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Commentary

Gγ-like (GGL) domains: new frontiers in G-protein signaling and β-propeller scaffolding

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Abstract

The standard model of signal transduction from G-protein-coupled receptors (GPCRs) involves guanine nucleotide cycling by a heterotrimeric G-protein assembly composed of Gα, Gβ, and Gγ subunits. The WD-repeat β-propeller protein Gβ and the alpha-helical, isoprenylated polypeptide Gγ are considered obligate dimerization partners; moreover, conventional Gβγ heterodimers are considered essential to the functional coupling of Gα subunits to receptors. However, our recent discovery of a Gβ5 binding site (the Gγ-like or "GGL" domain) within several regulators of G-protein signaling (RGS) proteins revealed the potential for functional GPCR/Gα coupling in the absence of a conventional Gγ subunit. In addition, we posit that the interaction between Gβ5 isoforms and the GGL domains of RGS proteins represents a general mode of binding between β-propeller proteins and their partners, extending beyond the realm of G-protein-linked signal transduction.

Keywords: G-gamma-like (GGL) domain; Guanine nucleotide-binding protein (G protein); Kinesin; PDE4D5; RACK1; Regulators of G-protein signaling (RGS) proteins

1. Introduction

Signal transduction controls a wide variety of cellular activities, ranging from release of hormones and neurotransmitters, modulation of transmembrane ion flux, and activation or repression of gene transcription, to integrated responses of cellular survival, proliferation, and differentiation. One major class of signal transduction pathways is that controlled by heterotrimeric "G-proteins" [1–3]. Loss-of-function and gain-of-function mutations to GPCRs and downstream regulators cause a variety of human diseases, including vision pathologies such as retinitis pigmentosa [4,5] and endocrine disorders such as pseudohypoparathyroidism and McCune-Albright syndrome [6–9]. Whooping cough and fatal diarrhea, characteristic of infection by *Bordetella pertussis* and *Vibrio cholerae*, respectively, are caused by direct effects on G-protein activity catalyzed by pathogen-produced exotoxins [10,11]. Perturbation of G-protein signaling is also central to the actions of many drugs, from anti-asthmatics and anti-hypertensives to anti-depressants and anti-psychotics [12,13]. Thus, a better understanding of the molecular machinery underlying G-protein-coupled signal transduction is key to its continued exploitation for drug discovery and the amelioration of human disease.

In the "standard" model of heterotrimeric G-protein signal transduction, serpentine cell-surface GPCRs are coupled to a membrane-associated, heterotrimeric G protein composed of α, β, and γ subunits (Fig. 1A). Upon binding

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Abbreviations: DEP, dishevelled/EGL-10/pleckstrin-related domain; DH, dbl-homology domain; GAP, guanosine triphosphatase-activating protein; GEF, guanine nucleotide exchange factor; GGL, G-gamma-like; GIRK, G-protein-gated inwardly rectifying potassium channel; GPCR, G-protein-coupled receptor; G protein, guanine nucleotide binding protein; GTPase, guanosine triphosphatase; mAChR, muscarinic acetylcholine receptor; MAPK, mitogen-activated protein kinase; PDE, phosphodiesterase; PDZ, PSD-95/Discs-large/ZO-1 related domain; PH, pleckstrin-homology domain; PI3Kγ, gamma isoform of phosphoinositide 3-kinase; PLC-β, beta isoform of phospholipase C; PTB, phosphotyrosine-binding domain; RACK1, receptor for activated C kinase type-1; RBD, Ras-binding domain; RGS, regulators of G-protein signaling; and SAPK, stress-activated protein kinase.

