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Molecular Glue: Kinase Anchoring and Scaffold Proteins

Minireview

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Introduction

Now that approximately 300 of the anticipated 2000 protein kinase and 1000 phosphatase genes have been identified, the challenge facing researchers is to figure out how these enzymes interface to control cellular events. Not surprisingly, regulation of kinase and phosphatase activity is a sophisticated process that is achieved on many levels. One emerging mechanism is that the subcellular distribution of several kinases and phosphatases is restricted by association with targeting proteins or subunits (reviewed by Mochly-Rosen, 1995). A variation on this theme is the formation of kinase–phosphatase signaling complexes to modulate the phosphorylation state of specific target substrates. Coordination of these complexes is achieved by multivalent binding proteins which serve as platforms for the assembly of these signaling units. The first signaling complexes identified centered around tyrosine phosphorylation events. The action of many tyrosine kinases (PTKs) and tyrosine phosphatases (PTPases) is coupled to downstream cytoplasmic enzymes through adapter proteins that contain SH2 and SH3 domains (reviewed by Pawson, 1995). Modular proteins like Grb2, p85, IRS-1, Crk and Nck consist of a single SH2 domain that recognizes certain phosphotyrosyl residues on signaling enzymes, and two SH3 domains that bind to a PXXP motif on a separate set of target proteins including cytoskeletal components or small molecular weight G-proteins (Pawson, 1995). Through these interactions signaling complexes are formed which generally emanate from receptor and nonreceptor PTKs and mediate cellular processes including growth factor signaling events, insulin action and platelet aggregation.

Signaling complexes of serine/threonine kinases and phosphatases are only now being identified. The molecular "glue" for these complexes is provided by two related but distinct classes of adapter protein. Scaffold proteins which simultaneously associate with several kinases of a signaling pathway, forming an ordered module that permits sequential activation of each enzyme (Figure 1A) and anchoring proteins which are tethered to subcellular structures and localize their complement of enzymes close to their site of action (Figure 1B). This minireview compares and contrasts the properties of scaffold and anchoring proteins which maintain kinase–phosphatase signaling complexes.

The Ste5p Complex

Ligand activated signal transduction pathways link cell surface receptors or activation of tyrosine kinases to changes in gene expression. In many cases this occurs through a series of protein-protein interactions that trigger the mitogen activated protein (MAP) kinase cascade. This pathway proceeds from the membrane bound guanine nucleotide-binding protein Ras, through the sequential activation of the cytoplasmic serine/threonine kinases Raf, MAP-kinase kinase (MEK), and MAP kinase (MAPK) and leads to specific gene expression in the nucleus. In the budding yeast *S. cerevisae*, there are at least five MAP kinase cascades. Each pathway is initiated by a distinct upstream regulator and individual MEKK-MEK-MAPK modules control mating, cell-wall integrity, pseudohyphal development and invasive growth, sporulation and osmoregulation (reviewed by Herskowitz, 1995; Levin and Errede, 1995). The kinases in these pathways may be segregated through association with scaffold proteins. The pheromone mating response is initiated through ligand induced changes in G-protein linked receptors to activate a kinase, Ste20p. This leads to the stimulation of Ste11p, a MEKK homolog, which phosphorylates and activates Ste7p, a MEK homolog, which in turn, phosphorylates and activates the MAPK homologs, Fus3p or Kss1p. This signaling pathway is tightly controlled because each enzyme associates with a scaffold protein called Ste5p (Figure 2). Sterile 5 was initially identified as a recessive mutation in the yeast mating pathway twenty years ago and has recently been shown to encode a 917 amino acid protein. Initial clues to its function came from yeast two-hybrid experiments reported by three groups which demonstrated that Ste11p and Ste7p bind to distinct sites on Ste5p while Fus3p and Kss1p compete for another site on the protein (Figure 2). These findings were confirmed through deletion analysis demonstrating that a Ste5p 1-336 fragment binds Fus3p or Kss1p whereas the Ste5p 336-917 fragment only binds Ste7p and Ste11p. Recent studies suggest that there may be additional components of the complex as the G protein β subunit, STE4 and possibly Ste20p interact with Ste5p (Leeuw et al., 1995, Whiteway et al., 1995) (Figure 2). On

