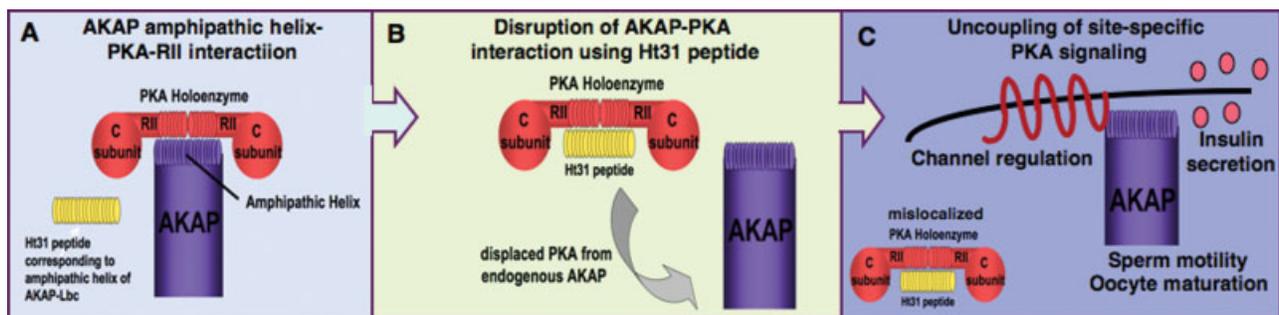
PKA ANCHORING

Most AKAPs contain a recognizable sequence that forms a binding site for the R subunits. This motif is a hallmark of all AKAPs and was first identified in the human thyroid anchoring protein, AKAP-Lbc. Biochemical studies showed that a 24-residue peptide (originally called Ht31) binds RII with nanomolar affinity (*6,21*). The Ht31 sequence is predicted to form an amphipathic helix, and structural studies indicate that the hydrophobic face of this region fits into a binding pocket formed by the N-terminal regions of the RII dimer of PKA (*22–24*) Cellular delivery of this peptide or related derivatives (using cell-soluble stearated forms or plasmid-based expression) has become a standard means to delineate a role for AKAPs in the coordination of cAMP-responsive events by disrupting PKA-RII anchoring inside cells (Fig. 2).

The utility of this peptide as a disruptor of PKA anchoring was first demonstrated in studies showing that perfusion of Ht31 into cultured hippocampal neurons uncoupled PKA from α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptors (*25*). This attenuates postsynaptic AMPA receptor currents and synaptic events. Similarly, the Ht31 peptide has been used to demonstrate that disruption of PKA anchoring uncouples cAMP-dependent regulation of the L-type Ca^{2+} channel (*26*). The importance of AKAPs in channel regulation relating to learning and memory and in cardiac physiology will be described later. The Ht31 peptide has also been used to demonstrate that sperm motility (*27*), oocyte matu-
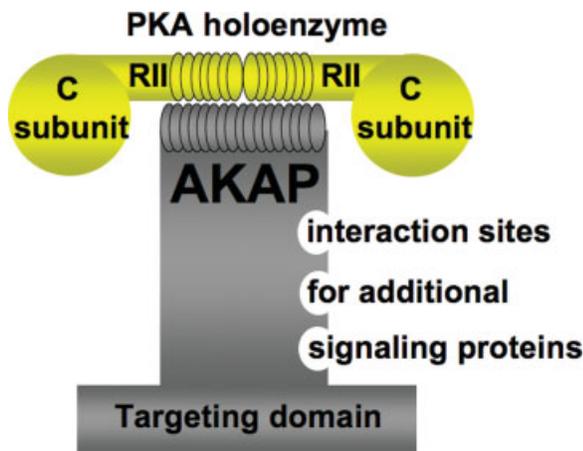
Figure 1. Properties of AKAPs. AKAPs bind to the regulatory subunit of PKA. AKAPs also bind additional signaling proteins (for example other protein kinases, protein phosphatases, phopshodiesterases, adenylyl cyclases, and small G proteins). AKAPs function to target signaling complexes to discreet locations inside a cell.

Figure 2. Study of AKAP function by peptide-mediated disruption of PKA anchoring. (A) The AKAP-amphipathic helix-PKA-RII interaction can be disrupted *in vivo* by introduction of a competing peptide called Ht31. (B) Ht31 will bind to PKA-RII, thereby displacing PKA from an AKAP inside a cell. (C) Mislocalization of PKA by displacement from an AKAP may lead to uncoupling of site-specific PKA signaling. This has been demonstrated in the processes of channel regulation, insulin secretion, sperm motility and oocyte maturation.

ration (28), and GLP-1-mediated insulin secretion (29) are AKAP-dependent processes. The roles of relevant AKAPs in these processes will be described later.

WHOLE ANIMAL MODELS; FLIES, FISH, AND MICE

Surprisingly, there is still little detail described for the physiological roles of AKAPs through the use of genetically modified model organisms. For many years Drosophila have been the most widely described genetically tractable organism, however there have been few studies involving AKAP function. Collectively, Drosophila studies have reinforced experiments carried out in cultured neurons indicating that AKAPs do indeed play a role in the subcellular organization of signaling components involved in learning and memory (30–32).

