GPCR SIGNALING

Focus Issue: New insights in GPCR to G protein signaling

Nancy R. Gough*

This Focus Issue highlights new discoveries at the level of the receptor, the α subunit, and the βγ subunit and spans research in yeast on polarized growth and G protein–coupled receptor (GPCR) trafficking, in mice on an orphan GPCR with constitutive activity, and a disease-causing mutation in an α subunit that results in inappropriate GPCR–G protein coupling.

G protein–coupled receptors (GPCRs) are the largest family of receptors in humans—encoded by more than 800 genes. Thus, unsurprisingly, they have roles in all aspects of physiology and are critical regulators of development. Many clinically available drugs target these receptors or their downstream signaling pathways. GPCRs belong to the larger family of seven-transmembrane (7TM) receptors and are so named because they activate heterotrimeric guanine nucleotide–binding proteins (G proteins). The importance of these receptors is evidenced by the nine Nobel Prizes awarded for GPCR signaling research. Although GPCRs can signal through at least two primary mediators, the G proteins and arrestins, this issue focuses on the G protein–mediated pathways.

G proteins consist of an α subunit with intrinsic guanosine triphosphatase (GTPase) activity, a β subunit, and a γ subunit, the latter two of which function as a heterodimer referred to as the βγ subunit. GPCR activation triggers guanosine 5′-diphosphate (GDP) for guanosine 5′-triphosphate (GTP) exchange on the α subunit, releasing the Gβγ subunit and enabling both the GTP-bound Gα subunit and the Gβγ subunit to signal to distinct downstream effectors [see the Animation (http://stke.sciencemag.org/highwire/filestream/202680/field_highwire_adjunct_files/0/G_Protein-Cycle_STKE.swf) in the Review by Preininger and Hamm]. The receptor interacts with the α subunit of the heterotrimer; thus, the α subunit determines which G protein is activated by a given receptor.

Although in humans there are 16 genes that encode α subunits, 5 that encode β subunits, and 14 that encode γ subunits, the G proteins and the GPCRs to which they couple are defined by the α subunit. Research by Masuho *et al*. indicates that this convention may be misguided, because GPCRs are more promiscuous than was previously thought. Their analysis demonstrated that many receptors can engage multiple G proteins with distinct efficacies and kinetics and that different classes of GPCR ligands and allosteric modulators bias the G protein–coupling signature. To make the situation even more complex, intracellular signaling modulators also affected the G protein–coupling profiles. Thus, GPCR–G protein coupling and the cellular response may be governed not only by external signals, but also by the relative abundance of the α subunits and other intracellular regulators.

Although the ligands and allosteric modulators are known for many GPCRs, the ligands for some remain unknown, and these GPCRs are referred to as orphans. Ligands for GPCRs can be full agonists, partial agonists, inverse agonists, antagonists, or allosteric modulators that do not directly affect receptor activity, but influence the response to ligands. In addition, the electrical potential of a cell, or membrane potential, can affect GPCR function. Rinne *et al*. found that the binding mode of agonists of the M_3 muscarinic acetylcholine receptor determines whether membrane-potential changes enhance or attenuate receptor signaling. This may explain how drugs that are agonists of specific GPCRs can have distinct effects in different cellular contexts.

GPCRs can also be activated by proteolysis to release a tethered ligand or expose a ligand-binding site. Fragments of the orphan receptor GPR37L1 are present in human cerebrospinal fluid, suggesting that this receptor may be activated by proteolysis. Unexpectedly, Coleman *et al*. found that the full-length unprocessed form of GPR37L1 was constitutively active and coupled to $G\alpha_s$, and that removing the N terminus inactivated the receptor. Thus, rather than activating the receptor, metalloprotease-mediated processing of the receptor turns it off. The idea of constitutively active components of the GPCR–G protein system is not without precedent. Bradford *et al*. found that not only were plant α subunits constitutively active (exhibiting rapid GTP binding and slow GTP hydrolysis), but so were α subunits from species that lacked GPCRs. Instead, in many species, G protein activity is limited by a 7TM protein with a regulator of G protein signaling (RGS) domain that functions as a GTPase-activating protein to stimulate GTP hydrolysis.