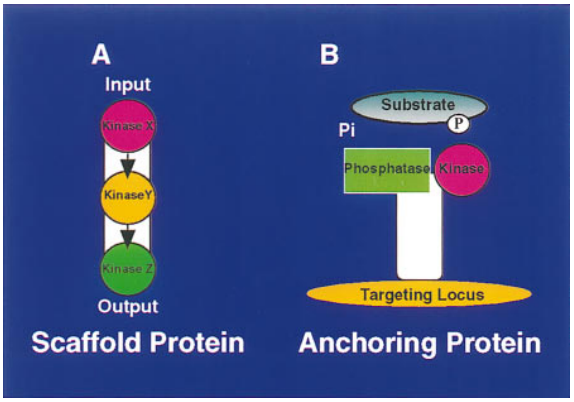

Figure 1. Localization of Kinases and Phosphatases through Scaffold or Anchoring Proteins

A) A schematic diagram depicting the topology of a prototypic kinase signaling scaffold and B) an anchored kinase–phosphatase complex.

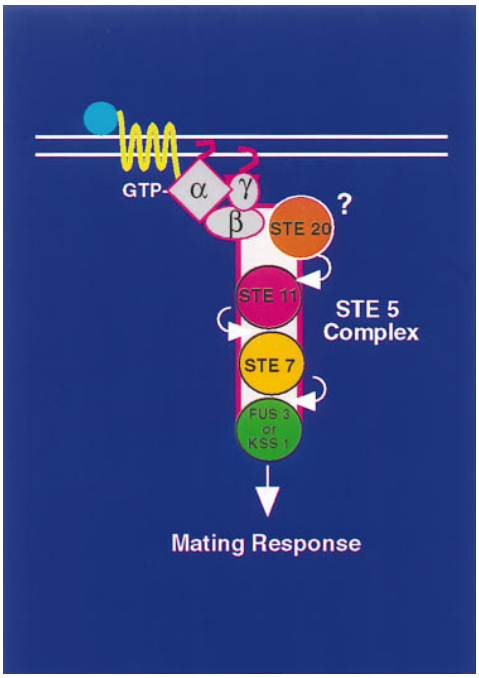

Figure 2. The Ste5p Signaling Scaffold

The activities of five signaling molecules, the G-protein β subunit, possibly Ste20p, Ste11p, Ste7p amd Fus3p or Kss1p are coordinated through their association with the scaffold protein Sterile 5. This complex includes the upstream components of the S. cerevisae pheromone mating pathway.

the basis of these and other biochemical studies with bacterially expressed proteins, it is clear that Ste5p possesses all the qualities of a "classic" scaffold protein as each enzyme binds to a distinct region of the protein (Figure 2). However, additional protein-protein interactions exist between each of the kinases in the absence of the scaffold protein, as two-hybrid studies indicate that Ste7p and Ste11p interact with either Fus3p or Kss1p in yeast strains lacking Ste5p.

Although components of the Ste5p complex have been identified, the regulatory role of this signaling scaffold remains to be determined. Obviously, the clustering of successive members in the MAP kinase cascade is optimal for the tight regulation of the pathway. Regulation could occur through assembly and disassembly of the complex or by dephosphorylation by a phosphatase such as Msg5p (Doi et al., 1994). It has also been proposed that scaffold proteins such as Ste5p may provide some selectivity by preventing cross-talk between functionally unrelated MAP kinase modules in the same cell. Indeed, a recent study using MEK gain-of-function mutations in *S. cerevisiae* has demonstrated that Ste5p limits interactions of Ste7p with other MAPK activation pathways, contributing to Ste7p specificity (Yasher et al., 1995). Ste5p is also able to maintain two different combinations of kinases Ste11p/Ste7p/Fus3p or Ste11p/Ste7p/Kss1p. An additional level of regulation could be obtained if the Ste5p complexes are differentially compartmentalized or additional enzymes associate with the scaffold. A likely candidate would be the phosphatase Msg5 which is known to dephosphorylate Fus3p (Doi et al., 1994). Clearly, the demonstration that members of a yeast MAP kinase cascade are physically associated through a scaffold protein begs the question of whether a similar signaling scaffold exists in mammalian cells.

