Is the model of signal amplification by GPCRs/GEFs activating multiple GTPases relevant to a broad spectrum of heterotrimeric and RAS superfamily GTPases?

Richard Kahn, Emory University

Journal Title: Cellular Logistics
Volume: Volume 4, Number 2
Publisher: Taylor & Francis | 2014-06, Pages e943602-e943602
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.4161/21592780.2014.943602
Permanent URL: https://pid.emory.edu/ark:/25593/rmmcc

Final published version: http://dx.doi.org/10.4161/21592780.2014.943602

Copyright information:
© 2014 Richard A Kahn. Published with license by Taylor & Francis Group, LLC
This is an Open Access work distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License (http://creativecommons.org/licenses/by-nc/3.0/).

Accessed January 31, 2020 4:16 PM EST
Is the model of signal amplification by GPCRs/GEFs activating multiple GTPases relevant to a broad spectrum of heterotrimeric and RAS superfamily GTPases?

Richard A Kahn*
Department of Biochemistry; Emory University School of Medicine; Atlanta, GA USA

Keywords: ADP-ribosylation factor (ARF), GTPase activating protein (GAP), G-protein coupled receptor (GPCR), Guanine nucleotide exchange factor (GEF), Receptor tyrosine kinase (RTK)

Concepts or models of biological processes shape how we think about them, discuss them, and design experiments to test aspects of them. Because of the importance of our models of cell signaling by regulatory GTPases and the desire to extend those models to related signaling modules, I have throughout my career been fascinated by the similarities and differences between the modeling of heterotrimeric G protein and monomeric RAS superfamily GTPases. Recent discussions with colleagues led me to conclude that there is a growing divergence in how researchers model the activation and signaling processes of monomeric and trimeric GTPases and also a surprising lack of consensus within each camp. This series of articles arose in response to these discussions and is intended to spark new ones.

At last summer’s FASEB meeting on ARFs/RABs I raised the question of whether there exists on membranes freely diffusible, activated GTPases, using a short history of (heterotrimeric) G proteins to contrast with my views of (monomeric) ARFs. This led to several interesting conversations, which continued after I returned to Emory. I am convinced that this is a fundamental question that shapes how we view GTPase signaling and design our experiments. As a result, I have asked a number of experts in the field of GTPase signaling to respond to the starting question, seen in my title. From Paul Liebman we have a historical perspective, which helps us appreciate the co-development of our models of biological membranes with those of G protein signaling, and also highlights the technical challenges encountered and lessons learned from the photoreceptor system. These issues are further refined and updated with newer technologies and data in the article from Arshavsky and Burns, which also focuses on rhodopsin—transducin—phosphodiesterase as the canonical GPCR/GEF—G protein—effector system that so strongly influenced early models and I think still colors our thinking in fundamental ways. Whether this canon can be appropriately extended to other or all GPCRs is explored in the articles by Ross and Hepler, which help us focus on existing data, key missing information, and well-reasoned suggestions as to how to obtain it. Articles from researchers focused on different families within the RAS superfamily were also solicited and currently include one from Martin Schwartz and Konstadinos Moissoglou, as a follow-up to their recent primary work on RHO family activation and translocation onto membranes with a clear role for diffusion modeled into the process. We again see in this article the close relationship between our understanding of GTPase activation/signalining with membrane biology and lipid signaling. And Cathy Jackson adds some keen insights into the discussions by detailing and emphasizing the likely roles that “downstream” effectors play through interactions with GEFs and GTPases and pointing out that some GEFs can bind GTPases at two different sites, one catalytic and the other regulatory, that allow feed-forward signaling that is yet another type of signal amplifier. I offer a bit of background information below as both context for the other articles and to focus on some of what I believe are key questions in the fields today.
I remember some seminal papers from the 70s that used a mixture of mathematical modeling and wet bench data to argue that each GPCR can activate a large number of GTPases, which diffuse away to activate effectors. The simplest model for GPCR actions as guanine nucleotide exchange factors (GEFs) for heterotrimeric G proteins is shown in the left of Figure 1, in which ligand binding leads to activation of a single Gα. I was lucky enough to have been present in the Gilman lab when others were developing the subunit dissociation model of G protein activation, which posits that the activated (GTP-bound) Gα has reduced affinity for Gβγ, promoting dissociation of α from βγ and leading to the generation of 2 different activators of effectors per trimer. Not shown in the GPCR model in Figure 1 is the early, compelling, and paradigm promoting evidence that one activated GPCR can activate many G proteins, which diffuse on the surface of the membrane to encounter and activate effectors, thereby providing a biologically important amplification step to the process (see following articles for details). This has certainly shaped my thinking over the years and is why I was so struck by the fact that ARFs do not appear to behave as predicted by these models.

