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Authors

Ghosh, Pradipta

Garcia-Marcos, Mikel

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Do all roads lead to Rome in G-protein activation?

Pradipta Ghosh[1,*], **Mikel Garcia-Marcos**[2,*]

[1]Department of Medicine and Cellular and Molecular Medicine, University of California, San Diego, California, 92093

[2]Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts, 02118

Abstract

High-resolution structural studies on GPCRs have flourished recently, providing long-sought insights into the dynamic process of G-protein activation. In parallel, analogous studies are starting to shed light onto how the same G-proteins are activated by non-GPCR proteins. Can we learn about common themes and variations in G-protein activation from them?

Keywords

GTPase; GPCR; GEF; allosterism

[Introduction]

Heterotrimeric G-proteins were identified in the late 70's as transducers of signals emanating from G-protein-coupled Receptors (GPCRs), but the structural basis for such coupling and activation has unraveled only within the last decade. The GPCR/G-protein signaling axis not only exemplifies evolutionary success that is conserved across eukaryotes, but also a paradigm of paramount biomedical importance representing the single largest class of targets in the 'druggable' human genome. The crux of this quintessential signaling mechanism is in how G-proteins become activated by GPCRs. GPCRs are Guanine-nucleotide Exchange Factors (GEFs) that loosen the grasp of G-proteins on GDP to permit subsequent binding of GTP to adopt an active state. Because GPCRs bind to G-proteins at a site that is far removed from the nucleotide binding pocket, it has been known for long that activation must be achieved through allosteric mechanisms, details of which have only begun to emerge in the recent years. Within the past decade, high-resolution structures have been leveraged and complemented by a variety of biophysical and computational approaches to collectively grasp the dynamic nature of the G-protein activation process [1–5]. In parallel, and also within the past decade, another evolutionarily conserved mechanism of G-protein activation has emerged; it too has important cellular functions and biomedical relevance [6, 7]. Within this alternative paradigm, activation is triggered by cytoplasmic

[*]**Correspondence to: Pradipta Ghosh, M.D**; Department of Cellular and Molecular Medicine and Medicine; University of California at San Diego; George Palade Labs, room 232; Phone: (858) 822-7633, prghosh@ucsd.edu. **Mikel Garcia-Marcos, Ph.D**; Dept. of Biochemistry, Boston University School of Medicine; 72 E. Concord St., Silvio Conte Bldg. (K), Rm. K208 (office)/Rm. K206 (lab), Boston, MA 02118., Tel.: 617-358-4396 (lab); Fax: 617-6384047 (office); mgm1@bu.edu.

proteins with GEF activity analogous to that of GPCRs. Despite their relatively recent discovery, understanding of the structural basis for how they bind/activate G-proteins has reached an advanced stage rapidly [8–10]. In this *Forum*, we summarize what has been independently learned about G-protein activation by GPCRs and by non-GPCRs; we highlight how the two classes of activators converge on a similar allosteric mechanism despite their divergent modes of physical engagement with G-proteins.

From the first atomic resolution structure of a GPCR/G-protein complex (β2-adrenergic receptor with Gs) reported in 2011 [1] and other holocomplex structures reported later (e.g., [11]), the most striking conformational rearrangement observed is a large separation of the Ras-like and helical domains of Gα. Such interdomain separation does not seem to be the principal trigger for nucleotide release, but rather, a sequel to allosteric mechanisms that trigger the release [4]; it facilitates the escape of the released nucleotide by providing an escape route. Two main allosteric routes communicating GPCR contact sites with the nucleotide-binding pocket have been hypothesized (Fig. 1A), both supported by computational, biophysical, and biochemical evidence in addition to high resolution structures [1–5]. Both routes involve changes in structural elements of the core of the Ras-like domain and share a common stretch that converges onto the P-loop and adjacent nucleotide-binding elements. The interaction of the P-loop with the β-phosphate of GDP contributes greatly to the overall nucleotide-binding energy; its disruption greatly favors nucleotide release.

The two proposed allosteric routes are initiated at opposite ends of the G-protein sequence that make direct contact with the receptor: the C-terminal region of the α5-helix and the αN/β1 loop [1–3, 5]. For the first route, the N-terminus of the α5-helix forms a loop with the β6-strand that makes direct contact with the purine ring of nucleotides. However, perturbation of this nucleotide binding loop does not seem to be the direct driver of nucleotide release. Instead, such perturbation leads to other alterations in the α1-helix within the hydrophobic core of the protein, which in turn is pivotal to alter other nucleotide binding elements such as the Sw-I and the P-loop [3]. The second route initiated from the αN/β1 loop communicates directly with the P-loop in the nucleotide binding pocket through the β1-strand [1, 2, 5]. Of note, Gβγ, which is an absolute requirement for GPCR mediated activation of Gα, appears to be important to allow the engagement of the αN/β1 loop [1, 2, 5]. A recent cryo-EM structure of Rhodopsin with transducin in the absence of any other stabilizing protein (e.g. nanobodies) suggests that Gβγ might have additional roles in facilitating the signal relay from the αN/β1 loop [11] by “pulling” the switch(Sw)-II of Gα away from the core of the Ras-like domain, which might in turn relieve constrains on the β1-strand imposed by the proximal Sw-II. Such a ‘pull’ is reminiscent of the “lever-arm” hypothesis proposed over 20 years to explain GPCR-mediated activation [12].

