A-kinase-anchoring proteins

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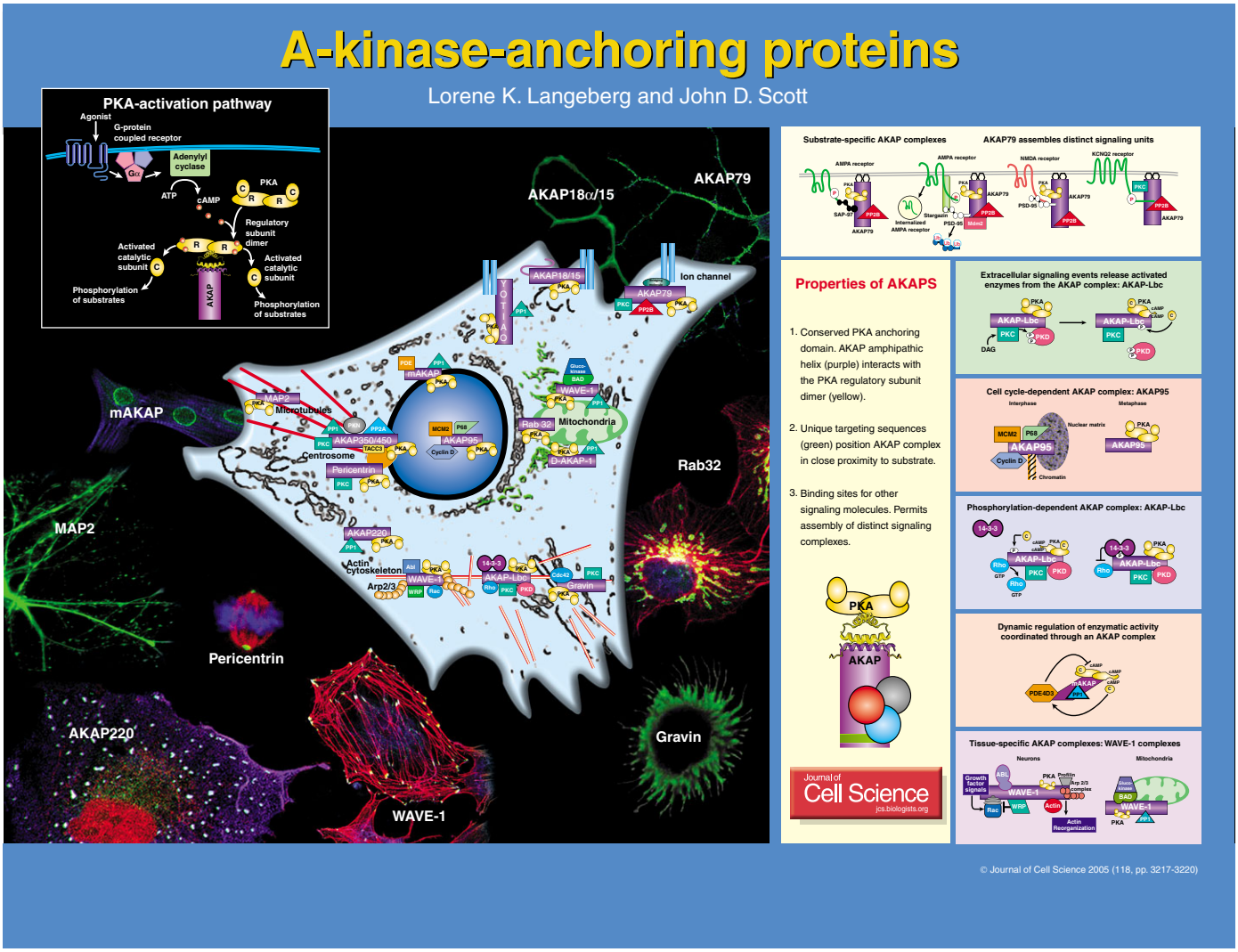
Highly organized molecular complexes determine the precise location and timing of the signal transduction events that occur downstream of cell surface receptor activation (Pawson and Nash, 2003). One set of scaffolding molecules that organize such complexes is a family of proteins known as A-kinase-anchoring proteins (AKAPs). AKAPs provide a framework for the coordination of phosphorylation and dephosphorylation events by sequestering enzymes such as protein kinases and phosphatases with appropriate substrates. AKAPs also bring together signal transduction and signal termination molecules in a convergence of signaling pathways. Indeed, the dynamic assembly of these AKAP complexes represents a paradigm of higher-order signal organization (Wong and Scott, 2004).