This was highlighted, I thought quite simply, when we found absolute specificity in pairing of cargo with adaptor for different cargos on the same membranes all apparently using the same set of ARF GTPases. This observation alone seems sufficient to argue against the cargo-stimulated production of activated ARFs, which diffuse away from the GEF to encounter adaptors/effectors, as it provides no source of specificity in adaptor recruitment. Instead, there is more likely to be direct involvement of the cargo, ARF GEF, ARF, effector (coat proteins in this example) and even ARF GAPs into a protein complex that together dictate the specific outcome (Fig. 1 and 2). In the model for cargo-dependent ARF activation shown in Figure 1 (right) it is important to realize what we do not know, as indicated by question marks. We do not know if there is a “ligand equivalent” that may bind and activate the transmembrane protein cargo, nor do we know how the presence of cargo at a membrane site leads to recruitment and/or activation of an ARF GEF (see Fig. 2). I show the presence of a hypothetical “adapter” (not to be confused with coat proteins that are also often termed adaptors) to indicate the potential/likely homology to the RAS activation process, shown in the middle of Figure 1. RAS activation by growth factor receptors, genetically termed receptor tyrosine kinases (RTKs) in Figure 1, involves recruitment to the membrane of the Grb2/SOS complex, the latter of which has RAS GEF activity, through binding of SH3 domains within Grb2 to specific phosphorylated motifs in the cytoplasmic tail of the RTK. Thus, while all regulatory GTPases are thought to require a GEF to activate them on the surface of the bilayer, G proteins use the heptahelical GPCRs that are intrinsic membrane proteins. In contrast, RASs and ARFs use GEFs that are recruited to membranes in a regulated fashion and may require distinct or concerted activation processes. Ligand binding to GPCRs leads to conformational changes that activate latent GEF activity. In contrast, ligand binding to RTKs promotes auto-phosphorylation that generates

![Figure 1. Contrasting, simplified models of activation of G protein (left), RAS (middle), and ARF families of GTPases.](image-url)

GPCRs are a very large family (~800) of heptahelical membrane spanning proteins that bind ligands on the outside of the cell, leading to conformational changes that activate latent GEF activity for heterotrimeric G proteins on the cytoplasmic surface, promoting release of GDP and binding of the activating GTP. GPCRs can act catalytically to generate many activated Gαs per activated receptor, though may also retain the bound G protein subunits to act in more of a scaffolding role. One model of RAS activation (middle) is through the binding of a growth factor to its receptor on the outside of cells, resulting in auto-phosphorylation of the cytoplasmic tail of the receptor, and recruitment of the RAS GEF, Grb2/SOS, which activates the RAS already present on the plasma membrane. Thus, the GEF is recruited by the Receptor Tyrosine Kinase (RTK). Note that other RAS GEFs use different mechanisms (not shown). Less well understood is the role of transmembrane Cargos (e.g., mannose 6-phosphate receptor, amyloid precursor protein, etc) in recruiting or activating specific ARF GEFs (e.g., GBF1, BIG1/2, etc) at different sites inside cells. Both the ARF GEF and the ARF itself are recruited to the site of action. Roles for a ligand, binding to the cargo, or of an adaptor to physically couple the cargo to the ARF GEF are speculative and are included to highlight predicted functional homologies to the other GTPase systems.
docking sites for the RAS GEF, Grb2/SOS. In addition, unknown ligands may bind to transmembrane protein cargos leading through unknown mechanisms to recruitment to the membrane of both ARF GEFs and the ARFs themselves to generate the activated ARFs and downstream signal.

One example of why I think it is so important to discuss and compare our models of GTPase activation and action is the following. If signal amplification via one GEF generating multiple activated GTPases with lateral diffusion on the surface of the bilayer is off the table, then instead our thinking should focus on temporal control or the proofreading aspect of GTPase signaling. That is, if the lifetime of the activated GTPase (G*) controls the magnitude of the output, then we have great opportunity for signal amplification. But if the output is stoichiometric, i.e., 1 GTPase:1 complex/effectector, then the lifetime of G* is more likely controlling the fidelity of the complex during assembly, and also imposing directionality to the assembly/disassembly processes through regulated GTP hydrolysis.