In addition to membrane-restricted GPCRs, cytoplasmic proteins can also activate heterotrimeric G-proteins. A subgroup of them, called Guanine-nucleotide Exchange Modulators (GEMs), does so through an evolutionarily conserved sequence dubbed the *Gα-Binding-and-Activating* (GBA) motif. The prototype member of this class of non-GPCR GEFs is GIV (a.k.a. Girdin) [8]. Two recent studies using complementary approaches that include x-ray crystallography, NMR, molecular dynamics simulations, hydrogen-deuterium

exchange, homology modeling, and extensive mutagenesis have determined key aspects of the molecular mechanism of G-protein activation by GIV [8, 9]. First, GIV acts on monomeric Gα instead of Gα-Gβγ complexes by binding to a site on Gα different from those used by GPCRs, a groove formed between the Sw-II and α3-helix (Fig. 1B). At the base of this groove, GIV makes direct contact with the β1-strand, which is consistent with the large perturbations in this element and the connecting P-loop detected by NMR [8]. Binding-induced rearrangement of the Sw-II leads to allosteric perturbations in Sw-I and other elements of the hydrophobic core of the Ras-like domain, including the α1-helix that is implicated in GPCR-mediated activation [2, 3]. Perturbations in the interdomain contact between Ras-like and helical modules are observed upon GIV action, suggesting that domain-opening during nucleotide release is a common feature of GPCR- and non-GPCR GEFs. Despite these commonalities, key differences exist. For example, although the α1- and α5-helix seem to change their relative positions for both GPCRs and GIV, the trajectories of such repositioning seem to go in different directions [3, 4, 9]. Also, unlike GPCRs, GIV does not seem to perturb α5/β6 loop that binds the nucleotide purine ring. On the other hand, G-protein activation by GIV has features that resemble how small G-proteins of the Ras superfamily become activated by their cognate GEFs (Box 1). It should be noted that in addition to GIV-like factors, there are other non-GPCRs with GEF activity towards Gα. The best characterized one is Ric-8A, which appears to have properties in common with both GPCRs and with GIV in activating G-proteins (e.g., it perturbs both the C-terminus of the α5-helix and the Sw-II) [10].

Some interesting ideas can be extracted from the comparison of the mechanisms mediated by GIV and GPCRs. One is that the studies on GIV confirm that direct disruption of the GDP contacts with the α5/β6 loop is not a driver of enhanced nucleotide exchange, as previously suggested for GPCRs [3]. Instead, disruption of other nucleotide binding contacts such as the P-loop and the Sw-I seems to be the conserved theme. Another is that GIV may recreate the effects of the GPCR activation route initiated at the αN/β1 loop by engaging some of the same elements, and in doing so, emulate the “lever-arm” role of Gβγ [11, 12].

In summary, GPCRs and non-GPCRs engage G-proteins in different ways but eventually converge to a set of shared alterations in the GTPase core that are executed by partially overlapping allosteric routes to promote nucleotide exchange. In the future, it will be important to keep probing into the allosteric mechanisms of G-protein activation by these two classes of GEFs, which can be mutually informative to advance our understanding of the basis and potential targeting of their respective signaling pathways.

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TEXT BOX 1-

Similarities between modes of action of non-GPCR GEFs for Gα-GTPases and GEFs for Ras-GTPases.

While GIV and related GEFs activate G-proteins by binding at different sites from those used by GPCRs, there are similarities with the binding mode of GEFs for small G-proteins of the Ras superfamily. For small G-proteins, GEFs bind to the Sw-I and Sw-II regions of Ras that are analogous to the Sw-I and Sw-II of Gα. In Ras, the GEF binding site overlaps extensively with the binding site for effectors and GTPase Activating Proteins (GAPs). Likewise, the binding site for GIV on Gα overlaps extensively with the binding site for effectors and GAPs; they also overlap partially with the binding site for Gβγ, which explains why GIV promotes Gαβγ heterotrimer dissociation and subsequently exerts its GEF activity on monomeric Gα. The main differences between GIV and Ras GEFs is that the latter make direct contact the Sw-I and also introduce residues in the nucleotide binding pocket to destabilize nucleotide binding. For GIV, perturbation of the Sw-I and other nucleotide binding elements is carried out allosterically. Thus, the mechanism of activation of Gα by GIV appears to be a variation on an ancestral theme of activation for proteins with a canonical GTPase fold.