Zebrafish are another genetically tractable organism. The combination of protein knockdown using morpholino-oligonucleotides combined with relative ease of analysis of embryogenesis make the use of this organism attractive for developmental studies. In particular, a recent study points to a role for gravin in gastrulation (33) (see Reproduction & Development section).

As for mice, there are relatively few reports of AKAP knockouts. This may be for several reasons including lethality or lack of obvious phenotype. Recent studies in our laboratory suggest subtle alterations in phenotype, which could in part be due to compensatory mechanisms. We also have experience with embryonic lethality of certain AKAP knockouts, suggesting a previously unappreciated role in development. There is also now a well recognized role for AKAPs in sperm and oocyte function that may also impinge upon successful generation of homozygous knockout mice.

Notable knockout or transgene studies include WAVE-1 (34) and AKAP150 (35) (see Learning and Memory section), AKAP149 (AKAP1) (28), AKAP82 (AKAP4) (36) (see Reproduction & Development section) and D-AKAP2 (37) (see Genetic Studies section).

REPRODUCTION AND DEVELOPMENT

Sperm Function

AKAPs play important roles in sperm function, including regulation of motility, sperm capacitation, and the acrosome reaction (Fig. 3). Several AKAPs have been identified in sperm, including AKAP84 (AKAP1), AKAP110 (AKAP3), AKAP82 (AKAP4), AKAP95 (AKAP8), AKAP220 (AKAP11), gravin (AKAP250; AKAP12), WAVE-1, and MAP2 (38). The sperm function of AKAPs involves targeting of other signaling molecules in addition to PKA, including PDE's [eg. PDE4A in the case of AKAP110 in flagella (39)] and protein phosphatases, for example PP1 in the case of AKAP220 (40). In addition to scaffolding PKA, AKAPs also bind to a group of four proteins that share homology to the RII dimerization/docking (R2D2) domain. R2D2 proteins are expressed at high levels in the testis. These proteins function in the regulation of flagella and cilia in-

dependent of PKA activity and, unlike RII do not bind cAMP (41). Recently it has been demonstrated that these proteins are expressed in a wide variety of tissues suggesting a more general function in both motile and primary cilia (41).

The Akap4 gene has been knocked out in mice. These (male) animals are infertile because of a lack of sperm motility. AKAP82 (AKAP4), also known as fibrous sheath component 1 is sperm specific and is the major fibrous sheath protein of the principle piece of sperm flagellum (36).

Oocyte Function

Studies have implicated that the PKA in the regulation of oocyte maturation and several AKAPs have been identified in oocytes. PKA activity maintains the oocyte in meiotic arrest in response to cAMP generated by ovarian follicle cells that is transmitted to the oocyte through gap junctions. Meiosis is arrested at the first prophase, corresponding to the G2 phase of the cell cycle. Reinitiation of meiosis occurs following a decrease in cAMP in the oocyte, in response to the pituitary leutenizing hormone (LH) (42). A change in PKA localization is also observed as the oocyte resumes meiosis (43). It is thought that AKAPs function in the initial maintenance of meiotic arrest and the subsequent irreversible progression to the polar body extruded stage by efficiently targeting PKA activity to specific sites and substrates in the oocyte (44). The major AKAP expressed in mouse oocyte is 140 kDa in size and is a product of the Akap1 gene. This gene (encoding AKAP1 also known as AKAP140/149 and other splice variants AKAP121, sAKAP84, or D-AKAP-1) has recently been knocked out in mice (28). Interestingly, although AKAP1 knockout males are fertile, the females exhibit infertility, or subfertility, suggesting a role in oocyte maturation. Further investigation demonstrates that PKA activity is disregulated in the knockout oocytes. Furthermore, these cells are more sensitive to cAMP-activated arrest because of a change in PKA localization. Results show that anchoring of RIIα-PKA on mitochondria through targeting by AKAP1 effectively prevents the holoenzyme from interfering with the maturation process once it is initiated. In addition, further studies using the Ht31 peptide in AKAP1 knockout oocytes suggest that PKA localization to an AKAP other than AKAP1 is essential at an early stage in order for cAMP to maintain the arrest of oocytes in meiosis I prophase. The identity of this AKAP is not known, however, it is postulated that it may interact with the protein phosphatase cdc25 and/or the protein kinase wee1 to facilitate phosphorylation of these substrates, thereby regulating these enzymes to inhibit oocyte maturation in response to cAMP.

