# A $G_s$-RhoGEF interaction: An old G protein finds a new job

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**The heterotrimeric G proteins are known to have a variety of downstream effectors, but $G_s$ was long thought to be specifically coupled to adenylyl cyclases. A new study indicates that activated $G_s$ can also directly interact with a guanine nucleotide exchange factor for Rho family small GTPases, PDZ-RhoGEF. This novel interaction mediates activation of the small G protein Cdc42 by $G_s$-coupled GPCRs, inducing cytoskeletal rearrangements and formation of filopodia-like structures. Furthermore, overexpression of a minimal PDZ-RhoGEF fragment can down-regulate cAMP signaling, suggesting that this effector competes with canonical signaling. This first demonstration that the G$\alpha_s$ subfamily regulates activity of Rho GTPases extends our understanding of G$\alpha_s$ activity and establishes RhoGEF coupling as a universal G$\alpha$ function.**

The canonical G protein pathway consists of a cell surface receptor, a heterotrimeric G protein, and an effector protein that controls signaling within the cells. This fundamental paradigm, familiar to every biologist, is rooted in discoveries by the laboratories of Sutherland, Rodbell, and Gilman, which in the 1970s and 1980s dissected biochemical mechanisms of adenylyl cyclase activation by hormones. Their breakthrough came after experiments showing that the G protein $G_s$ is essential to transfer agonist stimulation from the receptor to adenylyl cyclase (1). This G protein consists of the ~42-kDa $\alpha$ subunit, which binds and hydrolyzes GTP, and the permanently associated dimer of 35-kDa $\beta$ and ~10-kDa $\gamma$ subunits (G$\beta\gamma$). Their findings helped establish a canonical model in which the agonist-bound receptor causes the G protein to release GDP, and the heterotrimer dissociates into G$\alpha$-GTP and free G$\beta\gamma$; in this state, the G protein can activate its effector (*i.e.* G$\alpha_s$ will activate adenylyl cyclase until GTP is hydrolyzed). Although the rod photoreceptor G protein, transducin, was discovered by that time (2), the ubiquitously expressed $G_s$ can be considered the founding member of the G protein family.

The subsequent cloning and identification of the other three families ($G_i$, $G_q$, and $G_{12}$) completed the rough map of G protein–mediated transduction. These initial studies suggested that the $\alpha$ subunits were responsible for activation of one type of effector (*e.g.* G$\alpha_s$ for adenylyl cyclase and cAMP; G$\alpha_q$ for phospholipase C, phosphoinositides, and $Ca^{2+}$; and G$\alpha_i$ for ion channels and inhibition of adenylyl cyclase), whereas the free G$\beta\gamma$ complexes interact with a remarkably large number of binding partners, including some effector enzymes and ion channels (3). Later, G$\alpha_{12}$ and G$\alpha_{13}$ were found to regulate a distinct type of effectors, the RhoGEFs (4, 5). These multidomain proteins contain pleckstrin homology (PH) domains, which facilitate their membrane localization, and Dbl homology (DH) domains, which catalyze GDP-for-GTP exchange (guanine nucleotide exchange factor; GEF) in the Rho family of small (~20-kDa) G proteins. At the time, the $G_{12}$-RhoGEF pathway seemed odd as it contained two G proteins: the receptor-activated "large" $G_{12}$ class protein and the "small" Rho G protein, which is activated by RhoGEF. However, it was then discovered that G$\alpha_q$ could activate a RhoGEF called Trio (6), and that G$\beta\gamma$ complexes activate other RhoGEFs, indicating that this pathway, if unusual, is at least popular. G$\alpha_s$, however, mostly appeared to be faithful to its originally determined role—to stimulate adenylyl cyclase(s)—possibly contributing to the enduring perception that regulation of a second messenger–generating enzyme is the "real" function of a heterotrimeric G protein.

In the current issue of JBC, Castillo-Kauil *et al.* (7) force a reexamination of the existing canon, presenting data that show G$\alpha_s$ can also interact with a specific RhoGEF, in this case PDZ-RhoGEF (PRG). The authors made this discovery as part of an examination of the regulation of cell shape by the Rho family. They began by expressing a series of short constructs of three RhoGEF proteins, p115RhoGEF, PRG, and LARG, all of which activated RhoA as expected, promoting cell contraction. However, they noticed that the DH/PH domain of PRG also activated Cdc42 and induced filopodia-like cell protrusions. To investigate which G protein is responsible for activation of this Cdc42-mediated pathway, they overexpressed constitutively active mutants of different G$\alpha$ subunits. These mutants are stabilized in the active GTP-bound state due to substitution of the glutamine residue crucial for GTP hydrolysis. Surprisingly, the PRG-Cdc42 pathway was stimulated by G$\alpha_s$Q227L, the one G$\alpha$ subtype not known for interaction with RhoGEFs. Furthermore, they showed that binding of PRG to Cdc42 was promoted only by $G_s$-coupled receptors, and not by $G_q$- or $G_i$-coupled GPCRs. The authors then investigated the PRG site responsible for the interaction with G$\alpha_s$, narrowing it down to the isolated PRG DH and PH domains and their linker region. A construct encompassing these domains was able to inhibit (i) GPCR-mediated activation of Cdc42, (ii) the G$\alpha_s$Q227L-promoted interaction of PRG with Cdc42, and (iii) some protein phosphorylation events downstream of the canonical cAMP pathway. Taken together, their work identifies PRG as a novel effector for $G_s$; the G$\alpha_s$-PRG interaction mediates activation of Rho family protein Cdc42, leading to cytoskeletal remodeling.

