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Building unconventional G protein-coupled receptors, one block at a time

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Abstract

The structure, function, and dynamics of canonical activation heterotrimeric G proteins by the 7-transmembrane G-protein coupled receptors (GPCRs) has been illustrated in detail. However, emerging studies during the last decade have started to shed light onto how the same G-proteins may be accessed and modulated also by a diverse family of receptors that are not conventional GPCRs. Can we learn about common themes and variations in how cells assemble these atypical G protein-coupled receptors?

Keywords

GPCRs; Guanine-nucleotide Exchange Modulators (GEMs); Growth factors; Toll-like Receptors; Integrins; Frizzled

When it comes to G protein signaling, it's a GPCR world!

Trimeric G-proteins were identified in the late 70's as transducers of signals downstream of G-protein-coupled Receptors (GPCRs). 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*[1]. The crux of this quintessential signaling mechanism is in how G-proteins become activated by GPCRs (Figure 1A); these receptors serve as 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. To do so, GPCRs bind to G-proteins at a site that is far removed from the nucleotide binding pocket, and activation is achieved through long-range allosteric mechanisms, as detailed through high-resolution structures that have been leveraged and complemented by a variety of biophysical and computational approaches. These studies have collectively revealed the dynamic nature of the G-protein activation by the receptor GEFs, GPCRs [2]. These are very significant advances because

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the GPCR/G-protein axis constitutes a core mechanism of transmembrane signaling that has been a great evolutionary success and which also has broad implications for human health and therapeutics.

GEMs couple G proteins to receptors other than conventional GPCRs

A group of cytoplasmic proteins, i.e., Guanine nucleotide Exchange Modulators (GEMs) with an intrinsic ability of modulating G-proteins via a short, defined sequence motif was identified only about 10 years ago (Figure 1A)[3]. This motif is the unifying feature shared by all the 5 members of the GEM family that have been identified to date in eukaryotes (GIV, DAPLE, Calnuc, NUCB2 and PLCδ4b); however, the most well-studied of them is the prototypical GEM, GIV, a.k.a, Girdin. GEMs are bifunctional modulator of G proteins; both GIV and DAPLE have been shown to inhibit Gαs using the same motif that allows them to activate Gαi [4, 5]. As in the case of GPCRs, the mechanism of GEM-dependent of G protein activation is also evolutionarily conserved and has important functions in both normal physiological processes and as drivers of human diseases.

Despite their relatively recent discovery, the structural basis for how they activate G-proteins has been brought to an advanced stage rapidly to match the current state-of-the-art in the context of GPCRs. There is atomic-resolution structural information available (Figure 1B), which together with computational modeling, various solution-state techniques including nuclear magnetic resonance, and biochemical approaches has allowed to envision a plausible mechanism by which GEMs lead to enhanced nucleotide exchange on Gαi and suppression of cAMP in a GPCR-independent manner [6–8].

But perhaps the most unique aspect of GEM-dependent G protein signaling is the broad context of such activation. Unlike the canonical paradigm, where activation occurs exclusively in response to ligands of GPCRs, GEM-dependent activation has thus far been described downstream of diverse classes of receptors, e.g., numerous growth factor receptor tyrosine kinases (RTKs, e.g., EGFR, InsR, IGFR1, VEGFR, PDGFR, to name a few) [9], integrins [10], Toll-like receptors (TLRs), Frizzled receptors (FZDRs), and even, canonical GPCRs etc. Studies leveraging the powerful synergy of homology modeling approaches, combined with model-guided mutagenesis and use of such mutants in biochemical, biophysical and cell phenotypic studies have also defined the context in which GEM-dependent G protein signaling is triggered in cells. These studies have not only elucidated the common theme *via* which GEMs couple G proteins to the cytosolic tails of diverse classes of receptors, but also highlighted some key variations within such theme regarding the nature of such coupling (illustrated in Figure 1C). One of the common themes is that it is typically the C-terminus of the GEMs that engage with the receptors. The C-termini of GEMs, ~200–400 aa long, are predicted to behave as intrinsically disordered proteins (IDPs). Interactions with receptors are either mediated by ‘on demand’ folding into a SH2-like module, as happens in the case of ligand-activated tyrosine-phosphorylated cytoplasmic tails of RTKs [11], or are mediated *via* **s**hort **li**near **m**otifs (SLIMs), as happens in the case of integrins [10], TLRs [12] and Frizzled receptors [13]. As for the variations within the common theme, some receptor•GEM interactions occur constitutively (TLRs), whereas others are strictly ligand dependent (RTKs, integrins, and Wnt/FZDRs); some are direct

(RTKs, TLRs, FZDRs), whereas others are indirect, via key adaptor (Integrins, via Kindlin) (Figure 1C).