WAVE-1 is another AKAP demonstrated to play a role in oocytes. Specifically, immunofluorescence experiments show that WAVE-1 exhibits different cellular locations depending upon the phase of oocyte maturation. WAVE-1 redistributes from the cortex in germinal vesicle oocytes to the cytoplasm in oocytes arrested in meiosis II. Following fertilization, WAVE-1
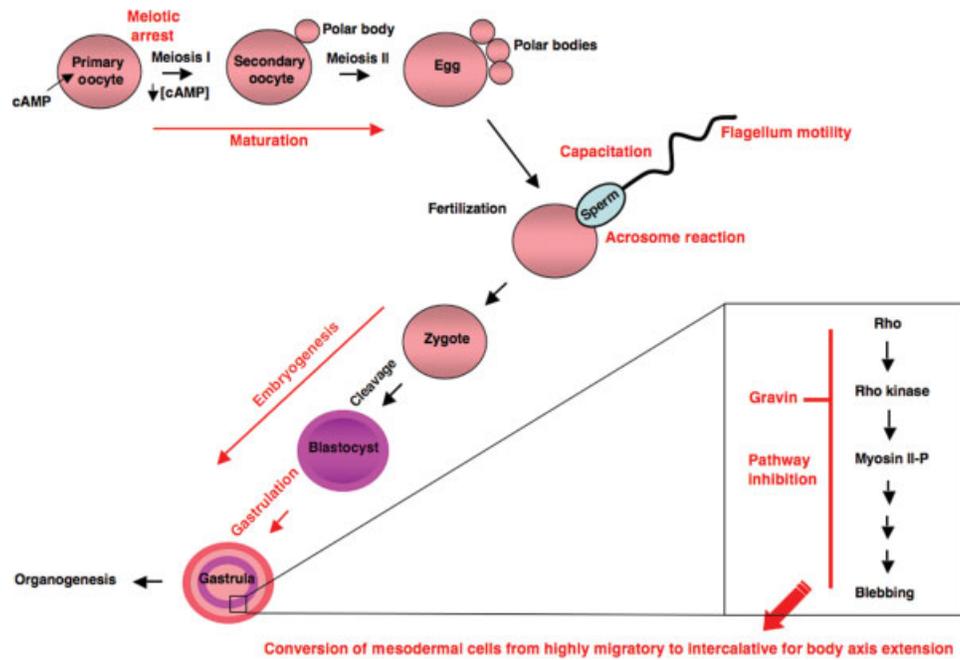
Figure 3. Development sperm function, oocyte maturation, and embryogenesis. AKAPs are highly abundant in testes, where they play important roles in sperm function including regulation of motility, capacitation, and the acrosome reaction. AKAPs also function in oogenesis where they function to regulate PKA localization maintaining initial meiotic arrest, followed by subsequent meiotic divisions to produce an egg ready for fertilization. Following fertilization, AKAPs play a role in embryogenesis. Specifically, gravin has been demonstrated to play a role in gastrulation, regulating the movement of germ layer cells to form an embryonic body plan.

relocalizes to developing male and female pronuclei. WAVE-1 then redistributes to the cytoplasm upon nuclear envelope breakdown at mitosis and concentrates at the cleavage furrow during embryonic cell division. Currently, although the localization of WAVE-1 in the process of oocyte maturation has been well documented, it is unclear as to the role of the anchored signaling proteins in the WAVE-1 complex. Association of WAVE-1 with PKA is detected in both meiosis II stage oocytes and pronucleate zygotes, but interaction with Arp2/3 is observed only in meiosis II stage oocytes (*45*).

Gastrulation

Gravin was one of the first AKAPs to be identified as a multi-functional kinase scaffold protein. In addition to PKA, gravin also binds PKC (*46*) and Src (*47*). Studies using cultured mammalian cells have implicated gravin in the regulation of cell morphology (*48*). Gravin is thought to function as a tumor suppressor (*49*). Orthologs of gravin (also known as SSeCKS in rat and mouse) have been identified in xenopus and zebrafish and studies with these organisms demonstrate a developmental role in embryo morphogenesis. Xenopus-gravin is zygotically expressed in a highly dynamic manner, beginning at the onset of gastrulation (Stage 10). Gastrulation involves the coordinated movement of germ layers, resulting in the development of an embryonic body plan. Convergent extension of the mesoderm is

a major process in this event (*50*). Recent studies in zebrafish show that gravin is required for the conversion of mesodermal cells from a highly migratory behavior to a medio-lateral intercalative behavior required for body axis extension. In particular, gravin functions to inhibit the blebbing movement of the mesodermal cells through inhibition of a Rho/ROCK/myosin II pathway (*33*). The precise mechanism of this inhibition relating to gravin remains unknown however. This observed role of gravin in embryogenesis has lead to speculation as to the tumor supressor function of this AKAP. It is conceivable that gravin may act to inhibit the blebbing movements observed in some tumors that may cause cell invasion and metastasis.

LEARNING AND MEMORY (CHANNEL REGULATION)

One of the first physiological roles identified for AKAPs was learning and memory.

AKAP79 (or the mouse ortholog AKAP150) can regulate synaptic plasticity through regulation of neuron channel response. This is accomplished by AKAP79 through scaffolding, targeting, and regulation of the signaling molecules PKA, protein kinase C (PKC) and protein phosphatase 2B (PP2B, calcineurin) at the postsynaptic membrane (*51*). Phosphorylation of specific channel subunits (including AMPA- and NMDA-glutamate receptors (*52,53*), the inwardly rectifying potassium channel

