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Classical models of receptor (GPCR) and G protein (Gαβγ) signaling based on biochemical studies have proposed that receptor stimulation results in G protein activation (Gα-GTP) and dissociation of the heterotrimer (Gα-GTP + Gβγ) to regulate downstream signaling events. Unclear is whether or not there exists freely diffusible, activated Gα-GTP on cellular membranes capable of catalytic signal amplification. Recent studies in live cells indicate that GPCRs serve as platforms for the assembly of macromolecular signaling complexes that include G proteins to support a highly efficient and spatially restricted signaling event, with no requirement for full Gα-GTP and Gβγ dissociation and lateral diffusion within the plasma membrane.

The question posed is whether or not there exists freely diffusible, activated GTPases on cellular membranes capable of signal amplification. As outlined in this series, the answer clearly depends on the GTPase in question and the related signaling system. In the case of G protein coupled receptor (GPCR) activation of heterotrimeric (Gαβγ) G proteins at the plasma membrane, Dr Ross1 summarizes previous reports clearly indicating that GPCR/G protein coupling is capable of supporting catalytic activation of multiple G proteins when reconstituted as purified proteins into phospholipid vesicles. Unclear, however, is how accurately these in vitro systems reflect the behavior of native G protein heterotrimers in the plasma membranes of live cells. As Ross points out, constraints on receptor and G protein diffusion certainly exist in a cellular context, thus limiting lateral diffusion and the opportunity for multiple G protein signaling events. Indeed, evidence now suggests that at least some GPCRs serve as signaling platforms for the assembly of a macromolecular complex of related signaling proteins (G proteins among them) for the purpose of a highly efficient and spatially restricted signaling event, with no requirement for Gα-GTP and Gβγ dissociation and lateral diffusion within the membrane.

Addressing this question requires the visualization and quantitative measurement of G protein subunit localization and movement in live cells. Along these lines, a number of studies have examined GPCR/G protein coupling, G protein activation, and heterotrimer dissociation in live cells using Resonance Energy Transfer (RET) techniques including Fluorescence-RET (FRET) and bioluminescence-RET (BRET). Together, these findings (as reviewed by Lambert2) support the idea that, while certain heterotrimeric G proteins fully dissociate following activation, others do not. These latter findings were unexpected and challenged established models3,4 of G protein activation/deactivation. In paradigm shifting studies, Loshe and coworkers5 provided unexpected yet compelling evidence that certain G proteins (Gi) appear to rearrange in situ rather than fully dissociate and diffuse away following receptor stimulation. Using complementary FRET probes fused to Gαi and either Gβ or Gγ, they examined FRET activity between Gα and Gβγ following receptor activation. If the G protein subunits dissociated and diffused away, then FRET signals would be expected to decrease. Quite unexpectedly, FRET signals between Gα and Gβγ increased following receptor stimulation, indicating the FRET probes moved closer together. When the FRET probes were moved to the opposite end of Gγ, the FRET signal decreased. Together, these findings are consistent with a rearrangement of inactive heterotrimer (Gα-GDP:Gβγ) to form an active heterotrimer (Gα-GTP:Gβγ) rather than dissociated and freely diffusible Gα-GTP + Gβγ. Independent studies have examined other Gβγ heterotrimers using either FRET or BRET approaches and come to similar conclusions.5,6

This idea runs counter to overwhelming evidence (as outlined here by Arshavsky and Burns7 and Liebman8) that one particular G protein Gt (transducin) does indeed dissociate from its partner Gβγ1 and diffuse locally. However, unlike other G proteins, Gt and its receptor rhodopsin are constrained within a highly specialized membrane compartment at extraordinarily high local concentrations for a specific function. By contrast, most GPCRs and G proteins outside of the visual system exist in very different membrane environments, at much lower local concentrations, and utilize different biochemical mechanisms to restrict their diffusion and dictate signaling. Each G protein heterotrimer is different, and some appear to dissociate more readily than others. Using fluorescence recovery after photobleaching (FRAP) to measure release of a defined Gβγ from different Gα in intact cells, Lambert and coworkers9 found that different G proteins dissociate more readily than others following receptor activation, with a rank order of Gα > Gi > Gs. However, this cellular behavior likely depends on the particular receptor and G protein under examination.
A variety of cellular factors dictate G protein heterotrimer formation and membrane association. In turn, these factors influence the behavior of G protein subunits (Go-­GTP and Gβγ) following receptor activation, and limit the capacity for subunit dissociation and diffusion within the plane of the lipid bilayer. These factors include: (1) lipid modifications and other membrane targeting domains present on the subunits; (2) heterotrimer composition; (3) local membrane environment; (4) other accessory binding partners such as RGS proteins or scaffolds; (5) the presence or absence of downstream effector(s); and (6) receptor coupling. Each Go subunit relies upon a unique combination of multiple N-­terminal covalent lipid modifications (myristoylation and/or palmitoylation) alone or in concerted action with adjacent charged polybasic patches to confer varying strengths of membrane anchoring and subunit targeting within the plasma membrane.11 G membrane anchoring and subunit targeting within the plasma membrane, and enable G proteins to remain part of a highly efficient GPCR macromolecular signaling complex.

