

PERSPECTIVE

Illuminating $G\beta_5$ Signaling

Corinne E. Zeller and Henrik G. Dohlman

Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

Received July 16, 2007; accepted July 16, 2007

ABSTRACT

G proteins are key intermediates in cellular signaling and act in response to a variety of extracellular stimuli. The prevailing paradigm is that G protein subunits form a heterotrimeric complex and function principally at the plasma membrane. However, there is growing evidence for localization at, and signaling by, G proteins at intracellular compartments. Moreover, different cellular pools of G proteins may be composed of distinct subunit subtypes, including some binding partners that function in the place of G protein γ subunits. An article in this issue

of *Molecular Pharmacology* (Yost et al., p. 812) describes the use of an innovative fluorescent cell imaging technique to study interactions of the G protein β_5 subunit with a panel of $G\gamma$ subunits as well as regulator of G protein signaling (RGS) proteins that contain a $G\gamma$ -like subdomain. The approach used here provides a new strategy to elucidate the spatial and temporal properties of G proteins, including a growing number of atypical $G\beta\gamma$ pairings.

Heterotrimeric G proteins normally consist of α , β , and γ subunits and are coupled to seven transmembrane receptors at the plasma membrane. Agonist binding to the receptor induces a conformational change of the $G\alpha$ subunit promoting the release of GDP and binding to GTP. This exchange triggers $G\beta\gamma$ disassociation from the $G\alpha$, freeing both components to modulate downstream signals. Hydrolysis of GTP to GDP by the $G\alpha$ results in reassociation of the heterotrimer and termination of the signal (Sprang, 1997).

So far, 23 $G\alpha$, 5 $G\beta$, and 12 $G\gamma$ subunits have been identified in the mammalian genome. Of the $G\beta$ isoforms, types 1 to 4 are highly conserved, sharing 80% sequence identity, but $G\beta_5$ is divergent, sharing only 50% identity. Like other β isoforms, $G\beta_5$ interacts with $G\gamma$ subunits; unlike the others, $G\beta_5$ can also interact with RGS proteins from the R7 family (RGS6, RGS7, RGS9, and RGS11) (Witherow and Slepak, 2003). Most RGS proteins regulate signaling by acting as GTPase-accelerating proteins, increasing the rate of GTP hydrolysis, causing a more rapid termination of the signal. Members of the R7 family of RGS proteins are defined as having a C-terminal RGS domain, a central $G\gamma$ -like domain,

and an N-terminal DEP (Dishevelled, Egl-10, Pleckstrin) domain. It is not clear why R7 RGS and $G\beta_5$ proteins interact; however, it has been shown that the interaction stabilizes the heterodimer against proteolysis (McCudden et al., 2005).

The RGS/ $G\beta_5$ complex could be thought of as a highly atypical $G\beta\gamma$ pair. Others are likely to exist (see below). With the identification of such atypical subunit complexes, new techniques are needed to ascertain their function within the cell. Bimolecular fluorescence complementation (BiFC) is one promising technique (Kerppola, 2006a). BiFC uses fragments of green fluorescent protein derivatives (YFP or CFP) each fused to interacting proteins. When not assembled, the individual fusion proteins do not fluoresce, but when associated, they produce a fluorescent signal. This technique allows for the detection only of proteins that are in complex, and so can be used to monitor the interaction of defined $G\beta$ and $G\gamma$ subunit subtypes. In addition, different pairs can be assembled to produce different color variants of GFP. Such multicolor BiFC allows for simultaneous visualization of two distinct protein complexes within a single cell. Using these techniques, complex formation can be measured in time and space.

In this issue of *Molecular Pharmacology*, Yost et al. (2007) report their use of multicolor BiFC to investigate the ability

Article, publication date, and citation information can be found at <http://molpharm.aspetjournals.org>.

doi:10.1124/mol.107.040055.

Please see the related article on page 812.