Kir2.1, (*54*) the β-adrenergic receptor (*55*), mGluR1/5 metabotropic glutamate receptor (*56*), and GABA receptors (*57*) at inhibitory synapses) may modulate synaptic efficiency either directly or indirectly by regulating receptor surface expression, which has been shown for AMPA receptors (*58,59*). Recently there have been a couple of studies published characterizing the knockout mouse ortholog of AKAP79 called AKAP150 (*60,61*). A knock-in mouse has been generated where AKAP150 has been replaced by a mutant form of this scaffold protein that is unable to bind PKA. Expression of PKA is highly reduced within postsynaptic densities (PSDs) of neurons cultured from these mice indicating that AKAP150 is the predominant AKAP responsible for targeting of PKA to the PSD in neurons. Further studies suggest that AKAP150-targeted PKA contributes to LTP in the adult hippocampus (*35*). In addition, AKAP150 null mice also exhibit deficits in motor coordination and strength, consistent with a role for AKAP150 in the cerebellum (*61*). Interestingly, AKAP79/150 can also tether adenylyl cyclase V/VI, thereby forming a protein configuration to regulate cAMP production. The anchoring of both an adenylyl cyclase (to generate cAMP) followed by the subsequent activation of PKA, which can inhibit the cyclase, leads to a negative feedback loop, which may be important in generating specific pools of localized cAMP (*62*). The physiological significance of this is currently unknown, but may be important in the context of characterizing the knockout mouse.

WAVE-1 (Wiskott-Aldrich syndrome, verprolin-homology domain containing protein) is another AKAP with important neuronal functions. Expression of this scaffolding protein is restricted to the central nervous system, where it functions to organize protein networks involved in the regulation of the cytoskeleton (*34*). In particular, this AKAP belongs to a family of proteins that link Rho GTPases to actin assembly (*63*). Interestingly, WAVE-1 is likely to exist in many different combinatorial complexes relating to its spatiotemporal function. For example, the RII-binding region of this protein overlaps with the actin-binding domain and *in vitro* binding experiments demonstrate that actin competes for the RII-binding site (*64*).

Synaptic remodeling, through actin polymerization, is believed to play a role in synaptic plasticity, learning, and memory. Insight into the role of WAVE-1 in this process comes from the characterization of WAVE-1 knockout mice, which display defects in hippocampal learning and memory (*34*). WAVE-1 knockout mice displayed defects in two tests (novel open arena and elevated zero-maze) used to determine anxiety levels associated with neural networks in the amygdala. Additionally, these knockout mice also displayed poor performance in tests for balance and coordination (rotarod, inclined screen and balance beam tests). The precise molecular mechanisms governing the observed phenotype of this mouse are not known. Interestingly, mutations in WRP (MEGAP/srGAP3) (a Rho GTPase) have been linked to 3p-syndrome mental retardation in humans, which also results in impaired learning and memory, poor balance, and reduced coordination (*65*). Further studies carried out in mice generated to express a form of WAVE-1 without the WRP binding sites indicate that the anchored pool of WRP associated with WAVE-1 plays a role in the control of cytoskeletal events that underlie normal neuronal development and synaptic plasticity (*66*). Thus, WAVE-1 localization of WRP is important for regulation of the actin cytoskeleton relating to processes of learning and memory. Currently, it is unclear if ablation of the WAVE-1 complex may also affect actin bundle formation through disruption of structural proteins involved in this process, such as the Arp2/3 complex and/or, if anchored PKA and/or other anchored signaling molecules such as Abl (a tyrosine kinase) may play a role in the aetiology of this phenotype. It is believed that dendritic morphology may be regulated by direct phosphorylation of WAVE-1 itself (*67*).

CARDIAC FUNCTIONS

To date, perhaps the most evidence for the physiological roles of a number of different AKAPs lies in the study of cardiac function (see summary Fig. 4). PKA phosphorylates numerous substrates that may influence contractility in cardiac myocytes, including the L-type Ca^{2+} channel, the ryanodine receptor, phospholamban, and troponin I (*68*). RIα is likely to play a role in cardiac physiology because it is the predominant isoform associated with the sarcolemma in cardiac myocytes (*69*). Mutations in the RIa gene are associated with both familial cardiac myxomas and Carney complex, implicating this isoform in cardiac growth and differentiation (*70*). However, in addition to RI, many AKAPs targeting type II PKA have been characterized in the heart. AKAPs play a critical role in modulating phosphorylation of numerous PKA dependent substrates that regulate cardiac function. Thus, altered expression of AKAP's would seem likely to result in drastic consequences. In particular, mAKAP (*71*), yotiao (*72*), AKAP18 (*73*), AKAP-Lbc (*74*), synemin (*75*), and myospryn (*76*) are all reported to have physiological functions in the heart. It should also be noted that the dual-specific AKAP2 (D-AKAP2), which targets both type I and type II PKA, plays an important role in the heart (*37*) and will be discussed later (see Genetic Studies).