AKAPs were first identified as a family of structurally diverse but functionally related proteins that share the capacity to bind protein kinase A (PKA) (Lohmann et al., 1984; Theurkauf and Vallee, 1982). The hydrophobic face of a conserved amphipathic helix within AKAPs anchors PKA through interaction with an N-terminal four-helix bundle in the regulatory subunit (R) dimer (Carr et al., 1991; Newlon et al., 1999). In addition, a distinct region of the AKAP contains a targeting sequence that serves to tether the complex to a specific subcellular compartment (Colledge and Scott, 1999). Anchoring of the kinase facilitates localized activation of the PKA catalytic subunit (C) following elevation of the second messenger cyclic AMP (cAMP). Importantly, it has been shown by many labs that, in addition to directing the action of PKA, AKAPs engage other signaling molecules (Colledge and Scott, 1999; Tasken and Aandahl, 2004). This enables the construction of enzyme complexes for the integration and dissemination of information at specific sites within the cell. These complexes are assembled in



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dynamic response to upstream signals and are often tethered close to specific substrate proteins. Combined, these factors provide an element of spatial and temporal regulation to AKAP-mediated signaling events.

AKAP79 and its ortholog AKAP150 maintain substrate-specific complexes in association with a variety of ion channels. Surface expression and modulation of AMPA-type glutamate receptor (GLuR) function in the hippocampus are regulated by AKAP-directed phosphorylation and dephosphorylation. In complex with SAP-97, AKAP79 facilitates PKA-mediated phosphorylation of Ser845 on GluR1 to potentiate AMPA receptor function (Colledge et al., 2000). Likewise, AKAP79-targeted protein phosphatase 2B (PP2B) may serve to dephosphorylate GluR1 and permit channel rundown (Dell'Acqua et al., 2002). Other AMPA-type channels bind to the related MAGUK protein PSD-95, which forms a bridge between AKAP79 and GluR1 via the protein Stargazin. This brings the AKAP-associated phosphatase PP2B into close proximity to dephosphorylate the PSD-95-associated ubiquitin E3 ligase Mdm2. Dephosphorylation of Mdm2 leads to ubiquitylation and degradation of PSD-95 and the concomitant internalization of AMPA receptors (Colledge et al., 2003). A PSD-95 bridge also links the AKAP79 complex to NMDA receptors, although less is know about the regulation of this interaction (Schnell et al., 2002). In sympathetic neurons of the brain, the association of AKAP79 with the KCNQ2 channel and AKAP79-directed phosphorylation of KCNQ2 by PKC primes the channel to allow a lower threshold for agonist-dependent inhibition (Hoshi et al., 2003). In the context of each of these ion channel complexes, the same anchoring protein serves as an adapter that brings together a specific subset of binding partners to regulate channel function. AKAP79-mediated phosphorylation and dephosphorylation events thus modulate ion channel function in a substrate-specific manner.

Intracellular targeting of AKAPs serves to position distinct signaling complexes at or on a given organelle. For example, AKAP220 targets PKA, protein phosphatase 1 (PP1), and glycogen synthase 3β (GSK3β) to vesicles (Schillace et al., 2001; Tanji et al., 2002). Similarly, Rab32, a member of the Ras superfamily of small GTPases, anchors PKA at mitochondria (Alto et al., 2002). Pericentrin and another anchoring protein, AKAP350/450, both target PKA and protein kinase C (PKC) to sites within the centrosome. However, the latter can also anchor the additional binding partners PP1, PP2B, phosphodiesterase (PDE), protein kinase N (PKN), TACC3 and CLIC4 (Diviani et al., 2000; Chen et al., 2004; Steadman et al., 2002). The repertoire of AKAP targeting may also be increased through transcriptional regulation. Alternative splicing gives rise to a family of AKAPs: AKAP350, AKAP450, CG-Nap and Yotiao (Witczak et al., 1999; Schmidt et al., 1999; Takahashi et al., 1999; Westphal et al., 1999). These AKAPs bring together kinases, phosphatases, phosphodiesterases, receptors and channels in important signaling pathways at three distinct sites within the cell: the centrosome, the Golgi, and the plasma membrane (Wong et al., 2001). By assembling various signaling partners, these AKAPs are able to modulate cell cycle progression, membrane trafficking and channel function.

