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MINIREVIEW

Organizing signal transduction through A-kinase anchoring proteins (AKAPs)

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A fundamental role for protein–protein interactions in the organization of signal transduction pathways is evident. Anchoring, scaffolding and adapter proteins function to enhance the precision and directionality of these signaling events by bringing enzymes together. The cAMP signaling pathway is organized by A-kinase anchoring proteins. This family of proteins assembles enzyme complexes containing the cAMP-dependent protein kinase, phosphoprotein phosphatases, phosphodiesterases and other signaling effectors to optimize cellular responses to cAMP and other second messengers. Selected A-kinase anchoring protein signaling complexes are highlighted in this minireview.

Introduction

Knowing how signal transduction cascades are effectively organized inside cells is key to understanding how cells communicate. Insight into how this is achieved has been forthcoming from research on anchoring and scaffolding proteins [1]. A number of protein kinases with broad substrate specificities associate with proteins that target them to precise sites inside the cell. Signaling events that are initiated by the second messenger cAMP involve the activation of discrete pools of anchored protein kinase A (PKA) [1]. The tetrameric PKA holoenzyme is composed of two regulatory R subunits and two catalytic C subunits. Multiple genes encode the PKA subunits. Accordingly, differential expression of the RIα, RIβ, RIIα, RIIβ, Cα and Cβ genes can generate a range of holoenzyme combinations with slightly different physiochemical properties [2]. PKA type II holoenzymes ($RII\alpha_2C_2$, $RII\beta_2C_2$) turn on with an activation constant (K_{act}) of 200–400 nM cAMP, whereas PKA type I holoenzymes ($RI\alpha_2C_2$, $RI\beta_2C_2$) are triggered with lower concentrations of the second messenger (50–100 nM) [3]. One clear distinction between these two isoenzymes is their

Abbreviations
AKAP, A-kinase anchoring proteins; β2-AR, β2-adrenergic receptor; ERK5, extracellular signal regulated kinase 5; HDAC5, histone deacetylase 5; HIF-1α, hypoxia-inducible factor 1α; PDE, cyclic nucleotide phosphodiesterase; PDE4D3, 4D3 isoform of phosphodiesterase; PHD, prolyl hydroxylase; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; PP2B, protein phosphatase 2B.

preference for interaction with A-kinase anchoring proteins (AKAPs) [4]. A majority of AKAPs associate with PKA type II, however, dual-specificity AKAPs have been identified [5]. Much less is known about PKA type I-selective anchoring proteins. PKA type II, hereafter referred to as simply PKA, binds via an RII dimer interacting with a 14–18 residue amphipathic helix within the AKAP [6]. Crystallographic analysis of this complex revealed that this interaction requires the formation of a groove on one face of a four-helix bundle formed between RII protomers [7,8]. Biochemical characterization of this complex has led to the generation of several valuable tools for determining the biological significance of these complexes. These include membrane-permeant peptides that bind RII with high affinity and therefore can be used to disrupt AKAP/PKA interactions inside cells [9,10]. This minireview focuses on some of the recent work elucidating the functions of selected AKAPs. Three anchoring proteins (AKAP150, mAKAP and AKAP-Lbc) and their interacting partners are discussed in detail (Table 1).

AKAP79/150 signaling complexes

To date, AKAP150 (the murine homolog of human AKAP79) remains the best-understood anchoring protein. In hippocampal neurons, AKAP150 positions PKA, protein phosphatase 2B (PP2B) and protein kinase C (PKC) at membranes proximal to α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA)-type glutamate receptors through its binding with synapse-associated protein 97 [11–13]. This complex permits the robust phosphorylation of AMPA receptors by PKA at key residues that enhance the flow of ions through the channel [11–13]. This effect is counterbalanced by AKAP150-targeting of the calcium/calmodulin-dependent protein phosphatase PP2B [14]. In the absence of PKA binding, PP2B dephosphorylates these ligand-gated ion channels resulting in decreased conductance [14]. The anchored PKC is inactive in this complex. However, AKAP150-anchored PKC plays an important role in another context. In superior cervical ganglion neurons, AKAP150 coordinates suppression of current through M-type channels in response to muscarinic receptors [15–17]. M channels allow the passage of potassium ions through the plasma membrane, and suppression of the current results in enhanced neuronal excitability. AKAP150 modulates the M channel by positioning PKC close to critical residues necessary for the passage of ions through the channel and silencing of AKAP150 reduces the M-current suppression by muscarinic agonists. The anchored PKA and PP2B remain inactive in this context [15–17]. The importance of AKAP150-coordinated signaling inside neurons is supported by evidence that mice lacking AKAP150 exhibit deficiencies in muscarinic suppression of M currents, motor coordination, memory retention and resistance to pilocarpine-induced seizures [18].

