G protein coupled receptor signaling complexes in live cells.

John Hepler, Emory University

Journal Title: Cellular Logistics
Volume: Volume 4
Publisher: Taylor & Francis | 2014, Pages e29392-e29392
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.4161/cl.29392
Permanent URL: https://pid.emory.edu/ark:/25593/mr22m

Final published version: http://dx.doi.org/10.4161/cl.29392

Copyright information:
© 2014 Landes Bioscience
This is an Open Access work distributed under the terms of the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/licenses/by/3.0/).

Accessed September 9, 2017 3:54 PM EDT
Cellular Logistics

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/kcll20

G protein coupled receptor signaling complexes in live cells

John R Hepler

Department of Pharmacology; Emory University School of Medicine; Atlanta, GA USA

Published online: 04 Jun 2014.

To cite this article: John R Hepler (2014) G protein coupled receptor signaling complexes in live cells, Cellular Logistics, 4:2, e29392, DOI: 10.4161/cl.29392

To link to this article: http://dx.doi.org/10.4161/cl.29392

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the “Content”) contained in the publications on our platform. Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Versions of published Taylor & Francis and Routledge Open articles and Taylor & Francis and Routledge Open Select articles posted to institutional or subject repositories or any other third-party website are without warranty from Taylor & Francis of any kind, either expressed or implied, including, but not limited to, warranties of merchantability, fitness for a particular purpose, or non-infringement. Any opinions and views expressed in this article are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor & Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

It is essential that you check the license status of any given Open and Open Select article to confirm conditions of access and use.
G protein coupled receptor signaling complexes in live cells

John R Hepler

Department of Pharmacology; Emory University School of Medicine; Atlanta, GA USA

Keywords: GTPases/G proteins, GEFs (guanine nucleotide exchange factors), GPCRs (G protein coupled receptors), GAPs (GTPase activating proteins), effectors, ARF, RAS, RAB

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 utilization 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 Gαβγ heterotrimers using either FRET or BRET approaches and come to similar conclusions.6,7

This idea runs counter to overwhelming evidence (as outlined here by Arshavsky and Burns8 and Liebman9) 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 photo-bleaching (FRAP) to measure release of a defined Gβγ from different Gα in intact cells, Lambert and coworkers10 found that different G proteins dissociate more readily than others following receptor activation, with a rank order of Go > Gi > Gs. However, this cellular behavior likely depends on the particular receptor and G protein under examination.

*Correspondence to: John R Hepler; Email: jhepler@emory.edu
Submitted: 05/05/2014; Accepted: 05/28/2014; Published Online: 06/04/2014
http://dx.doi.org/10.4161/cl.29392
A variety of cellular factors dictate G protein heterotrimer formation and membrane association. In turn, these factors influence the behavior of G protein subunits (G\(\alpha\)-GTP and G\(\beta\gamma\)) 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 G\(\alpha\) 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\(\alpha\) subunits, when bound to their inseparable partner G\(\beta\gamma\), also contain distinct combinations of C-terminal lipid modification (farnesylation or geranyl-geranylation). Together, these confer to the G protein heterotrimer capacity for membrane anchoring and selective coupling to GPCRs.13 The unique combination of lipids and G\(\alpha\) and G\(\beta\gamma\) pairing provide varying strengths of membrane anchoring as well as constraints on protein diffusion. In some cases, these also provide an “address label” of sorts that serves to target certain G\(\alpha\) to lipid rafts, highly specialized microdomains of the plasma membrane that can focus and restrict G protein signaling.12,13 Therefore, each of these factors limits and constrains G\(\alpha\) and/or G\(\beta\gamma\) lateral diffusion 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 for16-19 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 G\(\alpha\)-GTP and the other to G\(\beta\gamma\), would require full dissociation, as suggested.10 For example, the G\(\alpha\i\)-sensitive adenyl cyclase (ACII) and the G\(\beta\gamma\)-sensitive potassium channel Kir3.1 appear to form a stable complex with \(\beta_2\)-adrenergic receptor and G\(\alpha\i\beta\gamma\).23 Alternatively, a single effector that is sensitive to one or both G\(\alpha\)-GTP and G\(\beta\gamma\) (e.g., certain adenyl 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 GoLoco) motifs that selectively bind certain inactive G\(\alpha\i\) isoforms.26 Of interest are the findings that these GPR proteins bind G\(\alpha\i\)-GDP independently of G\(\beta\gamma\),27 and that some G\(\alpha\i\)-GDP:GPR protein complexes can be regulated by G\(\alpha\)-linked GPCRs in live cells.28-30 How G\(\alpha\i\)-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, G\(\alpha\i\) for specific signaling functions. Alternatively, they may engage and interact with classically defined GPCR/G\(\beta\gamma\) complexes. For example, their role may be to capture free G\(\alpha\i\)-GDP immediately following GTP hydrolysis, thereby serving to facilitate heterotrimer dissociation by redirecting G\(\alpha\) signaling and prolonging G\(\beta\gamma\) 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 G\(\alpha\) results in G\(\beta\gamma\) 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 G\(\alpha\) instead results in rearrangement of G\(\alpha\i\)-GTP and G\(\beta\gamma\) 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 G\(\alpha\i\)-GTP:G\(\beta\gamma\) heterotrimer is capable of engaging one or more adjacent effectors within this cage.3,4 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.22 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 proposed19,34). 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

References

that appear to freely move on and off of intracellular membranes have few parallels with this described system, and may be fully capable of catalytic signal amplification. Clarifying this question unambiguously for the variety of cellular GTPases will require advances in high resolution cell imaging and the development of novel biosensors (e.g., molecular nanobeacons) that can directly measure the behavior of native proteins in real-time, rather than our current reliance on overexpressed recombinant proteins fused to large fluorescent biosensors that in some cases can compromise protein behavior.35 Molecular beacons that recognize native mRNAs and microRNAs already exist,36 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.