

PERSPECTIVE

RGS Protein and G Protein Interactions: A Little Help from Their Friends

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G protein signaling pathways are essential for all aspects of cell and organ physiology, and the involved proteins have long served as primary drug targets. At the most basic level, these proteins include a signal-receiving G protein-coupled receptor (GPCR), a transducing heterotrimeric G protein ($G\alpha\beta\gamma$ subunits), and a signal-generating downstream target effector. These proteins work together to transmit signals across the plasma membrane. A neurotransmitter or hormone activated GPCR stimulates the exchange of GDP for GTP on $G\alpha$ to initiate heterotrimer dissociation and activation of effector proteins that, in turn, initiate a cascade of cellular signaling events. The regulators of G protein signaling (RGS proteins) participate in this process by binding directly to activated $G\alpha$ -GTP to serve as GTPase-activating proteins (GAPs), which limit the lifetime of $G\alpha$ -GTP and terminate signaling event(s).

RGS proteins are relatively new actors on this stage. All family members contain a signature RGS domain responsible for GAP activity. So far, more than 30 mammalian family members have been identified and classified into seven subfamilies based on sequence identity and functional similarities (De Vries et al., 2000; Ross and Wilkie, 2000; Hollinger and Hepler, 2002). Although many RGS proteins are relatively simple, others are more complex and contain multiple domains for binding various signaling proteins, and accumulated evidence now suggests that RGS proteins act as tightly regulated modulators and integrators of G protein signaling. Much has been learned in recent years about the biochemical mechanisms whereby RGS proteins stimulate the GTPase activity of $G\alpha$ (Ross and Wilkie, 2000). However, much less is known about how RGS function(s) are regulated in living cells (Hollinger and Hepler, 2002). After RGS proteins were first shown to act as $G\alpha$ GAPs, questions immediately emerged about which

RGS proteins talked to which $G\alpha$ subunits and how selectivity for these interactions is determined in a cellular context. Given that there are more than 20 $G\alpha$ subunits and more than 30 RGS proteins, early speculation predicted that $G\alpha$ and RGS proteins form discrete functional pairs. Surprisingly, this has not turned out to be true in most cases. Although certain RGS protein subfamilies do selectively bind and regulate the activity of a specific class of $G\alpha$ (for example, p115RhoGEF and $G\alpha_{12/13}$), this is an exception. Most RGS proteins are perplexingly promiscuous regarding which $G\alpha$ they can bind. In reconstitution assays using purified proteins, most can regulate the activity of many members of the G_{ai} subfamily or Gq (De Vries et al., 2000). So the question remains: exactly how do RGS proteins and $G\alpha$ subunits decide to pair up in living cells?

In this issue of *Molecular Pharmacology*, Roy et al. (2003) provide evidence that $G\alpha$ may receive critical help from their linked receptors to recruit a preferred RGS protein. The authors show that two simple RGS proteins, RGS2 and RGS4, are recruited to the plasma membrane by expressing either $G\alpha$ subunits (G_{ia} , $G_{q\alpha}$, or $G_{s\alpha}$) or linked GPCRs (M2-muscarinic cholinergic, AT1a-angiotensin, or β_2 -adrenergic, respectively). Not surprisingly, expression of G proteins initiates RGS protein membrane recruitment, whereas expression of RGS-insensitive G-protein mutants does not. However, among the remarkable observations the authors report is that RGS protein recruitment to membranes also occurs with receptors alone, is specific for receptors functionally linked to the target G protein, and is independent of the activation state of either receptor or G protein. Furthermore, RGS protein membrane recruitment mirrors RGS regulation of G protein function. Together, these findings suggest that GPCRs, either alone or in coordinated effort with their linked G proteins, can selectively recruit certain RGS proteins to the plasma membrane to determine their signaling functions.

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ABBREVIATIONS: GPCR, G protein-coupled receptor; RGS, regulator of G protein signaling; GAP, GTPase-activating protein; PDZ, PSD-95, Disc-large, ZO-1.