AKAP150 has also been identified in association with the L-type calcium channel subunit $Ca_v1.2$ in the brain, where a complex that includes β2-adrenergic receptor (β2-AR), $Ca_v1.2$, G proteins, adenylyl cyclase, PKA and PP2A plays an essential role in the modulation of Ca^{2+} signaling downstream of β2-AR stimulation [19,20]. Here the AKAP150-associated PKA is believed to phosphorylate Ser1928 on the central pore forming subunit $Ca_v1.2$ in response to beta-adrenergic stimulation and disruption of AKAP150 prevents this activation step [21]. Likewise in the heart, PKA anchoring to a similar complex plays an essential role in increasing cardiac rate and output in response to β2-AR stimulation. This physiological response requires modulation of L-type calcium channels, and Ser1928 on cardiac α1 subunits has also been identified as the key PKA phosphorylation site [22]. Interestingly, in another cellular context, AKAP150-mediated targeting of the kinase PKC to L-type calcium channels in arterial myocytes is necessary for stuttering

Table 1. Selected AKAPs and their binding partners. AKAP, A-kinase anchoring proteins; β2-AR, β2-adrenergic receptor; ERK5, extracellular signal regulated kinase 5; HDAC5, histone deacetylase 5; HIF-1α, hypoxia-inducible factor 1α; MAGUK, membrane-associated guanylate kinase; PDE, cyclic nucleotide phophodiesterase; PDE4D3, 4D3 isoform of phosphodiesterase; PHD, prolyl hydroxylase; PKA, protein kinase A; PKC, protein kinase C; PKD, protein kinase D; PP2B, protein phosphatase 2B; pVHL, von Hippel–Lindau protein; SAP97, Synapse-associated protein 97; Siah2, seven in absentia homolog 2.

	AKAP79/150	mAKAP	AKAP-Lbc
Interaction partners: signaling proteins, receptors and ion channels	PKA, PKC, PP2B, MAGUKs (SAP97, post synaptic density (PSD)-95), AC5, AMPA receptor, NMDA receptor, KCNQ2 channel, M1 muscarinic receptor, β-adrenergic receptor, L-type calcium channel, aquaporin channel	PKA, PDE4D3, Epac1, ERK5, HIF-1α, Siah2, PHD, pVHL	PKA, PKC, PKD, Rho, 14-3-3
Subcellular targeting	Membranes	Perinuclear membrane	Cytosol

persistent calcium sparklets and the regulation of myogenic tone and blood pressure [23,24]. Stuttering persistent calcium sparklets produced by the long openings and reopenings of L-type Ca^{2+} channels lead to increased calcium influx and vascular tone, and are regulated through the AKAP150-anchored PKC. Collectively, these studies highlight the role that cellular context and the differential assembly of specific AKAP150–enzyme complexes play in influencing the diversity of AKAP signaling events.

The mAKAP complex

In the heart, the muscle-selective anchoring protein mAKAP organizes different combinations of proteins to control diverse aspects of cardiomyocyte physiology that occur close to the nuclear membrane. Although initially described as an anchoring protein for PKA, mAKAP also interacts with the 4D3 isoform of phosphodiesterase (PDE4D3), the guanine nucleotide exchange factor Epac1 and the protein kinase, extracellular signal regulated kinase 5 (ERK5) [25,26]. This provides a locus for the control of cAMP and mitogenic signaling events (Fig. 1A–C). As local cAMP levels increase, the mAKAP-associated PKA is activated to phosphorylate PDE4D3 to enhance cAMP metabolism [27]. This mAKAP–PKA–PDE configuration forms a classic enzyme feedback loop because anchored PKA activity eventually leads to the termination of cAMP signals. Interestingly, the same AKAP