Another conclusion that emerged from my thinking on these issues is that signal amplification elicits different world views between the G protein and RAS superfamily camps. In the former, we see that the second messenger hypothesis was central to early models for G protein signaling and the importance of signal amplification. Binding of one ligand molecule on the cell surface generating huge changes in cAMP or Ca^{2+} in cytosol, came from those observations. In contrast, what is clearly emerging from studies of RAB biologies is “GTPase cascades” in which one activated RAB recruits to a membrane a GEF for the next GTPase which in turn recruits the GAP for the former one, and so on. With each activated RAB also recruiting or acting on distinct sets of effectors. This concatenation of activated GTPases results in signal amplification in the number of effectors affected by the sequential list of activated RABs. In the case of the RABs, whose functions are closely linked to the regulation of vesicular traffic, this cascade is closely tied to (perhaps even determines) vesicle maturation. Although several labs have contributed in important ways to this model it is the subject of a recent review from Mizuno-Yamasaki, et al. that does a far better job explaining the details than I can here. I find the G protein signal amplifier and RAB GTPase cascades to be useful models with divergent roles for each of the different components, despite the conservation of overall biochemical properties (GTP binding, GEF or GAP activities, etc). There are likely to be related or important variants of these models emerging from studies of these or other GTPase families and I encourage those of you with such views to share them by adding to this series.

Figure 2. Modeling GTPase output as either/both allosteric regulation of enzymes and scaffolding to regulate the assembly of multi-subunit protein complexes. GPCRs (top) may be best known for their roles in activation of G proteins, leading to allosteric regulation of adenylyl cyclase, phospholipase Cβ, or Rho GEF, but are also increasingly appreciated to act as scaffolds for recruitment of effectors, RGS proteins, arrestins, and associated proteins that themselves may signal inside the cell or promote internalization of the complex. RAS protein signaling (middle) is best known for roles in oncogenesis through allosteric regulation of key pathways that include Raf1-MEK-ERK kinases, PI 3-kinase, RasGEF, and others. ARF signaling was earlier known for actions as an allosteric activator of the ADP-ribosyltransferase activity of cholera toxin from the human pathogen, *Vibrio cholera*, as well as the lipid modifying enzymes phospholipase D1 (PLD1), PI 4-kinase and PtdIns 4P 5-kinase (PI4P5K). But today it is perhaps best known for its role in recruiting coat proteins or complexes (COPI, GGA1-3, AP-1/3/4, MINT3) to specific membrane sites to coordinate nascent carrier biogenesis/coating. The extent to which lipid modifying and protein coating are integrated and work toward a common endpoint has been the source of much speculation. This is expected to be a common topic in the future for all GTPase families; i.e., the extent to which allosteric enzyme regulation and scaffolding synergize or antagonize the actions of each GTPase.
Although key aspects of models for G proteins vs. RAS superfam-


ily members has been quite divergent, recent data suggest


more commonality than previously appreciated. While once we


thought of G proteins solely as allosteric activators or inhibitors


of enzymes (e.g., adenylyl cyclase, phospholipase C\textsubscript{B}, cGMP


phosphodiesterase, Rho GEF) at the plasma membrane, it is now


widely appreciated that GPCRs often act as signaling centers or


platforms (the term scaffold is often used in such contexts) that


coordinate interactions of a large and growing list of proteins


involved in a variety of aspects of cell signaling.\textsuperscript{5,6} In addition to


G proteins and their effectors there is evidence of direct binding

to Regulators of G protein Signaling (RGS) proteins, which pos-


sess GAP activity for G proteins, and arrestins to GPCRs. Sig-


naling by GPCRs, G proteins and their various interactors is also


understood today to traffic throughout the cell and need not be


limited to the cell surface. This is increasingly similar to a central


function of the ARFs, which are best known for their role in reg-


ulating vesicular traffic at the Golgi, endosomes, and cell surface


direct binding to a number of different coat proteins or


complexes (see Fig. 2) and also the RABs. Thus, perhaps all fami-


ilies of GTPases act in different ways, to allosterically regulate


both specific enzymes and the assembly of multi-subunit protein


complexes. Which of these types of output is viewed as the most

!important for any one GTPase or GTPase family will obviously


depend upon the context. But the fact that it is common for both


to be occurring, perhaps on the same membrane, and that there


is a finite pool of GTPases makes questions about specificity and


diffusion of GTPase signaling only more important to address


and model.