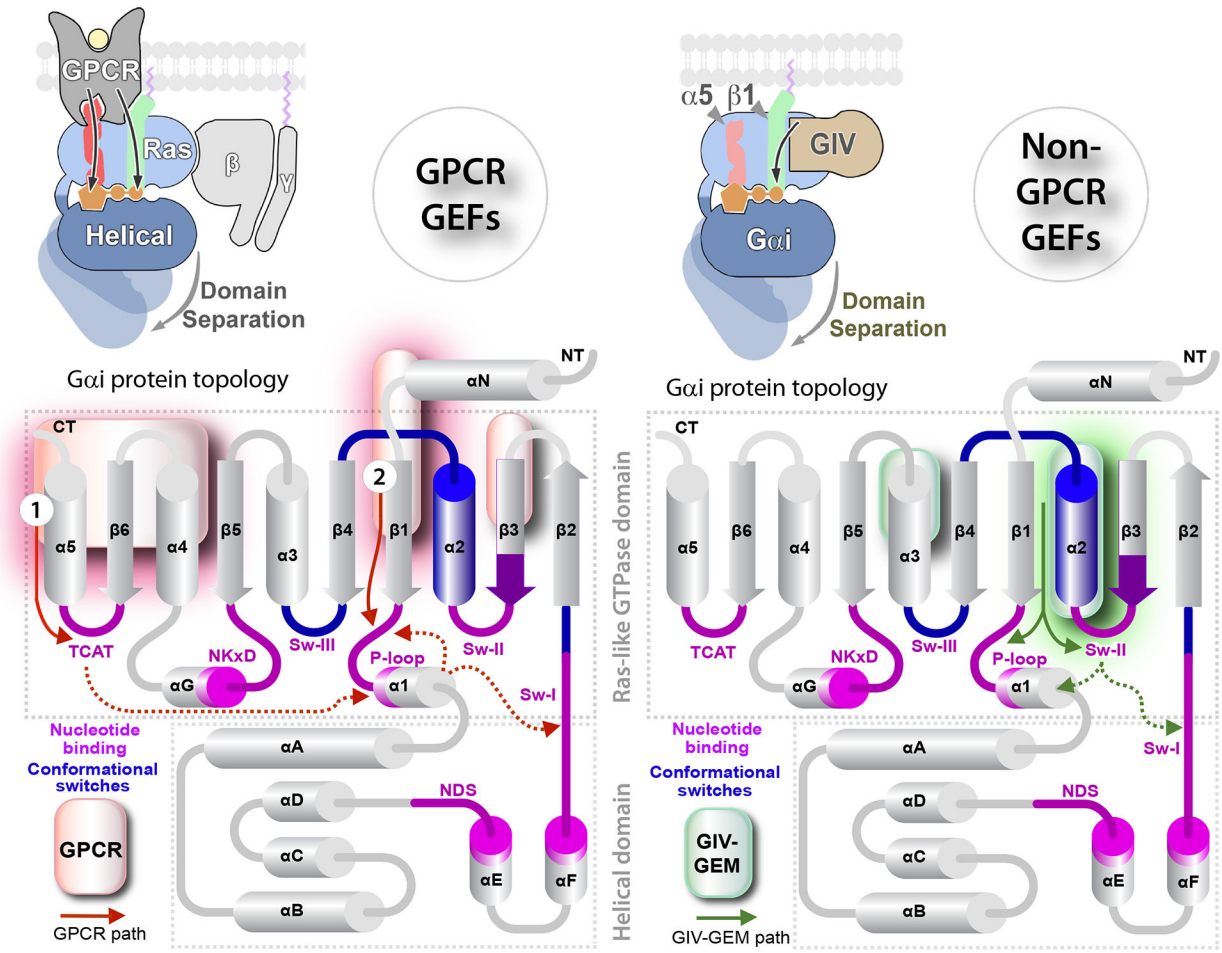

Figure 1. Comparison of allosteric routes utilized by GPCR and non-GPCR GEFs to promote nucleotide exchange in Gα.

Diagrams of secondary structure elements of Gα proteins are color coded to indicate the switch regions (blue) and nuclotide binding regions (purple). In panel A, the red shading indicates elements implicated in direct binding to GPCRs. The solid red arrows indicate the proposed primary alosteric routes linking GPCR-binding sites and nucleotide binding pocket (1 and 2), and the red dotted arrows indicate secondary allosteric routes involved in further perturbation of the nucleotide binding site. In panel B, the green shading indicates elements implicated in direct bdining to GIV. The solid green arrows indicate the proposed primary alosteric routes linking GIV-binding sites and nucleotide binding pocket, and the green dotted arrows indicate secondary allosteric routes involved in further perturbation of the nucleotide binding site.