These themes resemble the ‘LEGO brick’ metaphor, in which GEM’s modularity is a fundamental property that allows scaffolding of G proteins to cell surface receptors (either by assuming ‘on-demand’ conformations, allostery, or the induced-fit mechanisms). The ability of diverse receptors to ‘plug and play’ with the multi-modular GEMs and the G proteins is a classic example of how protein modularity generates plasticity, softness, and cross-talk that are essential for the coordinated execution of complex functions.

Consequences of unlikely coupling of G proteins with diverse classes of receptors

Regardless of the variations in the configuration, coupling of G proteins to diverse families of cell surface receptors *via* GEMs appears to have two major consequences. First, and the most important and shared consequence is the convergence of multi-receptor signaling on the core machinery (Gi → Adenylyl Cyclase) that regulates cellular cAMP. Consequently, diverse ‘input’ signals (not just GPCR ligands) can impact the ‘output’ (i.e., cAMP). This ability to modulate cellular cAMP largely represents a ‘gain’ of function for receptors that otherwise lack modularity to access/activate G proteins. A recent study that systematically compared GPCRs and the prototypical GEM, GIV/Girdin revealed divergence in their contribution to generating Gα-GTP and free Gβγ in cells directly measured with live-cell biosensors. Second, the recruitment of the GEM•Gαi complex at the receptor tail almost invariably impacts critical post-receptor decision-making, e.g., which adaptors are recruited, how much and when; those in turn impact major downstream signaling pathways.

Some interesting ideas can be extracted from the comparison of the GEM-enabled G protein coupled receptors. The C-terminus of the GEMs is responsible for receptor coupling, and in each instance, the specificity of each SLIM and its sensitivity to disruption *via* mutagenesis has been demonstrated. This raises the possibility that the disordered C-term of GEMs is designed to generate plasticity in signaling circuits by exposing when needed, and hiding when not, a catalogue of SLIMs. The exposed SLIMs may serve as ‘bar codes’ for coupling G proteins to a given receptor/pathway/cascade, thus, conferring spatial and temporal dynamics, context and functional plasticity in cellular signalling. Directed efforts in recent times have expanded the ‘toolbox’ available to study GEM-dependent signaling in cells using a combination of chemogenetics and optical biosensors [14–16]. These studies have the potential to illuminate the fundamental rules that govern GPCR-independent G protein signaling by GEMs.

In summary, receptors other than the conventional 7-TM GPCRs that engage G-proteins by binding to GEMs in different ways, eventually converge to trigger G protein signaling and suppress cellular cAMP. This raises the possibility that targeting the disordered C-terminus of GEMs may emerge as a promising therapeutic strategy to simultaneously target diverse cell surface receptors. As the GEM paradigm matures, it is conceivable that more and more unlikely sensors (of both intrinsic and extrinsic stimuli) may also converge upon G proteins *via* the GEM•Gα interface, leading to the further broadening of the importance

of this interface. Emerging evidence that this interface is 'druggable' [17, 18] and that its modulation can achieve desirable phenotypic resets in diverse chronic conditions [19], farther expands the already vast importance of G protein signaling in modern medicine and such concept has begun to gain traction [20]. Even though the details surrounding the exact extent, mechanism(s) and nature of the GEM-dependent G protein signaling are still emerging, and the differences in the observations made *in vitro* or *in cells* under physiologic conditions *versus* those made with overexpressed engineered probes [14] need to be urgently reconciled, one thing is clear—the paradigm of multi-receptor signaling *via* GEMs has permeated numerous fields, from immunology to cancer biology. Going forward, contributions from independent groups in these diverse fields will be critical to gain traction and fully explore the biological relevance and the translational potential of unconventional G-protein coupled receptors that eukaryotic cells assemble, using the multi-modular GEMs.

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Box1:

Various configurations for building G protein coupled receptors.

The multi-modular GEMs scaffold diverse classes of receptors (typically, non-GPCRs) to G proteins using variations of common themes of 'plug and play' aided by two key features of their carboxyl terminus: i) GEM's modularity and ii) intrinsically disordered property. For example, GIV, the first prototypical GEM, binds to the Tyr-phosphorylated cytoplasmic tails of ligand activated RTKs by folding its C-terminus into a SH2-like module [9]. In the case of integrins, a SLIM within GIV's C-terminus binds the key integrin-adaptor protein, Kindlin. Binding occurs at a site distinct from the site where '*NPxY*' sequences in the cytoplasmic tails of ligand-activated integrins bind Kindlin, and allosterically augments the latter, thereby altering the extent of integrin activation [10]. By contrast to RTKs and integrins, where the GEM/G protein system engages exclusively with ligand-activated receptors, in the case of TLR4 the binding is constitutive [12]. Here, yet another SLIM within GIV's C-terminus, a TIR-like BB-loop (TILL) motif, mediates binding of GIV-GEM to not just TLR4, but also other TIR modules-containing adaptor proteins that bind other TLRs. By showing that GIV uses conserved mechanisms to impact multi-TLR signaling, it was shown that it serves as a point of convergence of immune signaling of broader impact beyond TLR4. Finally, in the case of Daple, another GEM, direct binding to FZDRs allow these 7-TM receptors to trigger G protein signaling in cells, and the activation of unlikely pathways *via* G protein intermediates [13].