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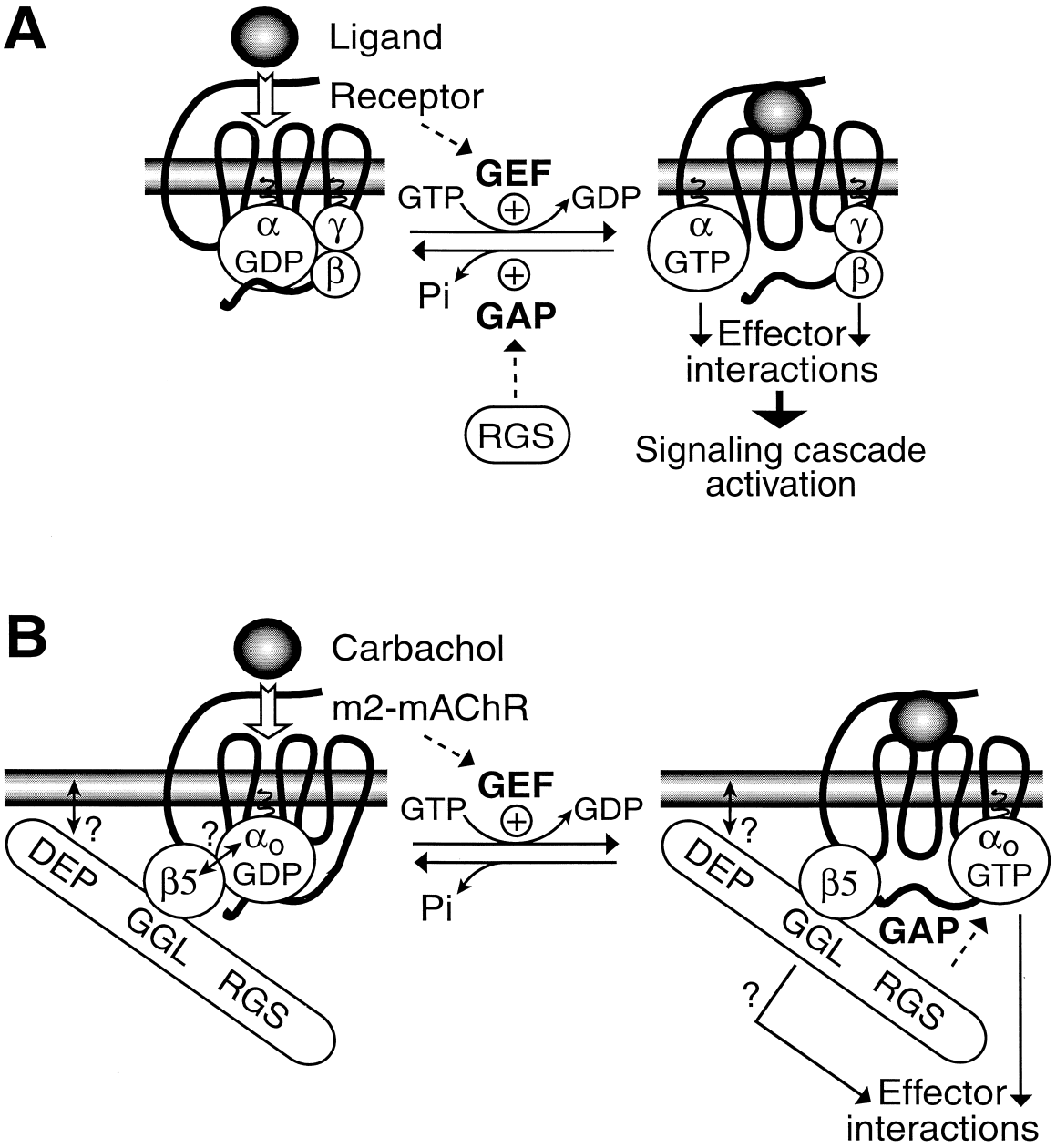


Fig. 1. (A) Standard model of GDP/GTP cycle governing activation of heterotrimeric G protein-coupled signaling pathways. Ligand-occupied cell-surface receptors stimulate signal onset by acting as GEFs for Gα subunits, facilitating GDP release, the subsequent binding of GTP, and release of the G$\beta\gamma$ dimer. RGS proteins stimulate signal termination by acting as GAPs for Gα subunits, thereby accelerating their intrinsic rate of GTP hydrolysis. (B) Possible modes of signaling and desensitization by a $G\alpha_o$/Gβ5/RGS9-coupled m2-mAChR. The DEP domain within the Gβ5/RGS9 heterodimer may serve to facilitate membrane recruitment in the absence of a conventional, lipid-modified Gγ subunit. Akin to conventional G$\beta\gamma$ subunits, the Gβ5/GGL moiety may associate with inactive, GDP-bound $G\alpha_o$; this association would presumably preclude any binding of $G\alpha_o$ with the RGS box as both binding events involve the same regions on the Gα subunit [14,15]. Upon carbachol binding, receptor-catalyzed guanine nucleotide exchange would switch $G\alpha_o$ into the active, GTP-bound state, releasing the Gβ5/GGL moiety to stimulate either conventional G$\beta\gamma$-effectors or novel effector proteins. GAP activity of the RGS-box within the Gβ5/RGS9 heterodimer would revert the system back to ground state (GDP-bound Gα), allowing rapid "re-interrogation" of receptor status and thus avoiding diffusional limitations to rapid signal kinetics [3,16].

extracellular ligand, the GPCR becomes a GEF through conformational changes in its intracellular loops, thus promoting replacement of bound GDP for GTP on the Gα subunit [17]. The binding of GTP changes the conformation of three "switch" regions within Gα [18,19], allowing its dissociation from G$\beta\gamma$. Both GTP-bound Gα and free G$\beta\gamma$ subunits initiate signals by interactions with downstream effector proteins, until the intrinsic GTPase activity of Gα returns the protein to the GDP-bound state (Fig. 1). Reassociation of G$\beta\gamma$ with GDP-bound Gα obscures critical

effector contact sites [20,21], thereby terminating all effector activations. In this manner, therefore, the duration of heterotrimeric G-protein-coupled signaling is controlled by the lifetime of the G-protein α subunit in the GTP-bound state.