The AKAP79 Signaling Complex

Several targeting proteins have been identified which anchor single kinases or phosphatases close to their specific substrates. For example, the type II cAMP-dependent protein kinase (PKA) is targeted by association of its regulatory subunit (RII) with *A-K*inase *A*nchoring *P*roteins, called AKAPs. PKC is tethered to the cytoskeleton or at submembrane sites through association with a family of substrate/binding proteins sometimes called Racks for Receptors for Activated C Kinase (Mochly-Rosen, 1995). The type 1 phosphatase is localized at glycogen particles, endoplasmic reticulum and nucleus through association with targeting subunits and the heterotrimeric phosphatase 2A is compartmentalized through interaction of its β subunit with microtubule and nuclear elements (Sontag et al., 1995).

In order to fulfill their function, it is likely that anchoring proteins contain at least two functional domains: an enzyme binding site and a targeting site responsible for anchoring to subcellular structures. Until recently, all of the evidence suggested that each kinase or phosphatase was targeted through association with its own anchoring protein. However, it now appears that at least one anchoring protein binds more that one enzyme at a time. In neurons, PKA is localized at postsynaptic densities by association with an anchoring protein called AKAP79. Biochemical studies show that AKAP79 also binds the Ca^{2+} calmodulin-dependent protein phosphatase 2B, calcineurin (Coghlan et al., 1995), as well as α and β isoforms of PKC (Klauck et al., 1996) (Figure 3). The structure of AKAP79 is modular and seems to resemble Ste5p in that deletion analysis, peptide studies and coprecipitation techniques have demonstrated that each enzyme binds to a distinct region of the anchoring protein. Targeting of the AKAP79 signaling complex to the postsynaptic densities suggests a model for reversible phosphorylation in which the opposing effects of kinase and phosphatase action are colocalized in a signal transduction complex by association with a common anchor protein (Figure 3). Potential substrates for the AKAP79 transduction complex are likely to be synaptic receptor/channels and may include AMPA/kainate receptors and Ca^{2+} channels which have recently been shown to be modulated by AKAP-targeted PKA, and NMDA receptors which are activated by PKC and attenuated by calcineurin (reviewed by Klauck and Scott 1995). The regulation of the AKAP79 complex is not fully understood, however, each enzyme is inhibited when bound to the anchoring protein suggesting that there is tight control of each enzyme when associated with the complex.

The apparent coordination of synaptic signaling by a modular anchoring protein raises the intriguing possibility that AKAP79 displays a similar function to the Ste5p scaffold. Both Ste5p and AKAP79 provide an additional level of regulation for protein phosphorylation events by restricting the action of kinases and phosphatases through protein-protein interactions. However, there are

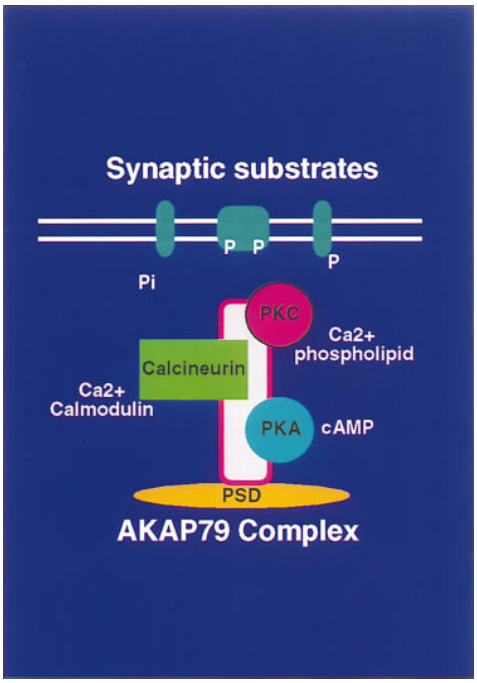

Figure 3. The AKAP79 Signaling Complex

The anchoring protein AKAP79 is attached to the postsynaptic densities and maintains PKA, PKC and calcineurin close to substrates in the postsynaptic membrane.

important differences. While AKAP79 dictates the subcellular location of two multifunctional kinases and a broad specificity phosphatase, Ste5p orchestrates the location and activation of three interrelated kinases. To insure efficient transduction of this signal, both intermediary kinases Ste11p and Ste7p have limited substrate specificity and bind to precise sites on the scaffold protein. AKAP79, on the other hand, restricts the location of PKA, PKC and calcineurin, enzymes with broad specificity. Another distinction is that the AKAP79 complex is able to respond to three distinct activation signals, whereas a single upstream event, the activation of Ste20p, is sufficient to transduce a signal from one kinase to the next in the Ste5p signaling scaffold. Despite these differences, the similarities between AKAP79 and Ste5p provide an oppportunity to speculate on other proteins involved in signaling that may also serve as a scaffold.