ABBREVIATIONS: RGS, regulator of G protein signaling; BiFC, bimolecular fluorescence complementation; RACK1, receptor for activated C kinase 1.

of G β_5 to interact with G γ subunits and RGS7 in live cells, with and without other binding partners [G α -GTP, G α -GDP, and R7 binding protein (R7BP)]. Using competition studies with heterologously expressed proteins, these authors demonstrate that G β_5 prefers to interact with G γ_2 over other G γ isoforms and that different G $\beta_5\gamma$ combinations activate phospholipase C β_2 in proportion to their abilities to form complexes, providing the first comparison of G $\beta\gamma$ complex formation with functionality in living cells. Having shown a strong capability of G β_5 to interact with G γ_2 , the authors next sought to determine whether G β_5 prefers to interact with G γ_2 or RGS7. Again using competition studies, they show that G β_5 prefers G γ_2 over RGS7; but when coexpressed with R7BP, G β_5 is able to form complexes equally well with both G γ_2 and RGS7. Both G $\beta\gamma$ and G β_5 -RGS complexes interact with G α subunits. The G $\beta\gamma$ interacts with inactive (GDP-bound) G α and the G β_5 -RGS interacts with activated (GTP-bound) G α . Both G α and G γ subunits contain lipid modifications that target the entire G $\alpha\beta\gamma$ heterotrimer to the plasma membrane. Using BiFC, the authors suggest that the activated G α is partially responsible for recruitment of the G β_5 -RGS7 complex to the plasma membrane, whereas inactive G α is complexed with G $\beta\gamma$ at the plasma membrane. Taken together, these data indicate that G β_5 associates with different partners depending on their relative abundance and the presence of secondary binding partners; these binding partners dictate cellular localization of the complex.

The issue of whether G β_5 interacts with both R7 family RGS proteins and G γ subunits has been controversial. The results of Yost et al. (2007) demonstrate that BiFC can be valuable for analyzing protein-protein interactions that have proven refractory to conventional biochemical methods. Although G $\beta_5\gamma_2$ can regulate effectors, G β_5 has thus far been copurified only with R7 proteins (Witherow et al., 2000). The instability of G $\beta_5\gamma_2$ under nondenaturing buffer conditions may explain this discrepancy (Yoshikawa et al., 2000; Jones et al., 2004). A current limitation of BiFC is that it may stabilize transient interactions, because the formation of the fluorescent complex is generally thought to be irreversible (Hébert et al., 2006; Kerppola, 2006b). However, it is possible that variants of the fluorescent fragments could be engineered that can associate reversibly. Nevertheless the BiFC technique will be very useful in identifying and localizing atypical G protein complexes in intact cells.

In the classic model of signaling by heterotrimeric G proteins, the α , β , and γ subunits are anchored to the plasma membrane (Neves et al., 2002). However, pools of G proteins have been found at intracellular compartments (Sorkin and Von Zastrow, 2002) and recent reports have demonstrated that G α subunits can transmit a signal from internal membranes and that atypical G β subunits can regulate signaling. In the *Saccharomyces cerevisiae* pheromone-response pathway, the G α protein is localized to the plasma membrane, but is also present at the endosome, where it activates production of the second messenger phosphatidylinositol 3-phosphate. The G $\beta\gamma$ remains at the plasma membrane and activates a

mitogen-activated kinase cascade. A second atypical G β is found at the endosome, where it functions as a regulatory subunit of the phosphatidylinositol 3-kinase (Slessareva et al., 2006). Additional examples of atypical G β subunits have been identified in fungi (Hoffman, 2007); examples include Gib2 in *Cryptococcus neoformans* (Palmer et al., 2006), Asc1 in *S. cerevisiae* (Zeller et al., 2007), and Gnr1 in *Saccharomyces pombe* (Goddard et al., 2006). Gib2 and Asc1 share sequence similarity with human RACK1 (Receptor for Activated C Kinase 1), and both function in glucose signaling through cAMP; Gib2 activates signaling by the adenylyl cyclase and Asc1 repress enzyme activity. Gnr1 functions as a negative regulator of the G α in the pheromone-response pathway. These findings in fungi suggest that the superfamily of G proteins may be far larger and more complex than previously recognized. With the identification of new G proteins and the abilities of some of these proteins to propagate signaling from intracellular compartments, the BiFC technique will undoubtedly prove useful in establishing their spatial and temporal signaling characteristics.

