Section 4: β-Cell Stimulus-Secretion Coupling: Metabolic Factors

Intracellular Targeting of Protein Kinases and Phosphatases

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Compartmentalization of kinases and phosphatases is a key determinant in the specificity of second messenger–mediated signaling events. Localization of the cAMP-dependent protein kinase (PKA) and other signaling enzymes is mediated by interaction with A-kinase anchoring proteins (AKAPs). This study focused on recent advances that further our understanding of AKAPs, with particular emphasis on the bidirectional regulation of signaling events by AKAP signaling complexes and their contribution to the control of actin reorganization events. *Diabetes* 51 (Suppl. 3):S385–S388, 2002

Extracellular signals, such as hormones, neurotransmitters, and growth factors, regulate a wide variety of cellular activities, including ion channel modulation, neuronal excitation, cell growth, cell differentiation, and insulin secretion events (1). Intracellular transduction systems receive these signals via receptors and transmit them quickly and precisely, resulting in the amplification of specific biological responses. However, cells often are exposed to several messengers simultaneously, and maintaining the fidelity of these networks is crucial in eliciting the appropriate physiological response. Doing so requires the accurate selection of effector molecules for regulated activation and deactivation, often by phosphorylation and dephosphorylation events. A principal strategy in achieving this selection specificity is compartmentalization of signaling enzymes (2–4). This study highlights the most recent advances in our understanding of compartmentalization of multivalent signaling complexes by A-kinase anchoring proteins (AKAPs) and the functional consequences they mediate.

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Abl, Abelson tyrosine kinase; AKAP, A-kinase anchoring protein; mAKAP, muscle-specific AKAP; NMDA, *N*-methyl-D-aspartic acid; PDE4D3, cAMP-specific type 4 phosphodiesterase; PK, protein kinase; PKA, cAMP-dependent protein kinase; PP, protein phosphatase; RyR, ryanodine receptor; SSeCKS, Src-suppressed C kinase substrate; WASP, Wiskott-Aldrich syndrome gene.

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CYCLIC AMP-DEPENDENT PROTEIN KINASE

One of the best-characterized signaling pathways involves the activation of the cAMP-dependent protein kinase (PKA). PKA is a serine/threonine kinase composed of two catalytic (C) subunits that are held in an inactive state by association with a regulatory (R) subunit dimer (5–8). The catalytic subunits are expressed from three different genes—Cα, Cβ, and Cγ—whereas the R subunits are expressed from four different genes—RIα, RIβ, RIIα, and RIIβ (9–12). The R subunit is a modular polypeptide containing an NH_2-terminal dimerization domain, an autophosphorylation site that serves as a principal contact site for the C subunit, and two cAMP binding sites. Activation of PKA is solely accomplished by the major, diffusible secondary messenger cAMP (13,14). Binding of cAMP to each R subunit relieves the autoinhibitory contact, allowing the C subunits to dissociate (15,16), thereby resulting in phosphorylation of local substrates.

Two forms of the heterotetrameric PKA holoenzyme exist: type I (RIα and RIβ dimer) and type II (RIIα and RIIβ dimer). Type I PKA is predominantly cytoplasmic, whereas type II PKA associates with specific cellular structures and organelles (17). Discrete localization of type II PKA within the cell is chiefly caused by association with nonenzymatic scaffolding AKAPs (3,18,19). This method of regulation ensures that PKA is exposed to cAMP gradients locally generated by adenylate cyclases and phosphodiesterases, thus allowing for efficient catalytic activation and appropriate substrate selection (20). Recent biochemical evidence suggests that a family of dual-function AKAPs that bind RI or RII may exist (21). Although the RI-AKAP interaction has an affinity within the physiological range, it is 100-fold lower than the RII-AKAP interaction and has yet to be demonstrated in vivo (22,23).

A-KINASE ANCHORING PROTEINS

The first AKAPs were originally discovered as contaminants of type II PKA holoenzyme preparations (24,25), and the family has since grown to include over 50 members. AKAPs are structurally diverse proteins that contain an amphipathic helix that functions to bind the amino termini of the PKA-RII dimer (26–28). Each also contains a unique subcellular targeting domain that restricts its location within the cell (19). Thus, their classic role is to control the intracellular localization of PKA.

