Making useful gadgets with miniaturized G proteins

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Kirill A. Martemyanov1 and Mikel Garcia-Marcos2

From the 1Department of Neuroscience, Scripps Research Institute, Jupiter, Florida 33453 and 2Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118

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G protein–coupled receptors (GPCRs) relay information from extracellular stimuli to intracellular responses in a wide range of physiological and pathological processes, but understanding their complex effects in live cells is a daunting task. In this issue of JBC, Wan et al. repurpose “mini G proteins”—previously used as affinity tools for structural studies—to develop a suite of probes to visualize GPCR activation in live cells. The approach is expected to revolutionize our understanding of the spatiotemporal control and mechanisms of GPCR signaling.

G protein–coupled receptors (GPCRs)3 are a large family of membrane proteins that initiate cellular responses to a diverse array of extracellular signals including neurotransmitters, hormones, and photons (1). The major mechanism employed by GPCRs to transduce signals involves activation of heterotrimeric G proteins (Fig. 1), which are broadly divided into four families (Gα, Gβγ, Gα11, Gα12/13) based on structural and functional similarity (1). The pathways activated downstream of GPCRs are critical for many physiological processes, and their dysregulation frequently leads to human disease. Accordingly, >30% of FDA-approved drugs target GPCRs for therapeutic benefits (2). Thus, the ability to monitor GPCR activity in live cells with high fidelity and precision is crucial not only for understanding mechanisms underlying the action of endogenous ligands, but also for the development of novel therapeutic agents.

GPCR activation is commonly monitored by following its effects on downstream signaling events, such as changes in levels of secondary messengers (e.g. cAMP, Ca2+), ion channels, or transcription. However, these indirect downstream events result from signal amplification and can be altered by cross-talk with other G protein–dependent and –independent pathways, which compromises the fidelity of the readouts as bona fide reporters of GPCR activity. A major advance in overcoming these limitations came from the development of fluorescence or bioluminescence resonance energy transfer (FRET or BRET)–based biosensors that measure G protein activity, a signaling event very proximal to GPCR activation (3–6). These sensitive and precise tools greatly enhanced our understanding of the mechanism of action of natural ligands and drugs. However, the activity of G proteins in cells is modulated not only by GPCRs, but also by an extensive network of regulatory proteins. Furthermore, G proteins are quite heterogeneous, with 16 possible Ga, 4 Gβ, and 12 Gγ subunits, allowing many unique combinations with distinct properties. Efforts have been made to develop tools to directly monitor GPCR activation instead of using G protein activity as a proxy, including FRET probes or nanobody-fused fluorescent proteins (e.g. Nb80) that monitor GPCR conformational changes (3, 7). However, these approaches tend to be noisy and technologically demanding, limiting their widespread use. Moreover, these approaches are specific for individual GPCRs, which number in the hundreds. Their adaptation to different receptors is labor-intensive and requires substantial customization.

In this issue of JBC, Nevin Lambert’s group describes the development of a new arsenal of tools and assays to monitor GPCR activation directly and robustly using standard equipment available in most laboratories (8). The new technology is based on repurposing the so-called “mini G proteins” (mGs) originally developed as structural biology tools by Carpenter, Tate, and colleagues (9, 10), who co-authored this paper. mGs consist of the Ras-like domain of Ga subunits, which have also been heavily engineered to gain stability and to become insensitive to conformational changes that cause GPCR disengagement during agonist stimulation (Fig. 1). In their report, Wan et al. (8) show that mGs fused with reporter probes can be used to quantify GPCR activation in multiple cell-based assay formats (see below). As long as the GPCR is active, the mG remains bound to it, thereby reporting the activation. Because different Ga subunits have unique properties, the identity of Ga mobilized by a GPCR reflects their distinct effects on cellular signaling and physiology. Remarkably, the authors were able to recapitulate intrinsic G protein coupling specificity of GPCRs using 4 different mGs, one from each of the major G protein families (mGαq, mGαi, mGαβγ, mGα12). They also show the work of mGs on several GPCRs, likely making their adaptation for the array of different GPCRs a straightforward task.

A powerful feature of mGs showcased in this paper is the versatility of assay formats in which they can be implemented. For example, mGs are diffusely distributed in the cytosol under resting conditions, but are rapidly recruited to cell membranes upon GPCR stimulation. Thus, tagging mGs with fluorescent

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2 Supported by NIGMS, National Institutes of Health Grants R01GM112631 and R01GM108733, American Cancer Society Grant RSG-13-362-01-TBE, and the Karin Grunebaum Cancer Research Foundation. To whom correspondence should be addressed: Boston University, 72 E. Concord St., Rm. K206, Boston, MA 02118. Tel.: 617-638-4387; E-mail: mgm1@bu.edu.
3 The abbreviations used are: GPCR, G protein–coupled receptor; mG, mini G protein; FRET, fluorescence resonance energy transfer; BRET, bioluminescence resonance energy transfer.
proteins allows tracking of the location of active GPCRs using imaging-based approaches. Using this strategy, the study beautifully documents detection of GPCR activation at various subcellular locations including plasma membrane, the Golgi apparatus, and endosomes (8). More quantitative and kinetic information on GPCR activation can be obtained when mGs and GPCRs are tagged with BRET pairs, yielding robust and reproducible ratiometric measurements in multiwell assay format on plate readers (8). What if you do not have access to sophisticated instruments or feel uncomfortable doing live-cell microscopy or BRET measurements? No problem. The authors have also adapted mGs to be used in bimolecular complementation luminescence assays that only require plain photon counting in a luminometer. mGs for the people!

Arguably, a tool is only as good as its ability to answer biologically relevant questions. One of the first things that comes to mind with GPCRs is pharmacology. In this regard, the authors explored by the authors is G protein specificity profiling, i.e. what G protein subtypes can be activated by a given GPCR. GPCRs are notorious for activating multiple signaling pathways and do so with superb selectivity for native heterotrimers.

Figure 1. mG technology for assaying GPCR signaling. Left, natively, binding of an agonist to a GPCR induces its conformational change creating a surface for association with cognate G protein heterotrimer consisting of α, β, and γ subunits. The association triggers nucleotide exchange on the α subunit replacing GTP with GDP. The α subunit consists of the helical domain (HD) and the Ras homology (RH) domain, which contains determinants for the association with GPCRs. The cycle is complete when Gα hydrolyzes GTP and reassociates with the Gβγ subunits. Right, mGs are engineered by removing the helical domain, introducing stabilizing mutations, and making them nucleotide-independent. mGs are tagged with various reporters for tracking their localization in cells or for detecting their association with GPCRs. Four mGs, one from each major subclass of the G protein family, recapitulate GPCR selectivity for native heterotrimers.

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