RGS-Receptor Interactions

This is only the latest evidence to support the idea that receptors contribute to selective RGS protein-G protein interaction in intact cells. A previous study demonstrated that closely related RGS proteins differentially and selectively regulate signaling through distinct GPCRs linked to the same G protein signaling pathway (i.e., Gq/11 directed Ca^{2+} mobilization) (Xu et al., 1999). A more recent study (Wang et al., 2002) shows that selective "knock-down" of mRNA and protein levels for RGS3 and RGS5 in target cells selectively regulates signaling through M3-muscarinic cholinergic and AT1a-angiotensin receptors, respectively. These findings are consistent with the conclusions of Roy et al. (2003) and suggest selective RGS protein regulation of receptor signaling. An important difference between these reports is in the approaches used. Whereas the previous studies examined the effects of RGS proteins on receptor and G protein signaling, the study in this issue focuses on the influence of G protein-linked receptors on the subcellular localization of the RGS protein.

One unexpected finding is that the activation state of either the receptor or the linked G protein does not seem to matter to the RGS protein; the mere expression of receptor or linked G protein is sufficient to provide membrane binding sites for RGS protein. Consistent with this observation, receptor- or G protein-independent activation of the relevant signaling pathways did not cause translocation of RGS proteins to the plasma membrane. These findings suggest that initial RGS protein association with the plasma membrane may be constitutive. In support of this idea, a previous report shows that the N terminus of RGS4 contains lipid modifications and positively charged patches that dictate membrane association (Srinivasa et al., 1998). In addition, RGS4 can spontaneously associate with anionic phospholipid vesicles, and this association is stabilized by addition of functionally

paired GPCR and G protein (M1-muscarinic and Gq or M2-muscarinic and Gi) (Tu et al., 2001). Still unresolved is whether RGS proteins associate directly with receptors or a receptor/G protein complex or somehow indirectly influence RGS protein membrane localization by intermediary proteins and/or lipids.

GPCR Scaffolding Complexes: Have RGS Proteins Joined the Party?

In recent years, GPCRs have been shown to recruit a growing list of non-G protein binding partners (Milligan and White, 2001; Brady and Limbird, 2002; Hall and Lefkowitz, 2002). Many of these proteins are regulatory in their functions, but others have established signaling roles of their own. As such, newly emerging models of GPCR signaling suggest that receptors can serve as platforms for scaffolding proteins that assemble multiprotein complexes. The clear advantage of such complexes is that they provide a cellular mechanism for dictating local organization of functionally related signaling proteins. One well-studied example of this is the InaD protein found in *Drosophila melanogaster*. In the fly, Gq and inositol lipid/ Ca^{2+} signaling mediate visual signal transduction. InaD is a scaffolding protein containing multiple PDZ domains that simultaneously bind the carboxyl-terminal tail of Gq-linked rhodopsin along with the functionally related signaling proteins phospholipase C, protein kinase C, and the transient receptor potential Ca^{2+} channel (Xu et al., 1998). The net effect of this complex is the assembly and tight regulation of all proteins necessary for a shared signaling task. Since the discovery of InaD, a number of novel binding partners and parallel signaling complexes have been identified for mammalian GPCR systems (Brady and Limbird, 2002; Hall and Lefkowitz, 2002). One complex RGS protein (RGS12) contains a PDZ domain which may bind a GPCR (Snow et al., 1998). However, no other RGS proteins contain PDZ domains, and the findings described here and elsewhere indicate that simple RGS proteins also participate in scaffolding complexes with GPCRs and proteins involved in a shared signaling task. As mentioned above, RGS4 association with artificial liposomes is enhanced by Gq-linked muscarinic receptors (Tu et al., 2001). Add to this the fact that RGS4 also can form a stable ternary complex with phospholipase C β , Gq α , and G $\beta\gamma$ (Dowal et al., 2001), and one could easily envision a scenario in which RGS4 is part of a larger multiprotein scaffolding complex of shared signaling function, as has been proposed (Sierra et al., 2000). Indeed, this may be the best explanation for the unexpected observation in this study that RGS2 is selectively recruited to the plasma membrane by expressing Gs-linked β 2-adrenergic receptors or Gs α . This was unexpected because RGS2 is not a GAP for Gs α but has been shown to bind certain isoforms of adenylyl cyclase to inhibit catalytic activity (Sinnarajah et al., 2001). Therefore, the emerging picture is that RGS proteins are recruited to the plasma membrane in cells by a GPCR-centered multiprotein complex to modulate the activity of the linked G α and/or effector (Fig. 1). If RGS protein functions are dictated by the receptor and not G α , then this would explain why RGS proteins are so promiscuous with regard to their G α interactions in vitro. In a cellular environment, RGS proteins would not be free to find any available G α . Receptors would selectively sort RGS proteins at the