The recent discovery of the "regulators of G-protein signaling" or RGS proteins [22–25] has added several new levels of complexity to this standard model of GPCR signaling [16]. At the simplest level, RGS proteins act via their hallmark, alpha-helical RGS-box [14,26,27] to accelerate the intrinsic GTPase activity of Gα subunits [28–30] and thus attenuate signals derived from GTP-bound Gα and free G$\beta\gamma$ subunits (Fig. 1A). While small RGS proteins such as GAIP, RGS1, GOS8/RGS2, and RGS4 encompass little more than an RGS-box [31–33], other RGS proteins are composed of multiple domains which bestow additional functionality. As one example, F-subfamily RGS proteins [34], typified by p115-RhoGEF [35] and PDZ-RhoGEF [36], bear DH and PH domains C-terminal to a G$\alpha_{12/13}$-specific RGS-box; these proteins not only accelerate G$\alpha_{12/13}$ GTPase activity, but also act concomitantly as Gα-effectors, since RGS-box occupancy by Gα-GTP stimulates the guanine-nucleotide exchange activity of the DH/PH tandem directed toward the monomeric G-protein Rho [36, 37]. The D-subfamily members, RGS12 and RGS14, are also presumed to play a role in coordinating cross-talk between heterotrimeric and Ras-superfamily G-proteins [38], given the recent identification of putative Ras-binding (RBD) and novel Gα-binding (GoLoco) domains within both RGS proteins [39–41], as well as PDZ [30] and PTB domains [16,42] unique to RGS12. However, the most radical affront to the standard model of GPCR signaling has come from the identification of the Gγ-like or "GGL" domain within the C-subfamily RGS proteins [43]—a discovery that has presaged not only the existence of novel G-protein subunit assemblies but also a potentially universal mode of interaction with β-propeller proteins.

2. Discovery of the GGL domain and its binding partner, Gβ5

In a continuing effort to identify and characterize novel RGS family members, we cloned the human *RGS11* cDNA and performed a detailed bioinformatic analysis of its encoded polypeptide sequence. Between N-terminal DEP [44] and C-terminal RGS-box domains, we observed a 64 amino-acid region with striking similarity to G-protein γ subunits [43]. This GGL domain was also noted to be present in the related RGS proteins RGS6, RGS7, RGS9, and EGL-10 [43] (Fig. 2), denoted the "C-subfamily" by Farquhar and colleagues [34]. In the standard model of heterotrimeric G-protein assembly, conventional Gγ subunits exist as extended alpha-helical polypeptides that form tightly-held heterodimers with Gβ subunits [51], both by interacting with the bottom of the WD-repeat β-propeller structure of Gβ and by forming a parallel coiled-coil with the Gβ N-terminus (Fig. 3B). We hypothesized a similar role for the GGL domain in binding Gβ subunits. Indeed, using *in vitro* co-translation/immunoprecipitation assays, we demonstrated robust and selective binding of RGS6, RGS7, and RGS11 to the neurospecific Gβ5 subunit (and its retinal-specific isoform Gβ5L); deletion, point mutation, and domain-swapping experiments have since confirmed the essential role of the GGL domain to Gβ5 binding [43, 54,55].