Yeasts have GPCRs and detect and respond to pheromones (mating signals) through a GPCR–G protein pathway. In this case, the βγ subunit is the primary effector transducing the signal. Indeed, Coleman *et al*. leveraged this pathway to screen for G protein coupling, using chimeric α subunits that could interact with the mammalian receptor and stimulate βγ to induce a reporter gene. Ismael *et al*. studied how yeast properly enhance the abundance of pheromone receptor at the side closest to the mating partner. The βγ subunit interfered with phosphorylation of the receptor in the region where the pheromone signal would be strongest (at the side nearest the closest potential mate) and thus prevented internalization of the receptor at that location. Neutrophils are immune cells that chemotax in response to signaling molecules that activate GPCRs. Surve *et al*. used a small molecule that released βγ without activating the $G\alpha_i$ subunit to show that active βγ subunits alone increased the intracellular concentration of the second messenger cAMP (adenosine 3′,5′-monophosphate) to such an extent that the cells stuck to coated surfaces. To enable movement, $G\alpha_i$ subunit activity had to balance this βγ-induced signal.

GPCRs also play many roles in the nervous system. The receptor GPR37L1 studied by Coleman *et al*. is involved in development of the cerebellum. Opioid receptors in the brain respond to the body's natural pain-relieving signals (endorphins) and are the target of opioid pain-relieving drugs. Halls *et al*. found that whether an agonist of the μ-opioid receptor triggered an increase in receptor movement in the plasma membrane or restricted receptor mobility determined the spatiotemporal dynamics of the response. The dopamine receptor D2 is linked to neuropsychiatric disease, and drugs targeting this receptor are used to treat schizophrenia. Likhite *et al*. found that arginine methylation of intracellular residues

Editor, *Science Signaling*, American Association for the Advancement of Science, Washington, DC 20005, USA.

***Corresponding author. E-mail: ngough@aaas.org**

was critical to signaling by this receptor. *Caenorhabditis elegans*, a worm, lacking the enzyme that performs this modification, had movement and behavioral defects that were similar to those in worms lacking D2. Thus, arginine methylation needs to be considered together with phosphorylation when investigating the posttranslational code that regulates GPCR signaling.

Dysfunctional GPCR signaling results in disease. Understanding when this is happening has important implications for drug screening and development. Villanueva *et al*. found that Smoothened (Smo), a 7TM receptor best known for its role in Hedgehog-mediated regulation of the transcription factors of the Gli family, also signals through $G\alpha_{i2}$ to promote cell proliferation in mammary glands. This activity of Smo may promote oncogenic growth, and thus drugs that target Smo should be screened for activity against this pathway, as well as the Gli pathway. Marivin *et al*. discovered how mutations in $G\alpha_{i3}$ cause auriculo-condylar syndrome (ACS), a condition that disrupts craniofacial development. In mice, this phenotype can be reproduced by disrupting endothelin receptor ET_AR signaling, which is coupled to $G\alpha_{q/11}$. The ACS-causing mutations in $G\alpha_{i3}$ resulted in this G protein aberrantly coupling to ET_AR, but also prevented the G protein from signaling. Thus, this represented a naturally occurring dominant-negative mutation in a G protein.

Although the examples mentioned here only provide a glimpse of the complexity and physiological importance of the GPCR–G protein pathways and do not even touch upon the other pathways through which 7TM receptors can signal, they show that even this arm of GPCR research remains an exciting and active field with broad implications for understanding physiology and treating disease.

Related Resources

Reviews

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- A. M. Preininger, H. E. Hamm, G protein signaling: Insights from new structures. *Sci. STKE* **2004**, re3 (2004). [Abstract]

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Teaching Resources

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