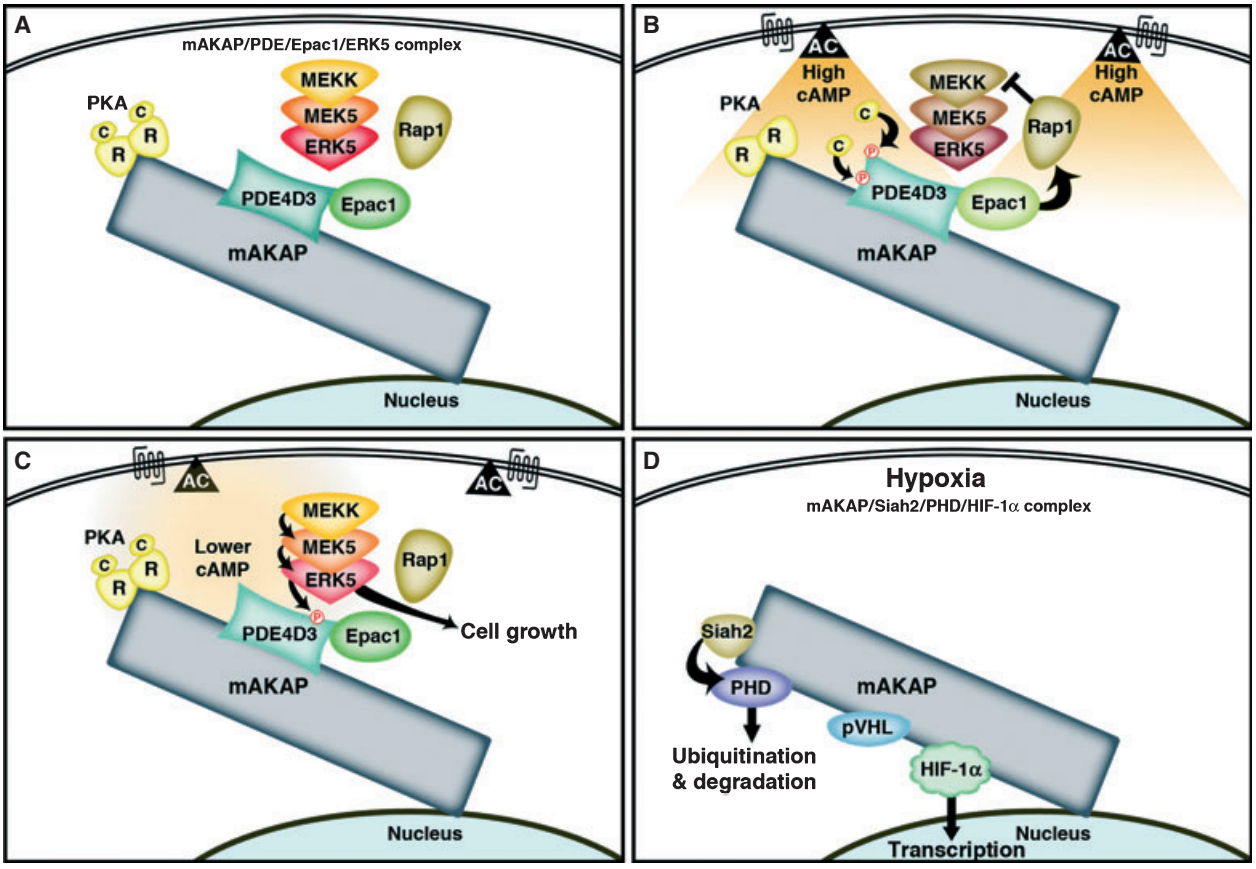

Fig. 1. mAKAP signaling complexes. (A) mAKAP assembles a cAMP-responsive complex of signaling enzymes at the perinuclear membrane in the heart. PKA, PDE4D3 Epac1 and ERK5 are brought together with other associated enzymes to control different aspects of cardiomyocyte physiology. (B) When intracellular cAMP levels are elevated the mAKAP-associated PKA phosphorylates the PDE4D3 in the complex at two sites, leading to increased metabolism of cAMP by the phosphodiesterase. Likewise, cAMP activation of Epac1 in the complex activates Rap1 to inhibit ERK5 signaling. (C) As cAMP levels decrease, the Epac1-mediated inhibition of ERK signaling is lost and mitogenic signaling favors cell growth. (D) mAKAP assembles an oxygen-sensitive signaling pathway that includes the ubiquitin E3 ligase seven in absentia homolog 2, prolyl hydroxylase, von Hippel–Lindau protein and the transcription factor HIF-1α. Under normoxic conditions, HIF-1α is continually degraded, however, when oxygen levels decrease, the mAKAP-associated PHD is degraded and HIF-1α accumulates and translocates into the nucleus.

complex contributes to cAMP-mediated regulation of an anchored ERK5 mitogenic signaling pathway. This is achieved through mobilization of an mAKAP-associated pool of cAMP-dependent Epac1, which activates the small G protein Rap1. Active Rap1 can, in turn, repress the ERK5 activity associated with the mAKAP-signaling network [26].