Although the question posed relates specifically to GTPase


activation, I cannot leave this introductory article without some


comparisons between the different models for termination of


GTPase signaling. Though most GTPases have intrinsic GTPase


activity that spontaneously hydrolyzes bound GTP, these are typ-


cally quite low and it is commonly assumed that GTPase signal-


ing in the cell is terminated as a result of hydrolysis promoted by


a GTPase activating protein (GAP) for RAS superfam-


ily members and termed RGS for G proteins. GAP/RGS proteins can


increase the rates of GTP hydrolysis by as much as five orders of


magnitude and thus promptly silence the signal output from any


substrate GTPase. But it is clearly a mistake to think of GAP/


RGS proteins as simply modulators or inhibitors of GPCR signa-


ling pathways with high specificity. I have for a long time been


convinced that the field sorely needs more and better detailed biochemical characteriza-


tions of the affinities and specificities of each GTPase for each


GEF, GAP, and effector, using the full length proteins and not


just the isolated GEF (e.g., SEC7), GAP, or GTPase-binding


domains. While such “simple” assays will certainly miss some


biologically important regulatory factors (e.g., lipids, other pro-


teins) they should form the basis for better models of GTPase


pathways and the specificities required in biology.


I conclude my thoughts with the observation that the G pro-


tein and RAS superfamilly fields began in different ways that I


think have colored their evolutions. G proteins were first pre-


dicted and then identified and purified as essential regulatory


components in hormone stimulated (notably β-adrenergic ago-


nists like adrenaline and isoproterenol) adenylyl cyclase activity


(apologies to the photoreceptor people). Those purifications, and


later cloning and homology searching, identified the family of


GTP-binding α subunits whose primary functions are coupling of


GPCRs to effectors. In contrast, the RAS superfamilly began in


the middle, with the GTPases (RAS itself, ARF, etc) at a time


when cloning and homology searching was more common and


led to the rapid realization of larger families of paralogs. In many


cases we didn’t have a biology associated with the GTPase under


study, and we have been identifying components in the pathways


ever since. I can point to over 20 effectors of ARF GTPases and


there are likely to be a similar number for RAS and other mem-


bers of the superfamilly. But I cannot yet describe for most of


those effectors how one is activated to the exclusion of the others.


Thus, what seems to me to be lagging behind in the RAS
superfamily fields are not only the sources of specificity in determining which effector(s) is in play in specific cases, but also how the GEFs are activated. We know many of the GEFs, the functional homologs of the GPCRs, but what are the “ligand equivalents”? What regulates the GEFs? I think in the case of ARFs, the GTPase-regulated pathways are much more likely to be constitutive in nature, tunable by mass action (e.g., changes in the levels of cargo at specific membrane sites or possibly of a lipid stimulator) rather than the acute changes in ligand concentration at the cell surface that require the rapid generation of an amplified signal that changes a biological outcome. This appears to be the situation with the RHO/RAC model described in the article from Schwarz and Moisoglou, as they point out the lack of point mutants but instead changes in GTPase expression levels showing correlations to diseases states. My goal here is simply to spark discussions that hopefully generate fruitful experimentation that continue the evolution of more comprehensive and specific models for GTPase signaling. It is a shame that as these fields have each gotten so large, we no longer have meetings that include G protein, RAS, RAB, ARF, RAN, RHO, etc researchers all together. I think we still have much to learn from one another, both in the commonalities and in the differences in the varied and critical cellular actions of our favorite signal transducers.

References

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
I thank the many colleagues who have discussed these topics with me over the years and in the generation of this Reasoned Debate series, too numerous to list. I readily admit to many sins of omission in models and citations in this article and figures as they are intended as summaries for discussion and are nowhere close to exhaustive. Many key findings and pathways were not included either due to my own ignorance or the need to stay focused on a few general points. There are many closely related topics that are interesting and extend or contradict statements made above. Not the least are novel roles for non-canonical GEFs for G proteins (e.g., Ric-8), membrane traffic of GTPases and their actions at different sites, and cross-talk between signaling by GTPases in different families (ARF-RAB, ARF-RHO, ARF-G proteins, etc).

Funding
This work was supported in part by a grant from the National Institutes of Health R01-GM-090158.