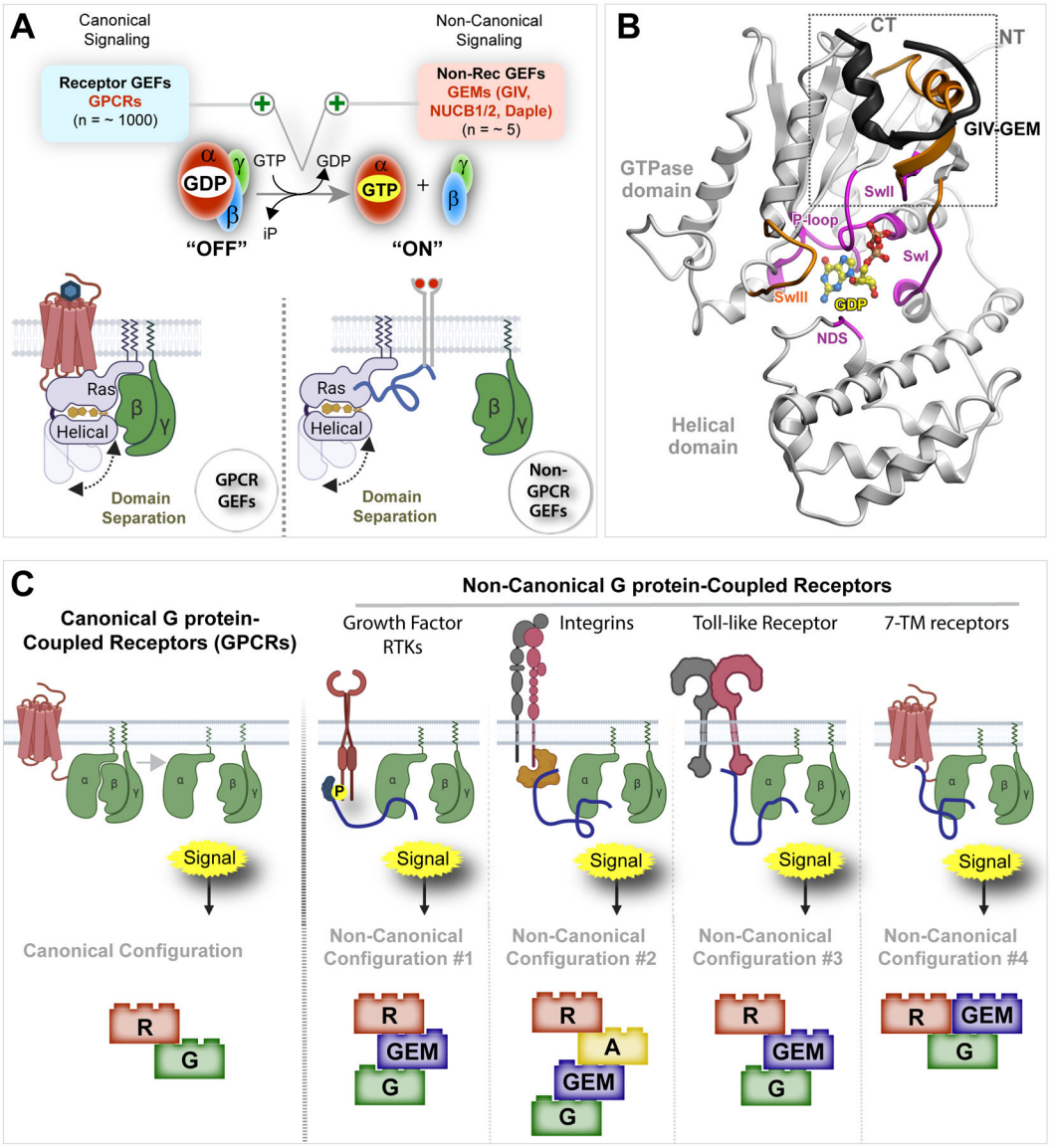

Figure 1. Diverse classes of receptors access and activate trimeric GTPases via GEMs.

A. *Top*: Schematic comparing canonical (left) and non-canonical (right) G protein signaling. *Bottom*: Regardless of the input signals (canonical, GPCRs; non-canonical G protein-coupled receptors), trimeric GTPases are activated via allosteric conformational changes (marked with arrows) that have similar consequences, i.e., rearrangement of the hydrophobic core of the Gαi-GTPase domain.

B. Crystal structure of Gαi with GIV-GEM peptide bound at Switch (Sw)-II. (PDB: 6MHF). Interrupted black box highlights the binding site for GIV, the prototypical GEM.

C. Schematic summarizing the various configurations *via* which GEMs couple diverse receptors to Gαi and trigger downstream signaling (detailed in Box 1). R, receptor; G, trimeric Gαi/s protein; A, adaptor protein, Kindlin.