A newly emerging duty for AKAPs is to coordinate signaling complexes by recruiting multiple signaling enzymes near potential substrates, effectively joining upstream activators with downstream targets (29). The prototypic AKAP79, yotiao, and AKAP220 have already been shown to function in this capacity. For example, in neurons, yotiao binds to the C1 exon-containing NR1 subunit of *N*-methyl-D-aspartic acid (NMDA) receptors. It also binds to protein phosphatase 1 (PP1) and to PKA, mediating the localization of the opposing functions required to modulate NMDA receptor function (30,31). This mechanism effectively and physically permits the association of an entire signaling complex with a specific substrate. Because of the large size of many other AKAPs, it is logical to postulate that they also function to coordinate different signaling complexes. For example, the 300-kDa muscle-specific AKAP (mAKAP) associates with PKA, the phosphatases PP1 and PP2A, a phosphodiesterase, and the ryanodine receptor (RyR) (32–34). The advent of this concept creates a need to investigate the composition of these scaffolds and the biological functions they mediate. Understanding these may allow us to determine whether molecular aberrations disrupting these complexes can be linked to the progression of various disease states. The following sections highlight the role of AKAP signaling complexes in the control of bidirectional signaling of physiological processes and precise regulation of specific cellular events, such as actin reorganization. We hope that these examples emphasize the utility of AKAP signaling complexes in the control of cellular events and introduce the anchoring hypothesis as a viable regulatory mechanism that coordinates complex signaling events such as protein trafficking and insulin secretion from islet β-cells (35,36).

BIDIRECTIONAL REGULATION OF SIGNALING BY AKAP SIGNALING COMPLEXES

A valuable feature of some AKAP signaling complexes is the presence of signal transduction and signal termination enzymes in the same network. This creates focal points of enzyme activity where the bidirectional regulation of signaling events can be controlled and the phosphorylation status of target substrates is precisely regulated. A common scenario is the clustering of protein kinase and phosphatase activities. For example, AKAP450/CG-NAP, a large centrosomal AKAP of unknown function, has been reported to bind three kinases (protein kinase [PK] A, C, and N) and two phosphatases (PP1 and PP2A) (37–39). Likewise, the simultaneous association of PP1 and PKA with anchoring proteins such as AKAP149/D-AKAP-1 and AKAP 220 undoubtedly contributes to the bidirectional regulation of phosphorylation events at mitochondria, endoplasmic reticulum, and vesicular organelles (40,41). Thus, the possibilities for coordinated phosphorylation and dephosphorylation events mediated by the enzymes associated with AKAPs are numerous.

Two recent reports have demonstrated that phosphodiesterases, the enzymes that catalyze cAMP metabolism, are present in signaling complexes with PKA (34). These findings add a novel twist to PKA regulation, as they indicate that an anchored pool of phosphodiesterase may tightly control local cAMP levels. Dodge et al. (34) found that the muscle-selective mAKAP directly binds PKA and a splice variant of the cAMP-specific type 4 phosphodiesterase (PDE4D3). Simultaneously, Tasken et al. (42) reported the interaction of PDE4D3 with AKAP450, a large centrosomal AKAP in testicular Sertoli cells. Biochemical and immunofluorescent analyses have indicated that both enzymes are constitutively associated with the centrosomes during the interphase of the cell cycle. The implications of both studies are that the role of PDE4D3 within these complexes is to depress cAMP levels within the vicinity of anchored PKA. At rest, PDE4D3 inhibits basal PKA activity associated with mAKAP, possibly acting to dampen noise and increase gain in the system. Furthermore, PKA phosphorylation is known to upregulate PDE4D3 activity two- to threefold, establishing a negative feedback loop that rapidly terminates the cAMP signal. It would appear that the close proximity of PDE4D3 and PKA in the mAKAP signaling complex facilitates this process, as peptide-mediated displacement of the kinase from the signaling complex prevents the phosphorylation and upregulation of the phosphodiesterase. Although PDE4D3 is a substrate for the kinase, it is clear that there are other PKA substrates associated with the mAKAP scaffold. For example, the regulation of RyR phosphorylation is important for maintaining contractility in response to α-adrenergic signaling and increases in intracellular Ca^{2+} concentration in the heart. Hyperphosphorylation of sarcoplasmic reticulum RyR leads to increased Ca^{2+} sensitivity of the channel and decreased sensitivity to α-adrenergic stimulation. These changes are manifest in human heart tissue undergoing heart failure, where changes in RyR phosphorylation are also detected (33). The abnormal regulation of RyR function may be attributable to several factors that regulate cAMP/PKA signaling in heart, including loss of phosphatase activity from the RyR complex and defects in regulation of cAMP levels by PDE activity associated with the complex. Thus, the composition and assembly of this signaling network may be altered in disease states.

