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important proteins whose functions depend on post-transcriptional control are being identified, this study provides a further motivation to apply tools for gene identification that take these modes of regulation into consideration.

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DEP Domains: More Than Just Membrane Anchors

The DEP domain is present in a number of signaling molecules, including Regulator of G protein Signaling (RGS) proteins, and has been implicated in membrane targeting. New findings in yeast, however, demonstrate a major role for a DEP domain in mediating the interaction of an RGS protein to the C-terminal tail of a GPCR, thus placing RGS in close proximity with its substrate G protein α subunit.

G protein-coupled receptors (GPCRs) transduce extracellular cues through their cognate G proteins, which consist of three subunits, α, β, and γ, that form a heterotrimeric complex (Hamm, 1998). Given the critical role of GPCRs in mediating divergent functions of cells and their adaptation to the environment, it is not surprising that GPCR signaling is subjected to stringent control. Whereas the binding of ligands to receptors initiates signals that are amplified through G proteins and effectors, it also activates negative feedback mechanisms to desensitize signaling. These negative feedback regulations act at almost every step of signal transmission, starting from the receptor. Key players in these desensitization processes are regulator of G protein signal (RGS) proteins, which decrease the intensity and limit the duration of G protein signaling by stimulating the intrinsic GTPase activities of Gα subunits (Abramow-Newerly et al., 2006).

The core RGS domain confers GTPase-activator protein (GAP) activity. It has been known for some time that many RGS proteins show little GAP selectivity toward Gα subunits in vitro but act in a GPCR-dependent manner in vivo. Recent studies indicate that the answer to these paradoxical findings may lie in additional domains of RGS proteins that bind to other cellular proteins and promote substrate specificity of the RGS GAP domain. In a study published recently in *Cell*, Ballon and colleagues demonstrated that the DEP domain contained in the N-terminal extension of a yeast RGS protein, Sst2, mediates the interaction of Sst2 with the C-terminal tail of its cognate GPCR Ste2, thus placing Sst2 in the vicinity of its substrate Gα subunit Gpa1 (Ballon et al., 2006).

The DEP domain is a stretch of ~90 conserved residues that was first identified in three proteins, Disheveled, EGL-10, and Pleckstrin (Ponting and Bork, 1996). Highly homologous domains are also found in a number of proteins involved in signal transduction, such as Epac2, yeast RGS protein Sst2, and the R7 subfamily (RGS6, 7, 9, and 11) of mammalian RGS proteins. Earlier studies in several proteins have pointed out a function for DEP domains in mediating membrane localization (Wong et al., 2000). Recent data indicate that the DEP domain of RGS9-2 directs its RGS activity toward D2 dopamine, but not M2 muscarinic receptor-mediated signaling pathways (Kovoor et al., 2005). However, the molecular mechanisms underlying this DEP domain-dependent selectivity are unknown.

Ballon et al. (2006) set out to identify binding partners of the DEP domain in Sst2, a yeast RGS protein. They screened a library of overexpressed genes to identify proteins that can suppress the defect in pheromone responses caused by a point mutation in the DEP domain of Sst2. Surprisingly, they pulled out the α-factor receptor Ste2. Using a combination of biochemical, genetic, and cell imaging approaches, they have confirmed an interaction between Sst2 and Ste2 and demonstrated that this interaction is mediated by the DEP

domain of Sst2 and the C-terminal tail of Ste2. More importantly, they have shown that the association of Sst2 with Ste2 is controlled by the activation state of the receptor. While Sst2 exhibits high-affinity binding to the C-terminal tail of Ste2 when it is unphosphorylated, agonist-stimulated phosphorylation of the tail prompts their dissociation. Thus, by association with the cytoplasmic tail of Ste2 prior to its activation, Sst2 is placed in close proximity to its substrate Gα subunits, readily available to turn off G protein signaling either due to spontaneous activation or pheromone stimulation.

The findings that the DEP domain contained in Sst2 is necessary and sufficient for the interaction of Sst2 with a GPCR have important implications for understanding the role of this functional motif in a number of signaling proteins. For example, it may explain how the DEP domain in RGS9-2 can target its RGS activity specifically to D2 dopamine, but not M2 muscarinic receptor-mediated, signaling pathways (Kovoor et al., 2005). In addition, it may suggest a mechanism for the signal transmission from seven-pass Frizzled receptors to DEP-containing Disheveled proteins in the Wnt signaling pathway. It is known that the Disheveled proteins play a key role in the transduction of the Wnt signal from the Frizzled receptor to three different downstream pathways, but the underlying mechanisms have not been identified (Malbon and Wang, 2006).