References

- Goddard A, Ladds G, Forfar R, and Davey J. (2006) Identification of Gnr1p, a negative regulator of G alpha signalling in *Schizosaccharomyces pombe*, and its complementation by human G beta subunits. *Fungal Genet Biol* **43**:840–851.
- Hébert TE, Gales C, and Rebois RV (2006) Detecting and imaging protein-protein interactions during G protein-mediated signal transduction in vivo and in situ by using fluorescence-based techniques. *Cell Biochem Biophys* **45**:85–109.
- Hoffman CS (2007) Propping up our knowledge of G protein signaling pathways: diverse functions of putative noncanonical Gbeta subunits in fungi. *Sci STKE* **2007**:pe3.
- Jones MB, Siderovski DP, and Hooks SB (2004) The G $\beta\gamma$ dimer as a novel source of selectivity in G-protein signaling: GGL-ing at convention. *Mol Interv* **4**:200–214.
- Kerppola TK (2006a) Design and implementation of bimolecular fluorescence complementation (BiFC) assays for the visualization of protein interactions in living cells. *Nature Protocols* **1**:1278–1286.
- Kerppola TK (2006b) Visualization of molecular interaction by fluorescence complementation. *Nat Rev Mol Cell Biol* **7**:449–456.
- McCudden CR, Hains MD, Kimple RJ, Siderovski DP, and Willard FS (2005) G-protein signaling: back to the future. *Cell Mol Life Sci* **62**:551–577.
- Neves SR, Ram PT, and Iyengar R. (2002) G protein pathways. *Science* **296**:1636–1639.
- Palmer DA, Thompson JK, Li L, Prat A, and Wang P. (2006) Gib2, a novel G β -like/RACK1 homolog, functions as a Gbeta subunit in cAMP signaling and is essential in *Cryptococcus neoformans*. *J Biol Chem* **281**:32596–32605.
- Slessareva JE, Routt SM, Temple B, Bankaitis VA, and Dohlman HG (2006) Activation of the phosphatidylinositol 3-kinase Vps34 by a G protein alpha subunit at the endosome. *Cell* **126**:191–203.
- Sorkin A and Von Zastrow M (2002) Signal transduction and endocytosis: close encounters of many kinds. *Nat Rev Mol Cell Biol* **3**:600–614.
- Sprang SR (1997) G protein mechanisms: insights from structural analysis. *Annu Rev Biochem* **66**:639–678.
- Witherow DS and Slepak VZ (2003) A novel kind of G protein heterodimer: the G beta5-RGS complex. *Recept Channels* **9**:205–212.
- Witherow DS, Wang Q, Levay K, Cabrera JL, Chen J, Willars GB, and Slepak VZ. (2000) Complexes of the G protein subunit G β_5 with the regulators of G protein signaling RGS7 and RGS9. Characterization in native tissues and in transfected cells. *J Biol Chem* **275**:24872–24880.
- Yoshikawa DM, Hatwar M, and Smrcka AV. (2000) G protein beta 5 subunit interactions with alpha subunits and effectors. *Biochemistry* **39**:11340–11347.
- Yost EA, Mervine SM, Sabo JL, Hynes TR, and Berlot CH (2007) Live cell analysis of G protein β_5 complex formation, function, and targeting. *Mol Pharmacol* **72**:812–825.
- Zeller CE, Parnell SC, and Dohlman HG (2007) The RACK1 ortholog Asc1 functions as a G protein beta subunit coupled to glucose responsiveness in yeast. *J Biol Chem*, in press.

Address correspondence to: Henrik G. Dohlman, Department of Biochemistry & Biophysics, University of North Carolina at Chapel Hill, 116 Manning Dr., CB 7260, Chapel Hill, NC 27599. E-mail: henrik_dohlman@med.unc.edu