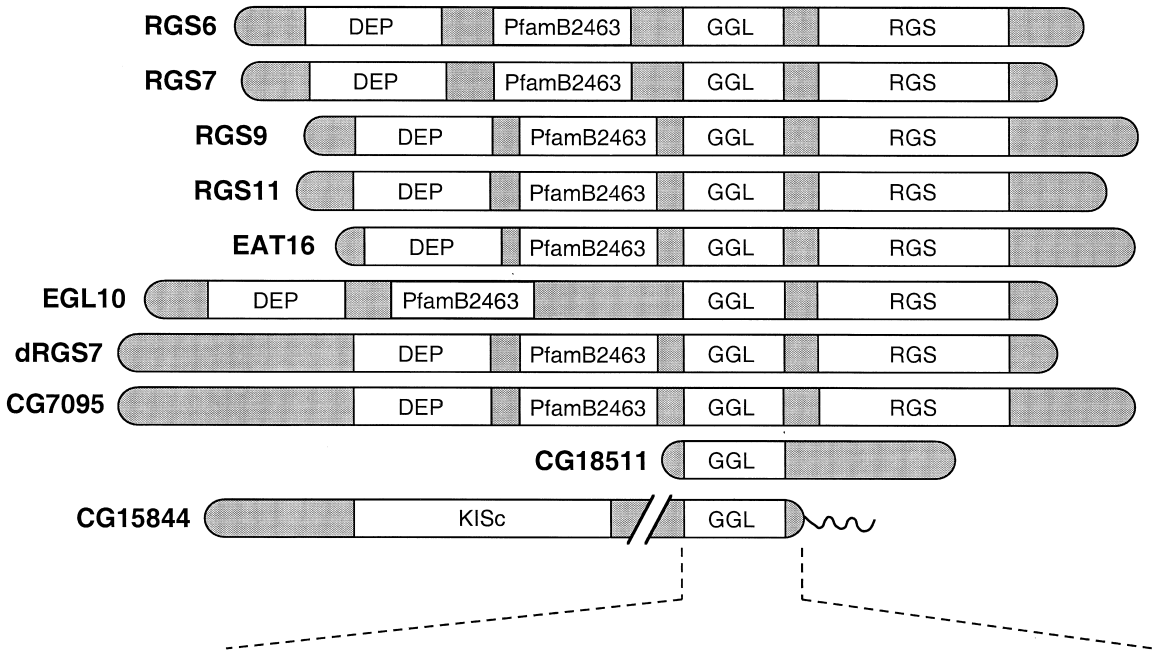
Specific formation of Gβ5/RGS heterodimers is also readily detectable after subunit co-expression in COS-7 and Sf9 cells, with Gβ5/RGS6, Gβ5/RGS7, and Gβ5/RGS11 heterodimers purified from the latter expression system exhibiting selective GAP activity toward Gα_o *in vitro* [43,54, 56]. Subsequent reports from several groups [55,57–59] describing the existence of native Gβ5/RGS heterodimers in brain and retinal tissue, as well as our own mutagenesis and molecular modeling studies [54], support the notion that the Gβ5/GGL complex is a structural analogue of conventional G$\beta\gamma$ dimers and, as such, its formation excludes concomitant binding of a Gγ subunit to Gβ5.

3. The true partner for Gβ5?

The discovery of C-subfamily RGS proteins as avid binding partners for Gβ5 brings into question the relevance of recent research exploring the signaling capacity of Gβ5 in complex with conventional Gγ subunits. In their papers describing the original identification of Gβ5 and Gβ5L, Simon and colleagues [60,61] suggested that Gγ2 is the most likely dimerization partner for both Gβ5 isoforms. However, this suggestion was based not on the frank isolation of Gβ5/Gγ2 dimers, but solely on an indirect measurement of conventional G$\beta\gamma$ dimer activity: the stimulation of PLC-β2 phospholipase activity [62] upon cellular co-transfection of Gγ2 and Gβ5 cDNAs. Gβ5/Gγ2 co-transfection can also cause other G$\beta\gamma$-like effects, such as the modulation of adenylyl cyclase activity [63,64] and the inhibition of N-type calcium channels [65,66], but cannot activate MAPK/ERK or JNK/SAPK signaling pathways [67] presumably because of an inability to activate PI3Kγ [68].

The observation that Gβ5/Gγ2 co-expression elicits only a subset of conventional G$\beta\gamma$ activities has been interpreted as reflecting the uniqueness of Gβ5, which shares only ~50% sequence identity with the other four Gβ proteins [60]. We suggest an equally plausible explanation: the Gβ5/Gγ2 heterodimer formed upon over-expression of both subunits is an unnatural and weakly-associated heterodimer that only inadvertently affects some conventional G$\beta\gamma$ effector systems.

While Simonds and colleagues have shown that Gγ2/Gβ5 co-transfection increases Gβ5 protein levels in COS-7