Considerable evidence now suggests that many GPCRs self-­associate into dimers and higher order oligomers in live cells.14,15 Whether these receptor oligomers are preassembled in complex with inactive G proteins is unclear, as evidence exists both for2,19 and against20,21 this idea. Preassembly of GPCR/G protein likely depends on the particular GPCR and G protein in question. Nevertheless, early kinetic analysis of signaling22 dictates that the G protein is certainly in near proximity and loose association with the receptor. The receptor oligomers serve as signaling platforms that recruit not only G proteins, but also functionally related signaling partners including one or more effectors, RGS proteins (GAPs), other modulators, and scaffolding proteins that help anchor the complex in close proximity. This macromolecular receptor-­centered protein complex constitutes a spatially restricted signaling nexus that is fine-­tuned for efficient and rapid signaling with all of the necessary signaling components nearby for activation, signaling, and deactivation. In this case, a preassembled (or loosely associated) and locally constrained GPCR/G protein complex is essential for rapid and efficient signaling, as well as repeated signaling. Under such circumstances, one could ask if full G protein heterotrimer dissociation and diffusion is necessary or even desirable? One can imagine that the presence of two different effectors, one sensitive to Go-­GTP and the other to Gβγ, would require full dissociation, as suggested.10 For example, the Goi-­sensitive adenylyl cyclase (ACII) and the Gβγ-­sensitive potassium channel Kir3.1 appear to form a stable complex with β2-­adrenergic receptor and Goi,βγ,23 Alternatively, a single effector that is sensitive to one or both Go-­GTP and Gβγ (e.g., certain adenylyl cyclase isoforms) would not necessarily require full dissociation. Even with full dissociation, lateral diffusion and the opportunity for signal amplification would be severely limited.

A new and puzzling wrinkle in this story is presented by certain members of the Activators of G protein Signaling (AGS) family of signaling proteins.24 Members of the group II subfamily of AGS proteins contain one or more -­20-­25 amino acid G protein regulatory (GPR, also known as GoLoco25) motifs that selectively bind certain inactive Go isoforms.26 Of interest are the findings that these GPR proteins bind Go-­GDP independently of Gβγ,27 and that some Goi-­GDP:GPR protein complexes can be regulated by Gi-­linked GPCRs in live cells.28-­30 How Goi-­GDP:GPR complexes fit into the G protein activation/ deactivation cycle under discussion here is unclear.31 One possibility is that these proteins represent newly appreciated G protein signaling complexes that form in parallel with, yet independent of, Gαβγ for specific signaling functions. Alternatively, they may engage and interact with classically defined GPCR/Gαβγ complexes. For example, their role may be to capture free Gα-­GDP immediately following GTP hydrolysis, thereby serving to facilitate heterotrimer dissociation by redirecting Go signaling and prolonging Gβγ signaling. At this point, this remains a matter of speculation and an active field of study.

Given these limitations on G protein subunit diffusion, more refined models of G protein activation/deactivation have been proposed.2,17,19 Classical models of G protein activation/deactivation based on the properties of purified G protein subunits propose that receptor-­stimulated nucleotide exchange and GTP binding to Go results in Gβγ release and full heterotrimer dissociation.3,4 In more refined models, G protein activation (GTP binding) and heterotrimer dissociation are distinct steps, and full dissociation is not necessary. GTP binding to Go instead results in rearrangement of Goi-­GTP and Gβγ in situ, the subunits held in place by lipids and receptor, and diffusion limited by a surrounding “cage” of related signaling proteins. This weakly bound yet active Goi-­GTP:Gβγ heterotrimer is capable of engaging one or more adjacent effectors within this cage.3,19 An RGS/GAP protein may be preassembled with the receptor/G protein complex where it is poised, ready to limit the life-­time of the signaling event.32 In some cases, a scaffolding protein (e.g., AKAPs, Arrestins, InaD-­Like, Homer, group II AGS proteins, others) may also anchor and orient functionally related signaling proteins (kinases, phosphatases) within the larger signaling complex.33 In this scenario, heterotrimer dissociation and subunit diffusion are not necessary, and diffusion resulting in signal amplification (multiple G proteins activating multiple effectors) is not likely.

In summary, growing evidence suggests that at least some (most?) GPCR/G protein signaling complexes serve as spatially constrained, highly efficient “solid-­state” signaling nodes on the cell surface (as proposed19,33). Thus, the heterotrimeric G proteins at the plasma membrane, in complex with their GPCR/GEF may not serve as a useful model for the behavior of the many Ras superfamily GTPases throughout the cell. Many monomeric GTPases
to large fluorescent biosensors that in some cases can compromise protein behavior. Molecular beacons that recognize native mRNAs and microRNAs already exist, and applying such techniques to signaling proteins in living cells will bring clarity to these questions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.