The muscle specific A kinase anchoring protein (mAKAP) is highly expressed in cardiac tissue and localized to the perinuclear membrane and junctional sarcoplasmic reticulum where it is in close proximity to a variety of substrates such as L-type Ca^{2+} channels and the ryanodine receptor (RyR) (*77*). By serving as a scaffold for a range of molecules such as PKA, PDE4D3, Epac1 and ERK5, mAKAP is able to assemble a highly structured network in which signals can be regulated and focused upon nearby substrates (*78*). It is well known that PKA phosphorylation of the RyR receptor results in opening of this receptor and increased cardiac function and contractility and this is in large part due to the mAKAP-mediated localization of PKA to the RyR (*79*). Tight regulation of cardiac contractility is critical for efficient function of the heart. mAKAP plays a role in this by scaffolding phosphodiesterases that are able to
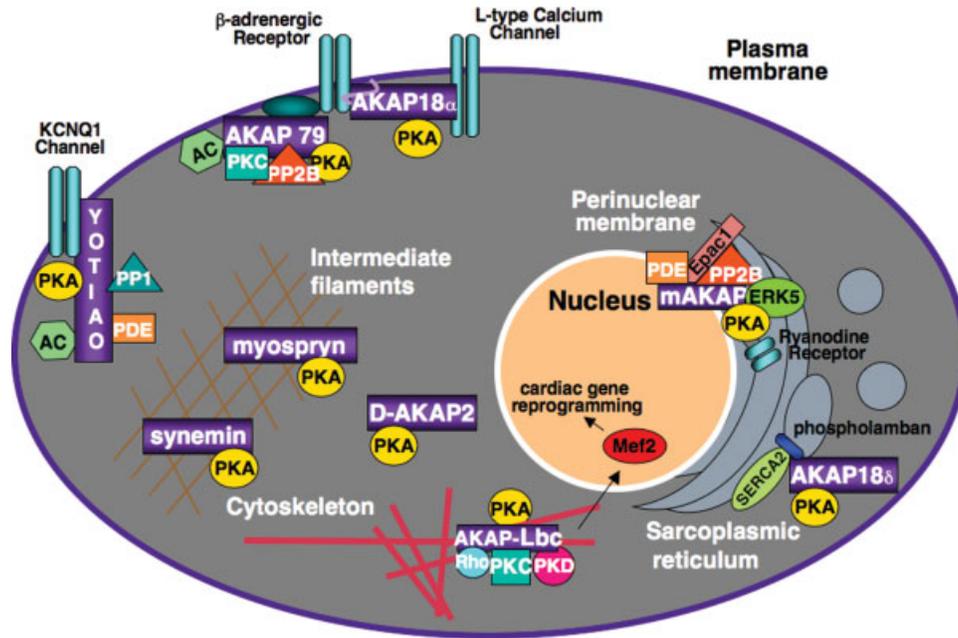
Figure 4. Cardiac AKAPs. AKAPs play a prominent role in the regulation of cardiac function. AKAP18, AKAP79, and yotiao fuction in channel regulation, whereas myospryn and synemin act to target PKA to intermediate filaments. mAKAP and AKAP-Lbc appear to play a role in the disease process of cardiac hypertrophy.

rapidly hydrolyze localized cAMP and restore PKA to an inactive state. In addition to a role in mediating cardiac contractility, mAKAP is also implicated in cardiac hypertrophy. Two components of the mAKAP complex that are involved in the hypertrophic response are the mitogen activated kinase, ERK5 (71), and the phosphatase PP2B (calcineurin) (80). Activation of ERK5 results in cardiac hypertrophy although ERK5 is readily dephosphorylated and inactivated by PP2B, thus once again demonstrating another feedback mechanism whereby mAKAP tightly regulates cardiac function.

AKAP15/18 is expressed in the heart and localizes to the plasma membrane (shorter isoforms α and β) because of an N-terminal lipid-anchoring domain (81,82).

Recently the long splice variant of the AKAP15/18 gene; AKAP15/18δ, which displays a cytoplasmic localization, was found to target PKA to phospholamban, which is a critical regulator of the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) (73). The interaction of AKAP15/18δ with phospholamban is necessary for PKA-mediated phosphorylation of phospholamban at Ser16, leading to the effects of adrenergic stimulation on calcium reuptake. Decreased SERCA expression/activity and phospholamban hypo-phosphorylation are intricately involved in heart failure, thus AKAP15/18δ plays a crucial role in maintaining cardiac function. Previous studies have demonstrated that expression of a pseudo-phosphorylated phospholamban (83) or enhanced expression/activity of SERCA (84) is sufficient not only to halt the progression of cardiomyopathy but can also restore cardiac function. As AKAP15/18δ mediates phospholamban phosphoryla-

tion and a subsequent increase in SERCA activity, it is intriguing to postulate that modulation of AKAP15/18δ may represent a novel pharmacologic target for restoring heart function.

Fractionation experiments suggest that this protein may serve as a scaffold for the β_2-adrenergic receptor (85) and the L-type Ca^{2+} channel (86). AKAP15/18 expression potentiates cAMP responsive Ca^{2+} currents while expression of the Ht31 peptide to disrupt PKA-AKAP interactions results in inhibition of PKA induced potentiation of the L-type Ca^{2+} channel (87).

In addition to AKAP15/18, AKAP79 also plays a role in the regulation of Ca^{2+}, specifically by targeting PKCα to the L-type Ca^{2+} channel in arterial myocytes. Recent studies demonstrate that AKAP150 knockout mice were found to lack persistant Ca^{2+} sparklets and have lower arterial wall intracellular calcium and decreased myogenic tone. These knockout mice were hypotensive and did not develop angiotensin II-induced hypertension (60).