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RGS6      254  RKTTKEDIRKQITFLNAQID.RHCLKMSKVAESLIAYTEQYVEYDPLITPA........EPSNPWISDDVALW      317
RGS7      251  KPPTEDELQQQIKYWQIQLD.RHRLKMSKVADSLLSYTEQYLEYDPFLLPP........DPSNPWLSDDTTFW      314
RGS9      215  QKQTVVAVKKEIMYYQQALM.RRSTVKSSVSLGGIVKYSEQFSSNDALMSGC........LPSNPWITDDTQFW      278
RGS11     219  MTKSADFHKREIEYFRKALG.RRTRVKSSVCLEAYLSFCGQRGPHDPLVSGC........LPSNPWISDNDAYW      282
EAT16     200  RRQNAQGYRCLMDRLRFAIKTKPWLKALKASDTMVTWVDQRAEFDPFLHPP........QPSNPWISDEASFW      264
EGL10     330  CTQVQDTLKLEIVQLNSRLSKNVLRTSKVVENYLAYYEQRRVFDPLLTPPGSQADPFQSQPNPWINDTVDFW      401
dRGS7     425  AKNPVEQLTRIIALRKQKLERRTIKVSKAAEALVAYYDQYNEFDYFITSP........ELPNPWQTDSTEMW      488
CG7095    417  KKKTVDDMQRDVDYLGKSLNRTRMKMSQACESLVCYSETFSEYDFFLQPA........LPSNPWVTEDIAFW      480
CG18511     7  QNMDRDALKKQIENMKYQASMERWPLSKSIAEMRSFIEENEKNDPLINAP.......DKKNNPWAEKG.KCPQH       72
CG15844   779  MAANLQQQRSINLQLRREVEMERMPVSVACSLMMKYMLEHEEEDCLLSGF.......TQKVNPFREKS.SCSIL*     844
  Gγ2       5  NTASIAQARKLVEQLKMEANIDRIKVSKAAADLMAYCEAHAKEDPLLTPV.......PASENPFREKKFFCAIL*      71
               αααααααααααααα       αααααααααααααα      αααα
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Fig. 2. Multi-domain structures of C-subfamily RGS proteins and *D. melanogaster* open-reading frames containing the GGL domain, and comparison of GGL domain polypeptide sequences to the conventional Gγ subunit, Gγ2. RGS6, RGS7, RGS9, and RGS11 are mammalian proteins [43], EAT16 and EGL10 are *C. elegans* proteins [25,45], dRGS7 is derived from *D. melanogaster* [46], and CG7095, CG18511, and CG15844 are predicted open-reading frames from the *D. melanogaster* Genome Project [47]. DEP, Dishevelled/EGL-10/Pleckstrin domain [44]; Pfam-B2463, block of conserved polypeptide sequence defined in the Pfam database [48]; GGL, Gγ-like domain [43]; RGS, regulator of G-protein signaling alpha-helical bundle [14,23]; KISc, kinesin motor catalytic domain [49]; wavy line, site of potential isoprenylation on the C-terminus of CG15844. Regions within Gγ2 of alpha-helical secondary structure, as assigned by crystallographic structure determination [50], are denoted by the α symbol below the primary sequence. The position of isoprenylated cysteine residue within Gγ2 (and predicted for CG15844) is indicated by a black arrowhead. Asterisk (*), C-terminal end of polypeptide chain.

cells, compared to transfection with Gβ5 alone, no co-immunoprecipitation data were presented [67]. We have chronicled our inability to isolate Gβ5/Gγ2 heterodimers in co-transfection/co-immunoprecipitation experiments [43,54], presumably due to the profound detergent sensitivity of the Gβ5/Gγ2 complex that is not apparent for either conventional Gβγ dimers or Gβ5/GGL complexes [54,69]. By accounting for such detergent sensitivity, the laboratories of Garrison and Nürnberg have described the purification of Gβ5/Gγ2 heterodimers from baculovirus-mediated Sf9 cell expression [68, 70,71]; this recombinant Gβ5/Gγ2 protein activates PLC-β2 *in vitro*, consistent with previous cell-based co-expression experiments [60,61,67]. However, several lines of evidence indicate that the Gβ5/GGL complex forms much more readily than the Gβ5/Gγ2 complex. First, co-translation studies have demonstrated that the formation of a Gβ5/GGL complex occurs *in vitro* even if the RGS protein is added to the reticulocyte lysate after Gβ5/Gγ2 dimer formation [55], presumably reflecting a higher affinity of the RGS protein for Gβ5. (Competition between Gγ2 and GGL domains for Gβ5 association could explain the ability of RGS6 and RGS11 to antagonize Gβ5/Gγ2-mediated inhibition of N-type calcium channels [66]; by use of RGS6 and RGS11 missense and deletion mutants, we have shown that this antagonism is dependent upon the GGL domain [66].) Second, immunoprecipitation and mass spectrometry experiments have failed to reveal the existence of native Gβ5/Gγ2 heterodimers within brain and retinal tissues, but have detected native Gβ5/RGS dimers [57, 59,69,72]. Finally, genetic ablation of the *RGS9* locus results in the loss of detectable Gβ5L protein in the mouse retina [73]. This is consistent with observations from our group [54] and others [72] that expression of Gβ5 in COS-7 cells dramatically increases the protein levels of co-transfected RGS6 or RGS7 and vice versa; collectively, these results suggest that stable *in vivo* expression of Gβ5 isoforms requires complex formation with C-subfamily RGS proteins.