Raf Activation and 14-3-3?

A pivotal event in the activation process of the MAP kinase cascade in mammalian cells is the membrane targeting of a Raf signaling complex and its transient association with Ras-GTP. One aspect of the Raf activation process could be the recruitment of a multienzyme signaling complex to the plasma membrane. Wartman and Davis (1994) reported that Raf exists as part of a membrane associated signaling complex which includes Ras, the molecular chaperones, hsp90 and p50, and MEK, the next kinase in the cascade. Hot on their heels, six groups simultaneously reported that Raf interacts with the 14-3-3 proteins, suggesting that 14-3-3 proteins may play a role in the coordination of signal transduction pathways. However, the role and significance of the Raf–14-3-3 interaction remains poorly understood. For example, functional studies in yeast suggest that 14-3-3 acts as a cofactor that is necessary, but not sufficient, for Raf activation (Irie et al., 1994), whereas others have reported that 14-3-3 does not directly activate Raf (Michaud et al., 1995). In addition, the 14-3-3 proteins, an abundant family of cytoplasmic proteins, have already been reported to play many diverse roles such as membrane targeting, possessing phosphlipase A2 activity, activation of tyrosine hydroxylase, and inhibition of PKC (Aitken 1995).

The recently solved crystal structure of 14-3-3 (Xiao et al., 1995; Liu et al., 1995) provides some clues to its role in association with cellular proteins. 14-3-3 is a dimer consisting of two subunits separated by a long negatively charged channel. All target proteins are believed to recognize a common binding groove lined with residues invariant in all 14-3-3 family members. Liu et al. (1995) suggest a general mechanism where binding occurs through an amphipathic helix allowing many diverse proteins to interact with 14-3-3. Furthermore, the large channel between the proposed binding sites may permit more than one kinase to bind leading Xiao et al. (1995) to hypothesize that 14-3-3 may function as a scaffold. It is possible that heterodimers of different 14-3-3 partners allow the formation of a complex between two distinct protein kinases. Another possibility is that the indiscriminate specificity of each binding site may accommodate many target proteins allowing two kinases to associate with a 14-3-3 homodimer. There are some additional reports that may be relevant to a role for 14-3-3 in Raf activity. PKC-α phosphorylation activates Raf and it is tempting to speculate that both kinases may be colocalized through association with 14-3-3. Indeed, recent evidence suggests that Raf and Bcr associate in a ternary complex mediated by the 14-3-3 dimer (Braselmann and McCormick, 1995). A ternary complex has also been reported between Ras, Raf, and 14-3-3 (Luo et al., 1995), and it will be of interest to establish whether MEK, which has been reported to be a loosely associated component of the Raf signaling complex, associates with 14-3-3 (Wartmann and Davis, 1994).

Conclusions

Although conceptually similar, there are distinct differences between all three signaling complexes. While Ste5p and AKAP79 both coordinate the access of their associated enzymes to substrates, AKAP79 interacts with three multifunctional enzymes that are activated independently by second messengers, whereas Ste5p coordinates the action of several kinases in response to a single activation event. Although it may seem counterintuitive to a classical view of signal transduction, signals passing through the Ste5p complex are not amplified. In fact, Ste5p may provide a framework that segregates one signaling event from the next, as there appear to be at least five distinct MAP kinase cascades in yeast. A unique feature of AKAP79 is that it localizes PKA, PKC, and calcineurin to the same subcellular sites, which potentially provides a mechanism to modulate the phosphorylation state of a single substrate protein in response to distinct second messenger signals. In contrast, the relaxed binding specificity of 14-3-3 allows

the formation of complexes with different combinations of target proteins and kinases. This may permit the integration of different activation signals depending upon which kinases are bound to the 14-3-3 dimer. Despite these subtle differences, a common denominator between these adapter proteins is that they restrict the movement and increase the intracellular organization of serine/threonine kinases and phosphatases. Both of these properties facilitate the diversity of signaling pathways that can be generated from connecting a few enzymes together.

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