AKAP-Lbc serves as a scaffold for PKA, PKC, PKD (88), and also contains a DH (Dbl-homology)-PH (pleckstrin-homology) domain that acts as a guanine nucleotide exchange factor (GEF) for the low molecular weight GTPase Rho (89) which is a known mediator of cardiac hypertrophy. As with many signaling pathways regulated by AKAP scaffolding, the Rho GEF activity of AKAP-Lbc is also tightly regulated. The GEF activity of AKAP-Lbc is activated via the $G_{12/13}$ family of heterotrimeric G-proteins, while it is inactivated by phosphorylation from the AKAP-Lbc bound PKA. It appears that phosphorylation of AKAP-Lbc by bound PKA results in oligomerization and recruitment of 14-

3-3 proteins, which prevent AKAP-Lbc from being able to activate Rho (90,91). In addition to its involvement in hypertrophy via the Dbl domain, AKAP-Lbc also serves to activate PKD through recruitment of its upstream activator, PKC (88). PKD has received much attention recently as a result of its ability to promote the nuclear export of certain class II histone deacetylases (HDAC's) thereby driving MEF2-dependent transcription of hypertrophic genes (92). Therefore AKAP-Lbc plays a central role in coordinating multiple pathways involved with the disease process of cardiac hypertrophy (93).

Synemin is a rather unique AKAP in that it is the first intermediate filament protein shown to bind RII and localize PKA. Synemin colocalizes in the heart at the Z line with RII and desmin. By localizing PKA near intermediate filaments, synemin may enhance PKA phosphorylation of substrates at the Z line such as desmin, vimentin, tubulin, and αB-crystallin. This anchoring protein may also serve to localize PKA near myofibril filaments for potential phosphorylation of substrates such as troponin I or myosin binding protein C. Localization of synemin at the Z line and the plasma membrane would make it ideally suited to be involved in cardiac remodeling following cardiac hypertrophy or even cardiomyopathies, as altered phosphorylation of PKA substrates is seen in these conditions (75).

In the mouse model of Duchenne Muscular Dystrophy (mdx) where dystrophin is knocked out, the AKAP myospryn (an α-actinin-interacting, costamere-localized protein expressed in striated muscle) and its anchored PKA is mislocalized in these mice. It is believed that this is because myospryn is unable to interact with dystrophin (94). Thus, PKA activity in the muscle may not be properly targeted and regulated in muscle tissue of patients exhibiting DMD. Furthermore, expression of mAKAP, RyR, and SERCA2A is decreased in these mice, resulting in disrupted Ca^{2+} homeostasis and cardiac hypertrophy. Prolonged cardiac hypertrophy can transition to cardiac failure in which there is downregulation of β-adrenergic receptors, decreased phosphorylation of RII, and as a result, there may be a shift in the localization of RII due to the different affinities of AKAP for phosphorylated and unphosphorylated RII (95).

Yotiao serves as a scaffold in the heart for localizing PP1, PDE4D3, and RII to the I_{KS} channel subunit hKCNQ1 by means of a C-terminal leucine zipper motif on hKCNQ1 (96). Mutations in this leucine zipper motif have been found in individuals with long QT syndrome and in particular the S1570L mutation has been found in 2% (1/50) individuals with long QT syndrome while being absent in over 1,300 individuals not exhibiting this syndrome. Computational analysis of this mutation reveals that it reduces cAMP dependent phosphorylation of the I_{KS} channel resulting in a prolonged action potential (97). (See Genetic Studies section for more detail).

GENETIC STUDIES

In the age of functional genomics mapping disease loci has become a very important method to identify potential proteins that may play a role in pathology, or define the aetiology of genetic disorders. In this regard, there is now significant genetic information coupled with suitable technology to identify single nucleotide polymorphisms (SNPs) in the DNA of individuals that may result in the coding of a mutant form of protein. A single nucleotide base change in a region of DNA coding for a protein may lead to the mutation of a single amino acid, or a completely novel protein sequence, or a prematurely truncated protein. All of these outcomes may have serious effects upon cell signaling pathways.

A mutation in the AKAP Yotiao has been identified in patients with familial long-QT syndrome (LQTS) (97). Long QT syndrome (LQTS) is a congenital disorder characterized by a prolongation of the QT interval on ECG and a propensity to ventricular tachyarrhythmias, which may lead to syncope, cardiac arrest, or sudden death. (The QT interval represents the duration of activation and recovery of the ventricular myocardium). LQTS is caused by mutations in the genes coding for cardiac potassium and sodium or calcium ion channels, such as KCNQ1, LQT1, KCNE1, and LQT5 (98). Yotiao forms a macromolecular complex with the slowly activating cardiac potassium channel I_{Ks}, which is critical for repolarization of the ventricular action potential in the heart (96). The S1570L form of yotiao disrupts this interaction and reduces the cAMP-induced phosphorylation of the channel. This eliminates the functional response of the I(Ks) channel to cAMP, thereby promoting a prolonged ventricular action potential in individuals inheriting this mutation (97).