So why are so many enzymes brought together by mAKAP at the same point in the cell? One explanation is that these multienzyme complexes create a situation in which subtle changes in the concentration of cAMP can have profound effects on the cellular processes that are active. As cAMP levels increase, anchored PKA works to deplete the second messenger by activating a local pool of PDE4D (Fig. 1B). Yet when cAMP levels decrease, Epac1-mediated inhibition of the ERK5 cascade is lost (Fig. 1C). The concomitant de-repression of ERK5 turns on mitogenic signals that favor cell growth (Fig. 1C). Thus these mAKAP complexes exemplify how distinct enzyme cascades constrained within the same macromolecular complex can respond and contribute to the ebb and flow of cAMP.

Recently, it has been discovered that mAKAP organizes additional and diverse signaling proteins [28]. This includes enzymes that coordinate the oxygen-dependent control of the transcription factor hypoxia-inducible factor 1α (HIF-1α) (Fig. 1D). Under normoxic conditions, HIF-1α protein levels are kept low by the action of prolyl hydroxylases (PHD), a family of oxygen-sensitive dioxygenases [28]. Hydroxylated proline residues in HIF-1α constitute a binding site for the von Hippel–Lindau protein, which is part of a multiprotein complex that ubiquitinates HIF-1α resulting in degradation by the proteasome. Under hypoxic conditions, HIF-1α protein levels increase as a result of two factors: (a) the enzymatic activity of the PHDs is reduced in the absence of oxygen; and (b) the ubiquitin E3 ligase, seven in absentia homolog 2 ubiquitinates selected PHDs. Together, these processes terminate the destruction of HIF-1α. The consequence of bringing these enzymes in proximity to their substrates was illustrated in cells lacking mAKAP. Gene silencing of mAKAP blunted hypoxia-induced HIF-1α-dependent gene transcription [28]. Delocalizing mAKAP from perinuclear membranes using a peptide corresponding to the perinuclear targeting domain of mAKAP reduced movement of HIF-1α into the nucleus and HIF-1α-dependent gene transcription [28]. Thus, mAKAP participates in response to oxygen tension by facilitating the proteasomal degradation or stabilization of the transcription factor HIF-1α.

AKAP-Lbc signaling complex

AKAP-Lbc is another multivalent anchoring protein that organizes PKA and PKC in a manner that favors activation of protein kinase D (PKD) [29,30]. An added feature of AKAP-Lbc is that it functions as a guanine nucleotide exchange factor for Rho, a small GTP-binding protein, thereby creating a point of convergence between the cAMP and Rho signaling pathways [31]. This anchored signaling complex interfaces with the cytoskeleton because AKAP-Lbc has the capacity to remodel actin upon activation of Rho [32,33]. Termination of AKAP-Lbc's Rho guanine nucleotide exchange factor activity involves homo-oligomerization of the anchoring protein and PKA mediated recruitment of 14-3-3 [34].

In the heart, chronic activation of PKD is associated with hypertrophy. In support of this notion AKAP-Lbc expression is increased $\sim 50\%$ in hypertrophic cardiomyocytes [35]. Reciprocal experiments demonstrated that cardiomyocytes lacking AKAP-Lbc are resistant to phenylephrine-induced hypertrophy [35]. Several lines of inquiry have implicated AKAP-Lbc as a co-factor in the mobilization of the fetal gene response that is emblematic of pathological cardiomyocyte hypertrophy [36]. A key event in this process is the PKD phosphorylation and subsequent nuclear export of class II histone deacetylases (HDACs) [35]. Using a combination of live cell imaging and gene-silencing approaches it was shown that depletion of AKAP-Lbc suppressed the nuclear export of HDAC5 and repressed transcription of the ANF gene, a marker for pathological cardiac hypertrophy [36]. These data provided some of the initial evidence that altered expression of AKAPs can influence the control of pathophysiological processes.

Perspectives

Considering the spatial and temporal distribution of intracellular signaling molecules is now recognized as an important determinant in the control of cell signaling. A defining characteristic of the AKAP family is the ability to shape the local environment through scaffolding both effectors and signal-terminating enzymes. This minireview has highlighted the advantage of AKAP signaling complexes in the organization of responses to second messengers. The examples we have used illustrate the utility of AKAPs as a family of cofactors that uphold the molecular organization of enzyme cascades and the fidelity of cell signaling events. Delineating these local environments will become increasingly more important to understanding

these pathways. Advances in mass spectrometry and the development and utilization of FRET-based reporters of kinase activity and second messengers inside living cells will greatly aid these efforts.

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