Bypassing GPCRs with Chemical Dimerizers

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G protein-coupled receptors (GPCRs) activate heterotrimeric G protein complexes. In this issue, Putyrski and Schultz (2011) describe a rapamycin-based system to bypass the GPCR by direct activation of a specific heterotrimeric G protein subunit, which induces downstream signaling cascades.

Heterotrimeric G proteins are activated by G protein-coupled receptors (GPCRs), encoded by more than 700 genes in the human genome. GPCRs are comprised of seven transmembrane (TM) regions, which are interconnected by three intra- and three extracellular loops. These receptors can perceive a wide variety of signals including light, hormones, ions, and neurotransmitters. Consequently, GPCR signaling is implicated in a wide variety of physiological processes (Wettschureck and Offermanns, 2005), and misregulation can cause cardiovascular disease, allergy, and cancer.

The GPCRs activate heterotrimeric G proteins consisting of one α, one β, and one γ subunit. The heterotrimer is a peripheral membrane protein due to lipid modification of the Gα and Gγ subunits. After activation of the receptor, the G protein complex undergoes a conformational change, allowing the Gα subunit to exchange GDP for GTP, which induces the activation of the Gα subunit and the dissociation from the Gβγ subunit. The activated Gα subsequently activates a second messenger-producing enzyme. Alternatively, the released Gβγ heterodimer can interact and modulate the activity of another effector enzyme (see Figure 1A).

There are approximately twenty different Gα subunits that are grouped in four classes based on structural homology and biological activity: Gαi/o, Gαs, Gαq, and Gα12/13. The Gβ subunit family comprises five members, whereas the Gγ subunit family has twelve members (Wettschureck and Offermanns, 2005). The numerous subunits give rise to a large number of possible heterotrimeric complexes, the make-up of which determines the activation profile of downstream effectors. Moreover, GPCRs usually activate a number of different Gα classes. Since the specificity of a GPCR and its interaction with the heterotrimeric G protein is poorly characterized (Oldham and Hamm, 2008) and the subunit composition of the heterotrimeric G protein is undefined in vivo, the output of an activated GPCR signaling cascade is complex and difficult to predict.

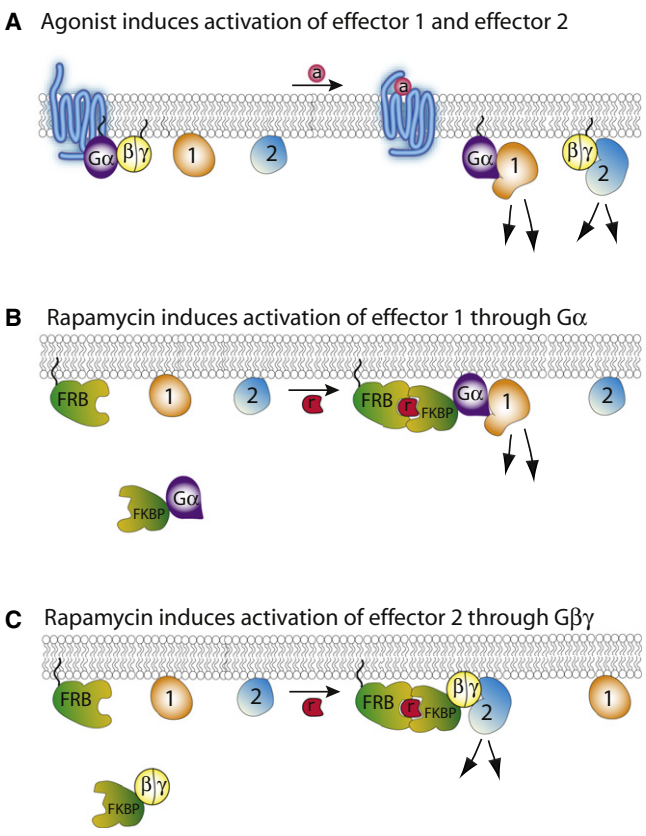

Figure 1. Bypassing GPCRs through Rapamycin-Induced Recruitment of Heterotrimeric G Protein Subunits
(A) Natural situation: agonist (a) stimulation of GPCR activates Gα and releases Gβγ, which activate effector 1 and effector 2, respectively.
(B) Addition of rapamycin (r) induces heterodimerization of lipidated, membrane-bound FRB and FKBP fused to Gα, thereby specifically activating effector 1.
(C) Conversely, recruiting the Gβγ subunit specifically activates effector 2.

