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When heterotrimeric G proteins are not activated by GPCRs: structural insights and evolutionary conservation

Vincent DiGiacomo, Arthur Marivin, and Mikel Garcia-Marcos*

Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118.

Abstract

Heterotrimeric G proteins are signal transducing switches conserved across eukaryotes. In humans, they work as critical mediators of intercellular communication in the context of virtually any physiological process. While G protein regulation by GPCRs is well established and has received much attention, it has become recently evident that heterotrimeric G proteins can also be activated by cytoplasmic proteins. However, this alternative mechanism of G protein regulation remains far less studied than GPCR-mediated signaling. This Viewpoint focuses on recent advances on the characterization of a group of non-receptor proteins that contain a sequence dubbed the “Gα-Binding and Activating (GBA) motif”. So far, four proteins present in mammals (GIV aka Girdin, DAPLE, Calnuc and NUCB2) and one protein in *C. elegans* (GBAS-1) have been described to possess a functional GBA motif. The GBA motif confers Guanine nucleotide Exchange Factor activity on Gαi subunits in vitro and activates G protein signaling in cells. The importance of this mechanism of signal transduction is highlighted by the fact that its dysregulation underlies human diseases, such as cancer, which has made them attractive new candidates for therapeutic intervention. Here we discuss recent discoveries on the structural basis for GBA-mediated activation of G proteins and its evolutionary conservation, and compare them with the better studied mechanism mediated by GPCRs.

Keywords

GIV; Girdin; DAPLE; GPCR; GBAS-1

Heterotrimeric G proteins are well known and highly utilized signaling nodes coupling to a truly remarkable number of downstream cascades. Since their discovery, the field has continued to reveal and refine the understanding of how G protein activation is achieved and controlled – the importance of which has commanded the attention of biologists since the 1980s. The picture that has emerged from decades of biochemical and structural insights involves the activation of Gα subunits by guanine nucleotide exchange factors (GEFs), proteins that facilitate the exchange of GDP for GTP. Gα activation is concomitant with Gβγ release, resulting in two signals that, independently or in combination, mediate the diversity of G protein controlled pathways by interacting with downstream effectors. The archetypical GEFs for Gα subunits are the transmembrane GPCRs but, as is the case for the

*Corresponding Author Boston University School of Medicine, Department of Biochemistry, 72 E. Concord St., K Building, Room 206, Boston, MA 02118. Tel: (617) 639-4037; Fax: (617) 638-5339; mgm1@bu.edu.

small GTPases, cytosolic GEFs can also activate trimeric G proteins (**Fig. 1A**). Among these non-receptor exchange factors, only those possessing a GBA motif have had their GEF activity ascribed to a well-defined structural element^{1,2}.

GBA motifs are short (~30 amino acids) segments that possess a core seven residue consensus sequence defined by Ψ -T- Ψ -x-[D/E]-F- Ψ (where Ψ denotes a hydrophobic residue and “x” is any residue)³. The modular motif is capable of activating G α i subunits and has thus far been found embedded within disordered regions of larger proteins such as GIV/Girdin, DAPLE, CALNUC and NUCB2³. These proteins have been linked to a variety of human pathologies, including cancer, liver fibrosis, congenital hydrocephalus, nephropathy and insulin resistance.

Though GPCRs and GBA proteins share a common function, the mechanism of activation varies amongst the different types of GEFs (**Fig. 1A**). Distinctions can involve the state of the G protein target or the route of activation (allostery). Regarding target state, for example, GPCRs are thought to exert their GEF activity most efficiently by acting directly on intact G $\alpha\beta\gamma$ heterotrimers, whereas GBA proteins act on G α monomers after displacement of G $\beta\gamma$. The mode of action of GBA proteins is reminiscent of the Ras superfamily of small G proteins, which also involves activation of monomeric proteins⁴. Similarity of the GBA proteins to the GEFs for small GTPases also extends to their binding site: a region in the Ras-domain of G α subunits also serving as the interface with effector proteins and other regulators (such as GAPs) – the GPCRs engage at a non-overlapping site⁴.

High-resolution structures of G proteins in complex with every major class of regulator (GPCRs, GAPs, etc.) have been used to study activation mechanisms with great success. Crystallographic studies of G α i1 bound to a synthetic GEF peptide similar to the GBA motif, however, showed minimal alteration of the nucleotide binding region compared to the inactive GDP-bound state. Conformational shifts that would ordinarily be induced by GBA binding may be lost during crystallization. The study of the allosteric details of GBA activation thus ultimately required approaches more suited to resolving the dynamic nature of protein states that are adopted during the process of nucleotide exchange⁵. A combination of biophysical (NMR), computational (modeling) and biochemical (mutagenesis) approaches helped to uncover fundamental differences in GPCR and GBA activation routes (**Fig. 1B**)⁵.

According to the predominant model of GPCR-based activation, GPCRs engage the C-terminal tail of G α subunits, displacing the α 5-helix and physically disrupting the pocket that binds the guanine moiety of the GDP nucleotide (the β 6- α 5 / β 5- α G loops) (**Fig. 1B**)^{6,7}. Disruption of the phosphate binding residues is subsequently thought to occur by secondary structural alterations following from a consequence of loosening guanine base binding. Loss of phosphate binding may also be aided by GPCR binding at the N-terminus, instituting a conformational change disturbing the proximal β 1 strand and the adjoining P-loop, which ordinarily secures the nucleotide phosphates.