A deleterious SNP that results in an amino acid change from Ile to Val in the dual-specific AKAP2 (D-AKAP2) gene was identified in an aging population of European-America individuals that may be predisposed to cardiac dysfunction (99). Further evidence for an important cardiac function of D-AKAP2 comes from a more recent study of cardiac myocytes that were derived from mouse embryonic stem (ES) cells in which the D-AKAP2 gene was disrupted, thereby affecting the last 51 amino acids of D-AKAP2 (ablating the PKA binding site and a C-terminal PDZ domain). An increased cardiac response to cholinergic signals was observed and mutant mice exhibited cardiac arrythmias and died prematurely (37). To date, specific PKA signaling pathways associated with D-AKAP are not known. However, the Val D-AKAP2 variant binds to the RIβ isoform of the PKA holoenzyme with a three-fold stronger affinity than the wild-type Ile D-AKAP2, thereby affecting compartmentalization of type I PKA. One function of D-AKAP2 is to target PKA to the mitochondria, thus an increase in the ability of the Val D-AKAP2 variant to anchor PKA at the mitochondria has been observed (99). Importantly, these studies suggest that factors affecting the compartmentalization of type I PKA may have significant pathological effects, possibly by affecting the phosphorylation state of PKA substrates.

Interestingly, this same IleVal SNP was also identified to be associated with familial breast cancer in an additional independent study (100). A SNP causing a Lys526Gln mutation in AKAP-Lbc was also identified in this study (100). This muta-

tion is not in the PKA anchoring domain or any other recognized domain of this protein and is of unknown function. AKAP-Lbc was also recently identified in a pharmacogenomic study designed to highlight gene expression markers predictive for a favorable response in acute myelogenous leukemia (AML) patients treated with the farnesyltransferase inhibitor tipifarnib (Zarnestra, R115777). Raponi et al. used microarray technology to monitor gene expression from bone marrow samples of relapsed and refractory AML patients undergoing treatment with tipifarnib. Several marker genes were identified that may predict a positive response to tipifarnib treatment, including AKAP-Lbc. Thus, when AKAP-Lbc is under-expressed, farneslytransferase inhibitors may exhibit greater antitumorigenic effects in AML patients. It was also demonstrated in this study that overexpression of proto and onco-Lbc increases resistance to tipifarnib, but not doxorubicin (a non-farneslytransferase inhibitor chemotherapeutic). This indicates that AKAP-Lbc plays a role in the progression of this disease, where increased AKAP-Lbc activity may lead to an increased cellular profile of transformation through a Rho-mediated pathway. The authors speculate that AKAP-Lbc may be a downstream target of the farnesyltransferase inhibitor, however, to date there is no primary evidence to support this.

Recently, mutations identified in the gene encoding the AKAP pericentrin (PCNT) were demonstrated to cause Seckel syndrome (101). Seckel syndrome is a disorder associated with defective ATR-dependent DNA damage signaling, resulting in a marked reduction of brain and body size. Although the mechanism underlying this disorder is not fully understood, the authors demonstrated a loss of expression of pericentrin. This was due to a homozygous single-base pair deletion in exon 12 leading to a frameshift, which was predicted to result in premature protein truncation after an additional 65 amino acids. An additional SNP was also characterized; an insertion in exon 18 resulting in a frameshift at codon 1190. Collectively, these mutations disrupt all mammalian isoforms of PCNT. Pericentrin (also known as kendrin) is a 360 kDa coiled-coil protein with a C-terminal PACT domain that targets it to the centrosome. This scaffolding protein localizes to the pericentriolar matrix, where it interacts with several structural centrosomal proteins including γ-tubulin as well as PKA and PKCβII. Pericentrin plays an important role in microtubule nucleation and spindle organization. Presumably, the loss of pericentrin from the centrosome results in the loss of regulated protein phosphorylation through PKA and PKC, functioning downstream of the ATR kinase in the DNA damage response pathway, including G2-M checkpoint activation.

CANCER

Pericentrin functions as an essential centrosomal protein that is necessary for directing centrosomal assembly (through interaction with dynein (102)) and microtubule nucleation (through interaction with γ-tubulin (103)). This protein plays a critical role in cell division, therefore disruption of this gene can have drastic consequences and irregularities in this AKAP are often seen in tumors in which there are defects in regulated cell division. Cells overproducing pericentrin develop extra centrosomes and spindle defects, display genomic instability and enhanced growth in soft agar. Pericentrin, is able to bind RII through a domain unlike the conventional domain found in other AKAPs (104). Disruption of PKA binding to pericentrin results in similar spindle abnormalities (105). Finally as dynein is a substrate for PKA, and inhibition of dynein function results in spindle abnormalities, it is possible that pericentrin anchored PKA regulates dynein function.

The AKAP gravin is localized in the cytosol as well as at the plasma membrane, where it is associated with β-adrenergic receptors and serves as a scaffold for PKA, PKC (106). The AKAP domain of gravin appears to be required for association at the plasma membrane with β-adrenergic receptors, and this association is involved in resensitization of the receptor (107). Gravin has recently been recognized as a tumor suppressor because of its ability to induce cell cycle arrest (via decreases in cyclin D1) and even apoptosis (via caspase 3 activation) in numerous cell types (108,109). Gravin is downregulated in a number of tumor types including prostate, ovarian, and breast cancer and is associated with metastatic progression of these tumors, thereby providing further evidence supporting the role of gravin as a tumor suppressor (49). It is thought that the hypermethylation of the gravin gene and its promoter may account for the downregulation of gravin seen in tumor cells (110).