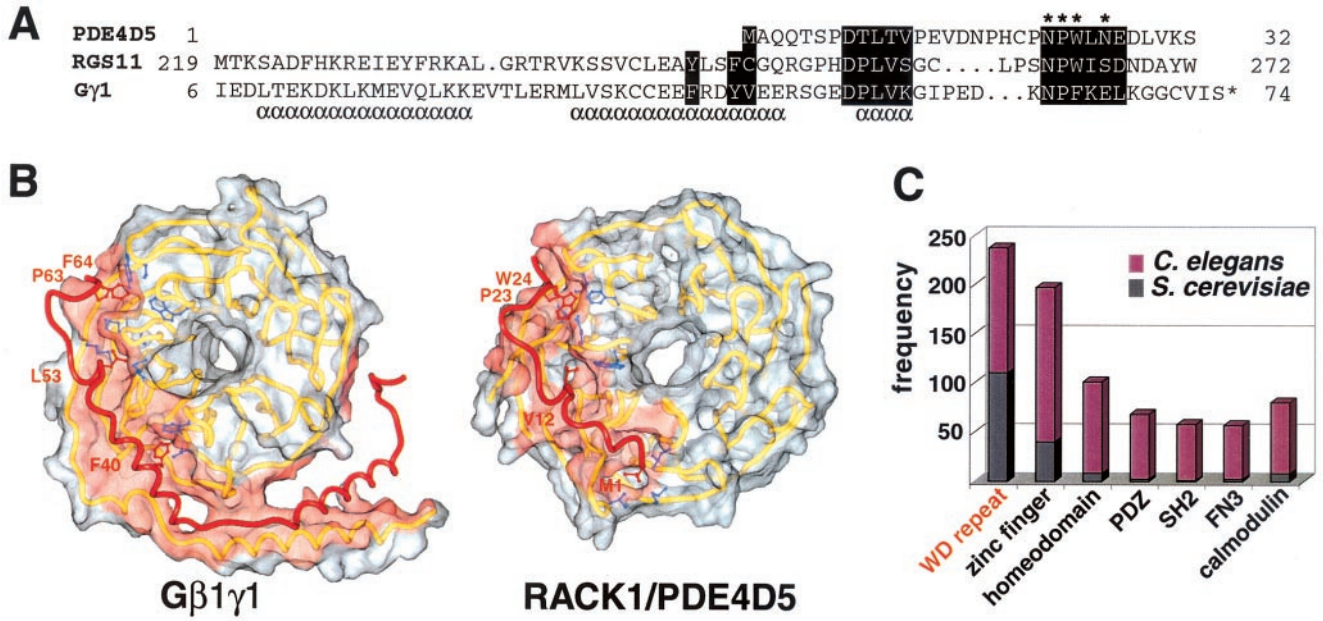

Fig. 3. Sequence and structural similarity between Gγ subunits, GGL domains, and the N-terminus of PDE4D5. (A) Representative GGL and Gγ sequences (from RGS11 and Gγ1, respectively) are aligned with the N-terminus of PDE4D5 shown to be necessary for interaction with RACK1. Conserved regions are highlighted with black boxes, and residues within PDE4D5 that, when substituted by alanine, abrogate binding to RACK1 [52] are denoted by asterisks. (B) Structural model of the interaction between RACK1 and PDE4D5 based upon the Gβ1γ1 dimer (PDB Accession Code 1TGB). A reasonable three-dimensional profile of the model was confirmed using the VERIFY function within INSIGHT (Molecular Simulations). Gγ1 and PDE4D5 N-terminus are colored red, and Gβ and RACK1 β-propellers are colored yellow; the surfaces residues within the β-propeller structures found within 4 Å of the Gγ or PDE4D5 polypeptide are shaded pink. (C) Proteins containing WD repeats are highly abundant in eukaryotic genomes (e.g., *Caenorhabditis elegans* and *Saccharomyces cerevisiae*) relative to more-thoroughly studied structural domains [53].

4. The true function of Gβ5/RGS complexes?

If the true *in vivo* partners for Gβ5 isoforms are GGL domain-containing RGS proteins, what function(s) in GPCR signaling is performed by these novel heterodimers? The RGS-box contained within such heterodimers has demonstrable GAP activity *in vitro* toward $G\alpha_t$ (in the case of RGS9 [57]) and $G\alpha_o$ (in the case of RGS6, -7, and -11 [43,56]); thus, a potential role for Gβ5/RGS complexes in accelerating GPCR signaling deactivation by enhancing GTP hydrolysis can be predicted and, indeed, has been observed recently in reconstituted mAChR systems [72,74].