Strategies that bypass the receptor by the direct and specific activation of heterotrimeric G proteins in cells are crucial for teasing out the contribution of each of the different isoforms in the complex intracellular environment of a cell. Classically, constitutive active (GTPase deficient) proteins are overproduced to evaluate their function. Biological assays are usually performed hours or days after introduction of the protein, and therefore this traditional approach lacks temporal control. Moreover, biological effects are usually blurred by processes that compensate for the prolonged presence of constitutive active G proteins. G protein (de)activators (e.g., toxins) have somewhat better temporal control, but often lack specificity.

Chemical biology approaches in which biomolecules are modified and combined in a way that creates molecular tools with novel biological function can be used to steer cellular processes and to

learn how cellular circuits operate (Lim, 2010). Such an approach for the direct activation of heterotrimeric G protein subunits is now reported by Putyrski and Schultz (2011). The system is based on relocalization induced by protein dimerization, which is controlled by a small molecule. The dimerizing module consists of the 11 kDa FRB protein domain from mTOR and the FK506-binding protein (FKBP12), two components that interact with high affinity when rapamycin, a small cell-permeant molecule, is present (Choi et al., 1996). By tagging one of the dimerizing components with a localization signal and the other component with a protein of interest, the subcellular localization of the protein of interest can be controlled by addition of rapamycin. Putyrski and Schultz replaced the natural plasma membrane localization signal of Gα or Gγ (lipidation) by a rapamycin-inducible localization signal (see Figures 1B and 1C). They report that the rapamycin-induced recruitment of Gq or Gs resulted in the activation of calcium or cAMP signaling, respectively. Moreover, it was shown that $InsP_3$ and protein kinase C (PKC) activity were elevated when Gq was switched on. This indicates that the inducible system triggers the relevant signaling cascade in living cells and that the signals are communicated to downstream processes within the cell. Rapamycin addition resulted in a half-maximal time for the relocalization of around 20 s for Gq. While this is relatively fast, it is slow compared to the half-time of Gq activation by receptors, which is 350 ms (Adjobo-Hermans et al., 2011). Still, rapamycin-activated Gq produced calcium oscillations that are similar to those triggered via agonist stimulation of endogenous purinergic receptors. Intriguingly, the washout of rapamycin did not dissociate the recruited G protein from the membrane and consequently did not stop calcium oscillations, indicating high stability of the dimerized complex.

The significance of the Putyrski and Schultz study is that the rapamycin-induced heterodimerization system effectively bypasses the GPCR and hence provides direct control and information about the role of individual G protein subunits. Furthermore, their results show that "forced" relocalization of G protein subunits suffices to trigger downstream signaling cascades. This elegantly demonstrates the significance of protein subcellular localization and interaction in controlling signal transduction. In other words: GPCR signaling is about "when," "where," and "with whom" and less about "how" or "how much."

While the rapamycin-approach already provides good temporal and some spatial control, new optical methods could substantially improve spatiotemporal control. Recently, a variety of processes in cells have been modulated with smart optical switches, as exemplified by the recent explosion of "optogenetics" (Toettcher et al., 2011). Several approaches to induce protein dimerization by light are conceivable, some of which have been demonstrated in cells. For instance, caged compounds can be used to release a small molecule by UV light. A caged analog of rapamycin has recently been described and shown to locally induce Rac activity (Umeda et al., 2011). Alternatively, light-sensitive proteins that alter their conformation or induce protein-protein interactions can be used (Toettcher et al., 2011). Improved spatiotemporal control will allow precise localized subcellular activation of heterotrimeric G proteins, which is of major interest for physiological processes in which spatially restricted activation of G proteins is important, such as cell division and cell migration. Undoubtedly, combining these tools with fluorescent biosensors and advanced multimode microscopy will shed novel light on the properties of signaling cascades that operate within single living cells.

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