While the findings of Ballon et al. have identified a new functional motif that mediates the interaction of RGS with a receptor, RGS proteins have been shown to increase their selectivity toward a particular Gα subunit by a variety of mechanisms (Abramow-Newerly et al., 2006). For example, direct binding to the intercellular loops of GPCRs has been shown for RGS2 and RGS12, while RGS2 and RGS19 may form a complex with receptors by association with scaffolding proteins spinophilin and GIPC, respectively (Figure 1). RGS proteins may also achieve their target specificity by cooperation with downstream effectors of the Gα subunit, as in the case of regulation of phototransduction where RGS9-1 cooperates with the effector protein PDEγ to turn off transducin signaling (Figure 1) (He et al., 1998). Interestingly, a splice variant of RGS9-1, RGS9-2, contains a C-terminal domain that bears sequence similarity to PDEγ and mimics PDEγ to provide an additional high-affinity interaction with its target Gαo subunit (Martemyanov et al., 2003). A similar scenario has been proposed for the action of RGS12 and RGS14, which contain a second Gα binding region (GoLoco) that may enhance the interaction of the RGS domain with its cognate Gα subunit (Figure 1) (Abramow-Newerly et al., 2006).

Although the work by Ballon et al. (2006) paves the way for understanding the function of DEP domains in signal transduction, much remains to be understood. For example, what is the structural basis for the recognition of a particular GPCR by the DEP domain? NMR structures for the Disheveled DEP domain have identified an electrostatic dipole that may mediate the interaction of the DEP domain with other proteins (Wong et al., 2000). Although Ballon et al. showed that mutating a residue within Sst2's dipole abrogates its interaction with the receptor Ste2, the dipole is either absent or not pronounced in the DEP domains of other RGS proteins (Civera et al., 2005). Moreover, the stoichiometric relationships of the DEP/GPCR interaction are unclear. There are two DEP-like domains in the N terminus of Sst2, and both are required for its RGS activities. Do both DEP domains bind simultaneously to one cytoplasmic tail of a GPCR, or is a single DEP domain sufficient to associate with a GPCR? If the latter is true, could the two DEP domains bind a GPCR dimer?

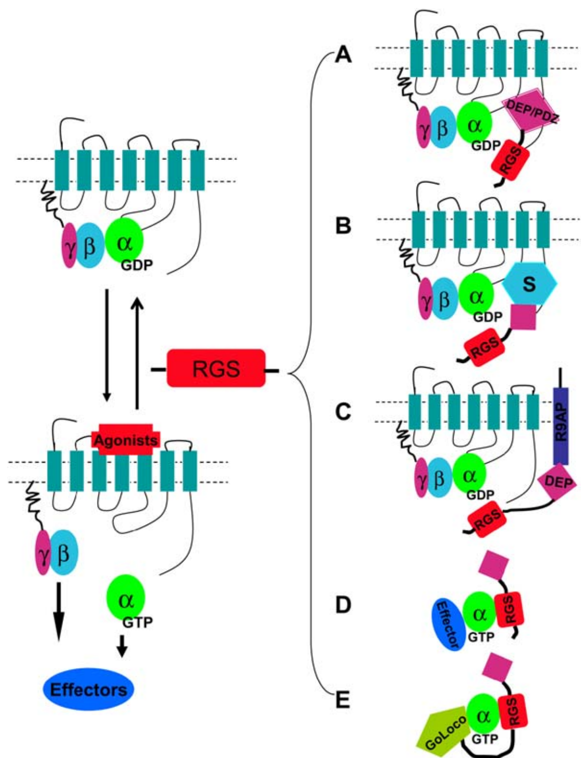

Figure 1. Schematic Representation of How RGS Proteins Regulate G Protein Signaling

In the inactive state, Gα subunits bind GDP and form a heterotrimeric complex with Gβγ subunits. Upon receptor activation, GTP exchanges for GDP on Gα subunits, leading to the dissociation of GTP bound Gα subunits from Gβγ subunits. Both free Gα and βγ subunits can activate downstream effectors. RGS proteins bind to GTP bound Gα subunits to enhance their intrinsic GTPase activities, thus accelerating signal termination. They may achieve their target specificity by: (A) directly binding to receptors through functional motifs such as the DEP and PDZ domains; (B) indirectly binding to receptors through scaffolding proteins (S) such as spinophilin or GIPC; (C) binding to membrane anchor proteins such as R9AP or R7AP; (D) cooperating with downstream effectors such as PDEγ; or (E) additional binding to Gα subunits through a second Gα-interacting domain such as GoLoco or the C terminus of RGS9-2.