Should we necessarily assume, however, that the Gβ5/GGL portion of such heterodimers exhibits any conventional Gβγ-like activities? To this point, the predictive value of the "standard" model of heterotrimeric G-protein signaling has been poor but not entirely useless. In the context of testing whether Gβ5/GGL is an effector activator, Posner *et al*. [56] have shown that Gβ5/RGS6 and Gβ5/RGS7 complexes do not share with conventional Gβγ dimers the ability to either modulate adenylyl cyclase activity or activate PLC-β isoforms. (The possibility still exists, however, that novel, as-yet-unidentified effectors are specifically targetted by Gβ5/RGS complexes). While Gβγ subunits bind avidly to GDP-bound Gα subunits and thus facilitate functional coupling of Gα to GPCRs, we and others have reported the inability to form heterotrimeric complexes *in vitro* between Gβ5/RGS dimers and GDP-bound Gα [43,56]. However, a role for Gβ5/RGS dimers in facilitating receptor/Gα coupling is suggested by the recent findings of Harden and colleagues[1] that the Gβ5/RGS9 heterodimer can support association of $G\alpha_o$ with phospholipid vesicles and agonist-stimulated nucleotide exchange on $G\alpha_o$ by m2-mAChRs (Fig. 1B). Such facilitation of receptor GEF activity could explain the accelerated kinetics of coupling of the m2-mAChR to GIRK channels that Lester and colleagues have observed upon co-expression of Gβ5 with RGS7 or RGS9 in *Xenopus* oocytes [74].

It is currently unclear what molecular mechanisms underlie the apparent discordance between the lack of Gα-GDP association with Gβ5/RGS dimers in solution and enhanced Gα/receptor coupling mediated by Gβ5/RGS proteins in phospholipid vesicles and transfected cells. One possibility is a role for the associated, N-terminal DEP domain [44] in enhancing recruitment of the Gβ5/RGS dimer to the membrane (Fig. 1B) and thus overcoming a weak affinity for Gα, which prevents detection of Gα/Gβ5/RGS trimer assembly in solution. There is currently no evidence that Gβ5 or C-subfamily RGS proteins are myristolylated at the N-terminus or lipid-modified at the C-terminus in a fashion similar to Gγ subunit isoprenylation [75], and so the DEP domain represents a likely membrane-

[1] Gilman AG and Harden TK, personal communication. Cited with permission.

anchoring module for the Gβ5/RGS complex, especially given recent reports of a membrane-localizing function for the DEP domains of Dishevelled and Epac1 [76,77]. Another possible means of membrane recruitment is palmitoylation of the RGS partner of Gβ5/RGS complexes, similar to that seen for other RGS family members [32,78]; while several candidate cysteine residues are present within C-subfamily RGS proteins, there is only one published report to-date regarding palmitoylation at these sites [79].

An additional possible function for Gβ5/RGS dimers is that of Gα effector. Such a function could be transacted by modulation, upon Gα association with the RGS-box, of some hitherto-uncharacterized enzymatic activity possessed by C-subfamily RGS proteins, in a manner similar to the activation of p115-RhoGEF and PDZ-RhoGEF by $G\alpha_{12/13}$ subunits [36,37]. Gα-dependent enzymatic activity might very well be encoded by the polypeptide sequence found between the DEP and GGL domains (Fig. 2), an uncharacterized region which is well-conserved among C-subfamily RGS proteins (Pfam-B-2463; Ref. [48]). Two additional models of effector function have been proposed by Guan and Han [80] to explain the function in *Caenorhabditis elegans* behavioral circuitry of the C-subfamily RGS protein EAT-16. Egg-laying by the nematode *C. elegans* is accelerated by signaling via $G\alpha_q$ and inhibited by signaling via $G\alpha_o$; in genetic studies, Koelle, Sternberg, and colleagues [45] found that loss of the $G\alpha_q$-specific RGS EAT-16 can suppress behavioral phenotypes caused by transgenic expression of activated $G\alpha_o$. This finding presents the possibility that EAT-16 might be a direct effector for $G\alpha_0$-mediated inhibition of $G\alpha_q$, such that $G\alpha_o$ activation somehow increases $G\alpha_q$-specific GAP activity of the EAT-16 RGS-box. Guan and Han envision two possible scenarios: (i) the binding of GTP-$G\alpha_o$ to EAT-16 activates the $G\alpha_q$-specific RGS-box, or (ii) the release of EAT-16 from a GPCR/GDP-$G\alpha_o$/Gβ5/EAT-16 complex upon receptor activation allows translocation of the RGS-box to $G\alpha_q$ [80]. However, formal biochemical evidence for either of these two scenarios has yet to be demonstrated. Moreover, as proposed by Koelle and colleagues [81], the antagonism between $G\alpha_o$ and $G\alpha_q$ signaling pathways may arise from their convergence at a point further downstream (e.g., at the level of second-messenger generation/destruction), and thus the only role for EAT-16 in this pathway may simply be to establish the baseline level of signaling from the $G\alpha_q$-coupled receptor(s).