In contrast, the allosteric details of GBA activation again parallel those observed for the small GTPases^{4,5}. Here, the GBA consensus sequence ostensibly adopts helical structure and

engages G α subunits near the Switch II region – one of the sites that undergoes a conformation change upon nucleotide exchange (**Fig. 1B**)⁵. Binding of GBA proteins at a cleft framed by the α 3-helix, Switch II, and the α 3- β 5 loop, triggers perturbations in the underlying β 1 strand, located posterior to the protein-protein interface. Similar to what occurs for GPCRs, disruption of β 1 may transmit changes to the contiguous P-loop, weakening the phosphate binding elements and facilitating nucleotide release⁵. Whereas GPCRs simultaneously perturb both the nucleotide base and phosphate binding elements, data surrounding GBA-based activation seems to champion the importance of the β 1-to-P-loop conduit and of abating phosphate binding as sufficient for GEF activity⁵.

The distinctions from GPCRs, both mechanistically and structurally, suggest GBA proteins evolved along different lines. Indeed, there is some evolutionary history to be gleaned from available data⁸. Though GPCRs and the G protein cycle components are present and conserved throughout all eukaryotic taxa, GBA proteins are a relatively new discovery, originally found to be present in proteins of the *ccdc88* family (e.g. GIV, DAPLE). Analysis of *ccdc88* orthologs across 87 metazoan species reveals a disjunction arising between vertebrates and invertebrates (**Fig. 2A**). The invertebrate to vertebrate transition not only marks the multiplication of *ccdc88* orthologs from a median of one to three, but also the widespread acquisition of the GBA motif⁹. Interestingly, GBAS-1, a *C. elegans* protein unrelated to the *ccdc88* family and with homologs only in closely related nematodes, was found to contain a putative GBA motif⁹, thereby suggesting its appearance by convergent evolution. Detailed biochemical and computational modeling confirmed that the GBA motif of GBAS-1 conserves both the GEF activity and binding site/mode of GBA motifs derived from *ccdc88* proteins for acting on its cognate nematode G α protein (i.e., GOA-1) (**Fig. 2B, C, D**)⁹. Preservation of these mechanics in an unrelated, invertebrate-exclusive protein demonstrates true modularity of the GBA motif and provides evidence that the GBA activation mechanism has been present for at least 300 million years.

With still much to be determined about the function of GBA proteins both physiologically and pathophysiologically, they present interesting objects for biological and therapeutic exploration.

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ABBREVIATIONS

GEF	guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
GBA	G α binding and activating
GIV	G α Interacting, Vesicle-associated protein

GAP	GTPase-activating protein
DAPLE	Dvl-associating protein with a high frequency of leucines
GBAS-1	Gα binding and activating and SPK domain containing protein 1

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Figure 1.

The activation mechanisms of GPCR and GBA GEFs. (A) GPCRs recognize GDP-bound Gαβγ trimers (1) and form a nucleotide-free GPCR-G protein intermediary complex (2) in which the Ras and Helical domains of Gα separate, favoring nucleotide exchange^{6,7}. Upon spontaneous loading of GTP, GPCRs and Gβγ dissociate from Gα (3). GBA proteins act on monomeric Gα subunits following Gβγ displacement (I), after which nucleotide exchange occurs via a nucleotide-free intermediary (II). Upon spontaneous loading of GTP, GBA proteins dissociate from Gα (III). (B) Comparison of the molecular mechanism of Gαi3 activation by GPCRs and GBA proteins. GBA binding (brown) at the Switch II/α3 cleft perturbs the β1 strand, instigating allosteric changes that favor GDP release by disrupting the phosphate binding P-loop without directly affecting the guanine base. In contrast, GPCRs act primarily by binding to the C-terminus and inducing perturbations in the α5 helix that are directly transmitted to the guanine base binding elements. GPCRs also perturb the phosphate binding P-loop, which is presumed to be transmitted by the β1 strand upon GPCR binding to the N-terminus region of Gα.

Figure 2.

Independent evolution of GBA motifs within ccdc88 proteins and GBAS-1. (A) GBA motif evolution timeline. Ccdc88 proteins arise in early metazoans, but GBA motif prevalence occurs along with ccdc88 gene duplication in vertebrates. A GBA motif within the Nematode protein GBAS-1 appears to have evolved convergently from a distinct lineage. (B) The GBA consensus sequence is present in multiple proteins. (C) Models show the GBA motifs of ccdc88 proteins (e.g. GIV, blue) and GBAS-1 (purple) engage their corresponding Gα subunits (Gαi3 and GαO-1, respectively) with highly similar binding poses, particularly at the anchoring phenylalanine residue (white arrow). (D) The major interface residues for both GBA motifs (blue, purple) and their target Gα subunits (black) are conserved between GIV/Gαi3 and GBAS-1/GαO-1 (GαO-1 residues in parentheses).