Another important question arising from Ballon's study is whether the interaction of the DEP domain with a particular GPCR is assisted or regulated by other proteins. It is known that RGS9 and its related R7 subfamily members bind via the DEP domain to membrane anchor proteins R9AP and R7BP (Figure 1) (Abramow-Newerly et al., 2006) and that these anchor proteins are necessary for the function and translocation of the RGS proteins onto cell membranes. Furthermore, the C-terminal tails of GPCRs have been shown

to interact with a diversity of proteins including Gα subunits. Although Ballon et al. showed in the study that Gα subunits do not compete for the binding of Sst2 to the cytoplasmic tail of Ste2, it remains to be determined if other proteins that interact with the cytoplasmic tail influence Sst2 binding. Finally, it is known that upon receptor activation, Sst2 and other RGS proteins may be phosphorylated. Whether this phosphorylation affects interactions with receptors also remains to be determined. Clearly, answering these questions will have significant impact on understanding the functional specificity of RGS proteins as well as the roles in signal transduction of other DEP domain-containing proteins.

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PALM Reading: Seeing the Future of Cell Biology at Higher Resolution

The inherent resolution limit of the light microscope has been a limiting factor in investigations of many fields of cell biology. A recent paper in *Science* by Betzig and coworkers describes a new method that can push the limit significantly lower.

Answers to many of the remaining mysteries in biology lie hidden somewhere between the cellular and the molecular scales. This is the domain of self-organizing supramolecular structures and molecular nanomachines that play roles in almost all of a cell's basic functions. Examples of these structures are kinetochores, vesicle budding sites at the plasma membrane and on trafficking organelles, cell adhesion sites, leading edges of migrating cells, and neuronal synapses. We still don't understand these structures and processes very well. Therefore, advances that would open new windows into this world are likely to give us important novel insights into cell biological mechanisms.

This world of tiny molecular machines is just below the resolution limit of the light microscope. Light microscopes can resolve two objects that are separated by about 200 nm, but images of objects that are closer than that are blurred into one fuzzy blob. The potential rewards for lowering this limit have motivated lots of work to try to develop methods to improve the resolution of light microscopy. Different approaches have been developed that can offer significant improvements in resolution (Betzig and Trautman, 1992; Donnert et al., 2006; Gustafsson, 2000, 2005; Willig et al., 2006).

In a recent issue of *Science,* Eric Betzig and his colleagues described a new super-resolution light microscopy method (Betzig et al., 2006). Their new method, photoactivated localization microscopy (PALM), is based on an innovative combination of two previously developed approaches: nanometer-resolution localization of single molecules and use of photoactivatable fluorescent proteins.

It turns out that the resolution limit of the light microscope does not actually prevent acquisition of spatial information below the limit of ~200 nm. It simply means that if two objects are closer than the resolution limit, they will appear as one object. However, it is possible to estimate the location of an isolated object with much higher accuracy, sometimes down to an accuracy of a few nanometers. This approach has been widely used in studies of single fluorescent molecules in vitro. When a solution of a fluorescently labeled protein is sufficiently dilute, the protein molecules can be detected as individual, clearly separated objects with a light microscope. Although each fluorescent molecule appears as a spot with a diameter of a couple of hundred nanometers, much larger than the actual molecule, the center of that spot, and the location of the molecule, can be estimated with high accuracy. This has been used, for example, for tracking nanometer movements of motor proteins (see e.g., Yildiz et al., 2003).

However, in cells, proteins are usually very concentrated, and thus single molecules cannot be resolved spatially. The method of Betzig et al. (2006) uses another dimension to separate individual molecules, namely time. To do this they use photoactivatable fluorescent proteins (PA-FP; Patterson and Lippincott-Schwartz, 2002; Lukyanov et al., 2005). A PA-FP fused to a protein of interest is expressed in cultured