5. The GGL domain as a modular β-propeller binding unit

We believe that the GGL domain represents a modular interaction site found within many different proteins that bind β-propeller partners, and not just a Gβ5 binding site restricted to certain RGS proteins. For example, we have recently identified two novel open-reading frames, CG15844 and CG18511, within the *Drosophila melanogaster* genome [47] that each possess a Gγ-like polypeptide sequence, yet lack an identifiable RGS-box (Fig. 2). While we have been unable to detect any additional functional domains within CG18511, the larger CG15844 open-reading frame appears to encode a member of the kinesin protein superfamily, a large collection of ATP-hydrolyzing, microtubule-dependent molecular motors involved in both the intracellular transport of vesicles and organelles and the assembly and movement in meiotic and mitotic spindles [49]. The GGL domain of CG15844 appears to be much more closely related to conventional Gγ subunits, given the presence of both an Asn-Pro-Phe (NPF) tripeptide sequence (rather than the Asn-Pro-Trp (NPW) tripeptide within all RGS-associated GGL domains; [54]) and an apparent C-terminal isoprenylation signal sequence (Cys-Ser-Ile-Leu; Fig. 2). In contrast, the N-terminal location of the GGL domain within the CG18511 open-reading frame presumably precludes isoprenylation of the Cys-69 residue even though it is conserved in position relative to the NPW motif (Fig. 2). We are currently testing whether either GGL domain binds the *Drosophila* homolog of mammalian Gβ5 (i.e., CG10763; GenBank Accession No. #AAF46336), but other Gβ subunits or β-propeller proteins are equally likely binding partners.

The strongest evidence supporting our contention that the GGL domain is a modular β-propeller binding unit comes from the recent report by Bolger, Houslay, and colleagues describing the interaction between RACK1 and PDE4D5 [52]. RACK1 like Gβ subunits, contains seven WD-repeats and, when expressed *in vitro*, exhibits hydrodynamic properties and trypsin resistance highly suggestive of a Gβ-like β-propeller structure [82]. PDE4D5 represents an isoform of cyclic AMP-specific phosphodiesterase derived from the human *PDE4D* gene; this isoform encodes a unique 88 amino-acid N-terminus that binds specifically to RACK1 in a yeast two-hybrid screen and in cellular co-immunoprecipitation and *in vitro* binding assays [52]. Through the use of deletions and point mutations to the PDE4D5 N-terminus, Bolger, Houslay, and colleagues identified a short polypeptide sequence, containing an Asn-Pro-Trp (NPW) motif (Fig. 3A), that is critical for RACK1 association. Given this evidence, we have created a model of the RACK1/PDE4D5 N-terminus interaction, starting from the atomic-resolution structure of the Gβ1/Gγ1 heterodimer [51] and using side-chain replacements as previously described [54].

Three distinct regions characterize the interface between Gβ and Gγ subunits, primarily involving the insertion of hydrophobic residues from Gγ between β-sheets of the β-propeller structure [51]. These structural characteristics are mimicked within the modeled interface between RACK1 and PDE4D5 (Fig. 3B). We have previously posited [54] that the structural constraints imposed by high-affinity interaction with Gβ subunits presumably give rise to the observed sequence conservation between Gγ subunits

and the GGL domains of RGS proteins. Given the presence of similar sequence motifs (Fig. 3A) within the N-terminus of PDE4D5 required for high-affinity interaction with RACK1, it is tempting to speculate that the subunit interface first characterized within G$\beta\gamma$ dimers, and most likely maintained within Gβ5/GGL and RACK1/PDE4D5 dimers, is a ubiquitous mode of interaction utilized by many of the numerous WD repeat-containing proteins (Fig. 3C) and their binding partners. Moreover, many proteins form β-propeller structures without the presence of WD-repeats (e.g., neuraminidases [83], YWTD-repeat proteins [84], and the N-terminal domain of clathrin heavy chain [85]) and so it is possible that some of these proteins and their binding partners also recapitulate the functional interface first described for G-protein β and γ subunits.

6. Conclusion

The discovery of the GGL domain as a novel Gβ binding partner is leading to a bifurcated view of G-protein-coupled signal transduction: a Gβ5/RGS heterodimer must now be placed into the context of GPCR/Gα/effector signaling alongside the conventional G$\beta\gamma$ subunit paradigm. Our recent identification of GGL domains in proteins outside the RGS C-subfamily suggests that it may be necessary to extend the concept of Gγ-like domains well past the current, narrow realm of heterotrimeric G-protein signaling.

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