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PERSPECTIVE

Illuminating Gβ_5 Signaling

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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 Gγ subunits as well as regulator of G protein signaling (RGS) proteins that contain a Gγ-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 Gα subunit promoting the release of GDP and binding to GTP. This exchange triggers G$\beta\gamma$ disassociation from the Gα, freeing both components to modulate downstream signals. Hydrolysis of GTP to GDP by the Gα results in reassociation of the heterotrimer and termination of the signal (Sprang, 1997).

So far, 23 Gα, 5 Gβ, and 12 Gγ subunits have been identified in the mammalian genome. Of the Gβ isoforms, types 1 to 4 are highly conserved, sharing 80% sequence identity, but Gβ_5 is divergent, sharing only 50% identity. Like other β isoforms, Gβ_5 interacts with Gγ subunits; unlike the others, Gβ_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 Gγ-like domain, and an N-terminal DEP (Dishevelled, Egl-10, Pleckstrin) domain. It is not clear why R7 RGS and Gβ_5 proteins interact; however, it has been shown that the interaction stabilizes the heterodimer against proteolysis (McCudden et al., 2005).

The RGS/Gβ_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 Gβ and Gγ 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

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

of $G\beta_5$ to interact with $G\gamma$ subunits and RGS7 in live cells, with and without other binding partners [$G\alpha$-GTP, $G\alpha$-GDP, and R7 binding protein (R7BP)]. Using competition studies with heterologously expressed proteins, these authors demonstrate that $G\beta_5$ prefers to interact with $G\gamma_2$ over other $G\gamma$ 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 $G\beta_5$ to interact with $G\gamma_2$, the authors next sought to determine whether $G\beta_5$ prefers to interact with $G\gamma_2$ or RGS7. Again using competition studies, they show that $G\beta_5$ prefers $G\gamma_2$ over RGS7; but when coexpressed with R7BP, $G\beta_5$ is able to form complexes equally well with both $G\gamma_2$ and RGS7. Both $G\beta\gamma$ and $G\beta_5$-RGS complexes interact with $G\alpha$ subunits. The $G\beta\gamma$ interacts with inactive (GDP-bound) $G\alpha$ and the $G\beta_5$-RGS interacts with activated (GTP-bound) $G\alpha$. Both $G\alpha$ and $G\gamma$ 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 $G\alpha$ is partially responsible for recruitment of the $G\beta_5$-RGS7 complex to the plasma membrane, whereas inactive $G\alpha$ is complexed with $G\beta\gamma$ at the plasma membrane. Taken together, these data indicate that $G\beta_5$ 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 $G\beta_5$ interacts with both R7 family RGS proteins and $G\gamma$ 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, $G\beta_5$ 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 $G\alpha$ subunits can transmit a signal from internal membranes and that atypical $G\beta$ subunits can regulate signaling. In the *Saccharomyces cerevisiae* pheromone-response pathway, the $G\alpha$ 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 $G\beta$ 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 $G\beta$ 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 $G\alpha$ 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.

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