AKAP95 is localized to the nucleus and serves to recruit PKA to the nucleus during mitosis where it plays an integral role in DNA replication (111). Upon initiation of mitosis, RII is phosphorylated by the CDK1-cyclin-B complex, which drives it to the nucleus to associate with AKAP95 (112,113). Once in the nucleus, RII bound AKAP95 plays a critical role in initiation and elongation of DNA by providing a scaffold for p68 RNA helicase and the minichromosome maintenance protein 2, and is furthermore involved in chromatin condensation by recruitment of the condensing complex (114). In addition, AKAP95 binds caspase-3 where it mediates apoptotic nuclear morphological changes (115). At the termination of mitosis, RII is dephosphorylated which causes it to dissociate from nuclear AKAP95 and translocate back to the centrosome-golgi area where it resides until the next mitotic cycle. The release of RII from AKAP95 releases inhibition of chromatin condensation and allows for chromosome decondensation as the nuclear envelope reforms. This phosphorylation dependent localization of RII to AKAP95 serves as a molecular switch for ensuring the proper timing of cell division (116).

A truncated form of AKAP-Lbc (AKAP13) missing both N- and C-terminal regulatory sequences, called Onco-Lbc (amino acids 1,923–2,347), was originally identified as an oncogene from myeloid leukemia patients (117). This oncogenic protein displays constitutive Rho-GEF activity and is able to induce cell transformation in a Rho-dependent manner. Another splice

variant of the Lbc gene, called Brx (amino acids 1,384–2,817) has been identified that is specifically expressed in testis and estrogen-sensitive tissues (118). Only the full-length version of AKAP-Lbc contains the PKA anchoring domain (amino acids 1,236–1,257).

DIABETES

Impaired insulin secretion is a characteristic of non-insulin-dependent diabetes mellitus (NIDDM). Insulin secretion can be stimulated by the hormone glucagon-like peptide 1 (GLP-1). One mechanism of GLP-1 action is through activation of PKA. A role for AKAPs in the regulation of hormone (GLP-1)-mediated insulin secretion was first identified in studies using the PKA-AKAP anchoring inhibitor peptide Ht31 (29). Results demonstrate that insulin secretion can be regulated by the reversible phosphorylation of β-cell proteins through the AKAP79 targeted effects of PKA and PP2B. However, the exact mechanism of this regulation is still not understood. It is believed that somehow AKAP79 facilitates a feedback loop whereby PKA activity promotes activation of PP2B. The reciprocal function of these enzymes in turn regulates the phosphorylation of substrates involved in insulin secretion pathways, for example ion channels and proteins involved in endocytosis, such as synapsin (119). Clearly more work is required in this area if specific cellular mechanisms are to be elucidated. This is one area where transgenic models would be very useful to facilitate in vivo studies.

EXOCYTOSIS

More recently the Rab27a GTPase effector protein MyRIP has been identified as an AKAP through a yeast two-hybrid screen for RII interacting proteins (120). MyRIP interacts with components of the exocyst complex, which is involved in protein trafficking and exocytosis. Specifically, the exocyst complex may function to facilitate the expression of the glucose transporter Glut4 at the plasma membrane (and therefore cellular glucose uptake) in response to insulin. MyRIP may play a role in this process by directing PKA to the exocyst complex.

MELANOSOME TRANSPORT

Most recently it has been demonstrated that the AKAP Rab32 is associated with melanosomes and links PKA to these organelles (121) as well as mitochondria (122). Melanosomes are found in melanocytes where the pigment melanin is synthesised and stored. Melanocytes are present in the skin and eyes of mammals whose primary function relates to photoprotection. Therefore exposure to potentially damaging UV radiation results in an increase in melatonin production. Melanosome transport is regulated by PKA and in particular PKA activation appears to stimulate melanosome aggregation in response to melanin production. Currently however, the exact molecular mechanisms of this process are unknown. Interestingly Rab32 and another

highly homologous small GTPase Rab38 (67% sequence identity) can functionally compensate for each other in the regulation of pigment biogenesis. A recent study showed that Rab32/38 regulate a critical step in the trafficking of melanogenic enzymes, in particular tyrosinase from the trans golgi network to melanosomes (123). The authors do not comment on whether PKA activity is required in this process or whether Rab38 also interacts with PKA. Given the sequence similarity between Rab38 and the mapped PKA anchoring region (amino acids 178–192) on Rab32 it seems likely that PKA will interact with Rab38, performing an important function in this pathway.

In summary, we hope this review provides the reader with an insight into some of the physiological/pathophysiological processes facilitated by this interesting and well-characterized family of scaffold proteins. With the combination of large functional genomic screens and the study of tissue or whole animal models it is likely that we will gain further understanding into the physiological roles of these scaffold proteins. Hopefully these studies may lead to improved aspects of human health through the integration of protein function with precise molecular mechanisms of action.

ACKNOWLEDGEMENTS

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