The unexpected results of Castillo-Kauil *et al.* open up new opportunities to explore this mechanism at different levels of biology. The experiments described in the paper were performed *in vitro* using cultured cells, imaging, and pulldown of

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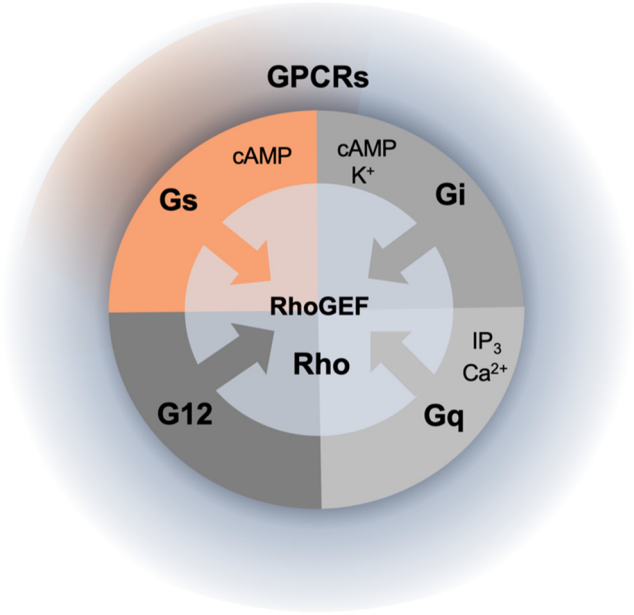


**Figure 1. Activation of the Rho family by heterotrimeric G proteins.** The Rho family of small GTPases is activated by RhoGEF proteins, some of which can be stimulated by heterotrimeric G proteins. Of four families of heterotrimeric G proteins, three ($G_{12}$, $G_q$, and $G_i$, shown in *shades of gray*) were known to activate certain RhoGEFs. The new results (highlighted in *orange*) (7) show that $G_s$, the G protein known to stimulate production of cAMP, can also stimulate a particular RhoGEF; this suggests that the Rho GTPases can potentially be stimulated by the multitude of signals from the entire class of GPCRs, including those coupled to $G_s$. *IP$_3$*, inositol 1,4,5-trisphosphate.

protein complexes containing the overexpressed Gα$_s$ Q227L mutant. Considering the multitude of $G_s$-coupled receptors and RhoGEFs in the body (8, 9), it will be important to understand the physiological context where the new $G_s$-mediated pathway plays a significant role. This will require experimentation *in vivo* and possibly reevaluation of the phenotypes associated with known pathogenic mutations in Gα$_s$ (*GNAS*) and other relevant genes. At the molecular level, it would be important to delineate the biochemical mechanisms of Gα$_s$ interaction with PRG. For example, at what stage of the GTP/GDP cycle does Gα$_s$ bind to PRG: in the GTP-bound state, which also activates adenylate cyclase, or in the transition state (*i.e.* just before the terminal phosphate of GTP is removed)? Indeed, there is precedent for proteins that bind preferentially with the transition state—specifically RGS proteins, which accelerate the GTPase reaction. Another possibility is that, by analogy with p115RhoGEF, which stimulates GTPase activity of Gα$_{12}$ and Gα$_{13}$, PRG (and other RhoGEFs with similar DH-PH sequences) can influence interaction of Gα$_s$ with nucleotides, Gβγ, and other partners.

Since defining the receptor, G protein, and effector as the three essential members of the G protein pathway, researchers have discovered many additional proteins that regulate the amplitude and duration of the stimulus and/or participate in cross-talk with other signaling circuits. These "new" proteins include arrestins, receptor kinases, nonreceptor exchange factors, GTPase-activating proteins, special chaperones, etc. Thus, in a way, discovering a novel binding partner for a signaling molecule is not as surprising as it would have been 20 years ago. However, the new partner identified by Castillo-Kauil *et al.* makes the result of extra significance; until now, we knew that three of four G protein subfamilies could regulate Rho GTPases by activating RhoGEFs: $G_{12}$ and $G_q$ via their α subunits and $G_i$ via the Gβγ subunits (10). The demonstration that the $G_s$ subfamily is no exception shows that activation of RhoGEFs by heterotrimeric G proteins may be a truly universal mechanism (Fig. 1). The significance of this insight is that the multitude of biological processes regulated by Rho-GTPase networks can potentially respond to the entire repertoire of GPCR-mediated stimuli.

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*Abbreviations*—The abbreviations used are: PH, pleckstrin homology; DH, Dbl homology; GEF, guanine nucleotide exchange factor; PRG, PDZ-RhoGEF; GPCR, G protein–coupled receptor.

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