TARGETING PKA TO THE ACTIN CYTOSKELETON

The actin cytoskeleton mediates a variety of essential biological functions in all eukaryotic cells, including the establishment of cell shape, polarity, motility, and division (43). A fundamental question is how cells integrate signals from a variety of pathways to control the precise location and timing of actin polymerization. A key role is played by members of the Rho family of small GTPases (Rho), which have emerged as the principal mediators of signals emanating from transmembrane receptors to actin filament nucleation (44). The most extensively characterized members are Rho, Rac, and cdc42, which control the formation of actin stress fibers, lamellipodia, and filopodia, respectively. These distinct actin remodeling events are the consequence of the selective interaction of the activated Rho GTPases with specific effector proteins. Recent evidence that actin-binding proteins such as gravin, an antigen for the autoimmune disease myasthenia gravis, and WAVE, a member of the Wiskott-Aldrich syndrome protein (WASP) family of adapter proteins, are AKAPs (45,46).

Gravin originally was identified as a cytoplasmic antigen recognized in sera from patients with myasthenia gravis, an autoimmune degenerative disease that primarily affects

transmission at the neuromuscular junctions (47). Furthermore, cloning and a more complete biochemical characterization revealed that gravin is a multivalent kinase scaffold protein of 250 KDa that interacts with PKA, PKC, and actin (45). Gravin shares significant homology with Src-suppressed C kinase substrate (SSeCKS) (also called clone 72), a cell cycle-regulated myristylated PKC substrate that also binds to PKA and actin (48). Ectopic expression of SSeCKS in NIH-3T3 fibroblasts has been shown to cause significant cell flattening, the loss of actin stress fibers, and the elaboration of SSeCKS-associated filopodia-like projections. In addition, SSeCKS has been implicated in the process of cell migration during mouse embryogenesis (48). Recently, gravin has been shown to interact with the β2-adrenergic receptor in human epidermoid carcinoma cells (49). Inhibition of gravin expression in these cells using antisense oligonucleotides disrupts recycling of the β2-adrenergic receptor after agonist-induced desensitization (49).

Other AKAPs also participate in the regulation of actin remodeling events (4). The WASP family of proteins currently consists of five members: WASP, N-WASP, Scar-1, and three WAVE isoforms. WASP, the founding member of the family, is mutated in Wiskott-Aldrich syndrome, an X-linked human immunodeficiency disease (50). The WASP homolog N-WASP is expressed ubiquitously in vertebrate cells and causes the formation of filopodia when co-expressed with cdc42 (51). Scar-1 was discovered in *Dichtyostelium*, in a genetic screen for proteins downstream of the chemotaxis receptor for cAMP, cAR2 (52). More recently, three mammalian orthologs of Scar-1, termed WAVE-1, WAVE-2, and WAVE-3 that are involved in Rac1-induced actin reorganization, have been cloned.

Recently, we showed that WAVE-1 binds to both PKA and the Abelson tyrosine kinase (Abl) (46). Abl binding appears to be a common characteristic of the WAVE family, as WAVE-2 and WAVE-3 also interact with the Abl SH3 domain. To the contrary, only the WAVE-1 isoform binds to PKA. Interestingly, the RII binding region of WAVE-1 overlaps with a verprolin homology (VPH) domain that previously has been characterized as a binding site for G-actin. In vitro competition experiments have shown that actin competes for the RII-binding site (46). This might provide a mechanism for the regulation of PKA anchoring at sites of actin reorganization where the local actin concentration may be sufficient to displace the anchored PKA. Another interesting property of the WAVE isoforms is their ability to homo- and heterodimerize. This provides an additional level of organization, as signaling units containing various WAVE isoforms may be nucleated at distinct sites of actin reorganization. Finally, assembly of the WAVE-1 signaling complex is dependent on extracellular stimuli. Activation of Rac upon application of platelet-derived growth factor (PDGF) results in a rapid redistribution of WAVE-1, PKA, and Abl to lamellipodia and actin ring structures at the periphery of the cell (46). Dynamic assembly of WAVE signaling complexes may represent a sophisticated mechanism to coordinate the location and action of PKA and Abl, in response to extracellular signals.

CONCLUSIONS

Control of signal transduction specificity is crucial for eliciting proper physiological responses. Compartmentalization of PKA by AKAPs provides an important molecular mechanism to ensure specific activation and appropriate substrate selection. However, although we have a significant appreciation for the biochemical role that AKAPs play in PKA signaling, we are still on the cusp of understanding the extent to which AKAPs coordinate other signaling and adaptor proteins. The findings also have generated new insights into the dynamics of the AKAP-coordinated signaling complexes. The many ways in which AKAPs create intracellular signaling specificity are highly sophisticated. Undoubtedly, a variety of genetic approaches to generating relevant biological models as well as proteomic approaches to identifying new AKAP interactors will be instrumental in defining the precise functional roles of AKAPs in many physiological processes.

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