AKAPs expressed in different parts of the body can assemble tissue-specific signaling networks. A signaling complex in the brain organized by WAVE-1 directs a Rac-mediated actin reorganization that is involved in neurite outgrowth (Machesky, 2000; Westphal et al., 2000). In addition to PKA and actin, this neuronal WAVE-1 complex includes Abl, Arp2/3 and the Rac-terminating GTPase-activating protein (GAP) WRP (Soderling et al., 2002). Interestingly, in liver and sperm, the same AKAP, WAVE-1, nucleates a very different mitochondrial-sheath-targeted complex that includes the BCL2-antagonist of cell death BAD, glucokinase, protein phosphatase PP1, and PKA (Danial et al., 2003; Rawe et al., 2004).

The complement of AKAP-binding partners is dynamically responsive to and regulated by intracellular signals. For example, AKAP-Lbc synchronizes PKA and PKC phosphorylation events that lead to the activation of protein kinase D (PKD). Diacylglycerol-mediated activation of PKCη in the AKAP-Lbc complex leads to phosphorylation and activation of the co-anchored PKD (Carnegie et al., 2004). In parallel, cAMP-dependent phosphorylation of AKAP-Lbc Ser2737 by PKA reduces the affinity of the interaction between the AKAP and PKD, releasing the activated PKD from the complex. AKAP-Lbc brings together the essential modulators of this PKD activation cascade downstream of the extracellular signaling events.

Dynamic regulation of enzymatic activity is coordinated through AKAP complexes. The muscle-specific AKAP (mAKAP) tethers both PKA and the cAMP-specfic phosphodiesterase PDE4D3 to the nuclear membrane of cardiac myocytes (Dodge et al., 2001). As local cAMP levels rise, the activated C subunit of PKA is released. Phosphorylation of PDE4D3 by the kinase enhances the binding affinity between PDE and mAKAP and increases the metabolism of cAMP by the phosphodiesterase twofold. As PDE4D3 activity lowers cAMP levels, this feedback loop generates localized pulses of PKA activity. Similarly, another AKAP maintains a phosphorylation-dependent pathway to regulate Rho activity. AKAP-Lbc possesses Rho-specific guanine nucleotide exchange factor (GEF) activity. Phosphorylation of AKAP-Lbc at Ser1565 by its associated PKA creates a phospho-specific 14-3-3-binding site. The subsequent interaction of 14-3-3 with this site inhibits the Rho-GEF activity and thus the activation of Rho (Diviani et al., 2004; Jin et al., 2004).

Proteins that have been identified as AKAPs can have scaffolding functions that, at times, are temporally distinct from their PKA-targeting function. In interphase cells, the nuclear AKAP95 primarily associates with the nuclear matrix whereas a small fraction complexes with chromatin (Coghlan et al., 1994; Eide et al., 2002). During mitosis, as the nuclear structures breakdown, AKAP95 interacts with the RII subunit of PKA. As the daughter cell nuclei reform, AKAP95 appears once again to segregate from the kinase. Cyclin D, p68 RNA helicase, and the minichromosome

maintenance 2 protein (MCM2) have all been shown to interact with the interphase AKAP95, and nuclear signaling complexes may contain subsets of these binding partners (Akileswaran et al., 2001; Arsenijevic et al., 2004; Eide et al., 2003). Consequently, AKAP95 serves as a scaffold for coordinating assembly of nuclear complexes for replication and transcription and has a role in PKA targeting at distinct stages of the cell cycle.

AKAPS thus provide more than just an address for PKA, and our understanding of their roles has moved from identification of them as mere kinase-targeting molecules to platforms for the convergence and integration of highly organized signaling pathways.

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