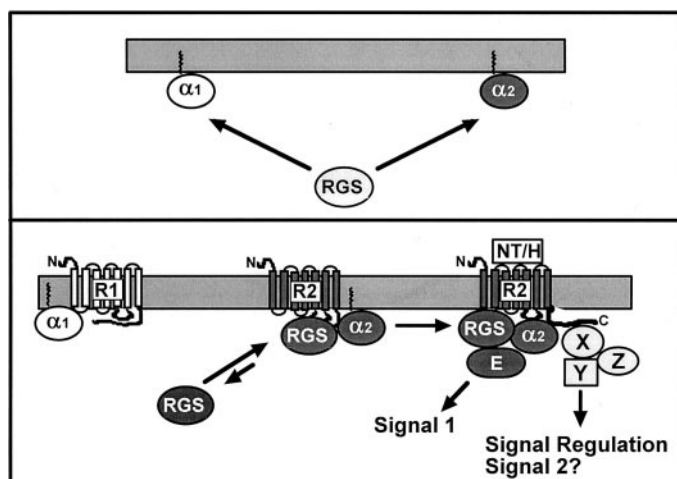


Fig. 1. Do receptors determine specificity for RGS protein/G α interactions in cells? Top, many RGS proteins are capable of interacting with multiple G α subunits ($\alpha 1$ and $\alpha 2$) in vitro and when expressed in intact cells. Bottom, G protein-coupled receptors (R1 and R2) may recruit specific RGS proteins to the plasma membrane to interact with receptor-coupled G α subunit(s), thereby determining specificity for RGS protein/G α interactions in cells. After receptor activation by neurotransmitter (NT), hormonal (H), or pharmacological agonists, RGS proteins may participate in a larger receptor-centered scaffolding complex involving proteins with shared signaling functions including effector (E) and/or other regulatory proteins (X, Y, and Z) bound to the receptor carboxyl terminus.

plasma membrane to orient and optimize their GAP activity toward the linked $G\alpha$. Where and how RGS proteins and receptors interact at the plasma membrane is still unclear. Recent evidence suggests that certain GPCR and linked proteins with shared signaling function assemble within specialized microdomains at the plasma membrane known as lipid rafts and in cavolin-enriched rafts termed caveolae (Steinberg and Brunton, 2001; Ostrom, 2002). It is conceivable (although not yet demonstrated) that some RGS proteins and receptors may colocalize within lipid rafts to modulate signaling events. Many RGS proteins contain multiple binding domains for various protein binding partners. Once bound to their target receptor, RGS proteins also could serve as secondary scaffolds that recruit other signaling proteins in much the same way that β -arrestins do with β 2-adrenergic receptors (Hall and Lefkowitz, 2002). Add to this our new appreciation that GPCRs can oligomerize and couple to multiple G proteins (Angers et al., 2002), and the possibilities for future study (despite the bewildering complexity) become very exciting.

Implication for RGS Proteins as Potential Drug Targets

Since their initial discovery, RGS proteins have been eyed as important new drug targets (Zhong and Neubig, 2001; Neubig and Siderovski, 2002). GPCRs and their linked signaling pathways are the direct targets for a large number of currently used drug classes. What makes many RGS proteins such attractive new drug targets is their unique capacity to modulate G protein signaling combined with their highly regionalized localization, most notably within the central nervous system (Gold et al., 1997). Small molecules that inhibit RGS protein/ $G\alpha$ interactions have been proposed as novel drugs to potentiate the actions of endogenous neurotransmitters in various disease states such as Alzheimer's and others. Alternatively, such therapeutic agents could be used to boost the effects of existing GPCR-directed drugs by decreasing the therapeutic dose needed while increasing the agonist's regional specificity, thereby reducing unwanted side effects (Neubig and Siderovski, 2002). If RGS proteins are recruited to specific receptor signaling complexes, then identifying which receptor(s) are paired with which RGS proteins warrants further investigation. The design of small molecules that block or mimic RGS protein/receptor interactions could become a highly specific therapeutic tool that is effective only in those cell types in which both the RGS protein and the receptor are localized. Identifying binding partners and specific sites of RGS/receptor interaction will be important both for a better understanding of G protein signaling and for future drug development.

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