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The Expanding Roles of Gβγ Subunits in G Protein–Coupled Receptor Signaling and Drug Action

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Abstract——**G$\beta\gamma$ subunits from heterotrimeric G proteins perform a vast array of functions in cells with respect to signaling, often independently as well as in concert with Gα subunits. However, the eponymous term "G$\beta\gamma$" does not do justice to the fact that 5 Gβ and 12 Gγ isoforms have evolved in mammals to serve much broader roles beyond their canonical roles in cellular signaling. We explore the phylogenetic diversity of G$\beta\gamma$ subunits with a view toward understanding these expanded roles in different cellular organelles. We suggest that the particular content of distinct G$\beta\gamma$ subunits regulates cellular activity, and that the granularity of individual Gβ and Gγ action is only beginning to be understood. Given the therapeutic potential of targeting G$\beta\gamma$ action, this larger view serves as a prelude to more specific development of drugs aimed at individual isoforms.**

I. Introduction

Heterotrimeric G proteins composed of Gα and G$\beta\gamma$ subunits relay signals from G protein–coupled receptors (GPCRs) to a wide range of downstream effectors, including adenylyl cyclase isoforms, phospholipase isoforms, ion channels, protein tyrosine kinases, and mitogen-activated protein kinases (MAPKs), among others. Originally, the G$\beta\gamma$ dimer was thought to be necessary primarily for inactivation of Gα subunits, allowing them to reassociate with the receptor for subsequent rounds of signaling. In this sense, G$\beta\gamma$ was viewed as a negative regulator of Gα signaling, and was thought to decrease the signal-to-noise ratio by preventing spontaneous Gα activation in the absence of receptor stimulation [reviewed in Neer (1995)]. The first evidence for a direct role of G$\beta\gamma$ dimers in cellular signaling came in 1987, when it was shown that purified G$\beta\gamma$ subunits from bovine brain were able to activate a cardiac potassium channel normally activated by muscarinic receptors following acetylcholine release (Logothetis et al., 1987). A large body of work subsequently revealed that G$\beta\gamma$ subunits can also modulate many other effectors, via direct interaction, that are also regulated by Gα subunits, including phospholipase Cβ (Camps et al., 1992), adenylyl cyclase isoforms (Tang and Gilman, 1991), and voltage-gated calcium channels (Ikeda, 1996; Zamponi et al., 1997). In this review, we focus on the diversity of Gβ and Gγ subunits, their unique roles in the regulation of both canonical and novel effectors, their implications in disease, and their potential as therapeutic targets. In addition to work described here, a number of other recent reviews focus on different aspects of G$\beta\gamma$ function in greater detail, especially in the context of the G protein heterotrimer (Smrcka, 2008; Lin and Smrcka, 2011) and GPCR signaling and ontogeny (Dupré et al., 2006, 2009; Robitaille et al., 2009a).

II. Diversity and Phylogeny of G$\beta\gamma$ Subunits

The presence of diverse, yet sequence-similar Gβ and Gγ subunits may be the result of an evolutionary process reflecting the emergence of distinct functions.

ABBREVIATIONS: AC, adenylyl cyclase; AEBP1, adipocyte enhancer-binding protein; AP-1, activating protein-1; β_2AR, β_2-adrenergic receptor; BRET, bioluminescence resonance energy transfer; CCT, cytosolic chaperonin complex; CGS-21680, 3-[4-[2-[[6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl]amino]ethyl]phenyl]propanoic acid; CK2, casein kinase 2; DPDPE, [D-Pen2,D-Pen5]-Enkephalin; DRiP78, dopamine receptor interacting protein 78; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2; GFP, green fluorescent protein; GGL, Gγ−like; GPCR, G protein–coupled receptor; GR, glucocorticoid receptor; GRK, G protein–coupled receptor kinase; GRK2-ct, C terminus of GRK2; HA, hemagglutinin; HDAC5, histone deacetylase 5; HEK293, human embryonic kidney 293; IP$_3$, inositol 1,4,5-triphosphate; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEF2, myocyte enhancer factor 2; OPL, outer plexiform layer; PH, pleckstrin homology; PhLP1, phosducin-like protein 1; PI3K, phosphoinositide 3 kinase; PIP$_2$, phosphatidylinositol (4,5)-bisphosphate; PKC, protein kinase C; PKD, protein kinase D; PLC, phospholipase C; R7BP, RGS7-binding protein; RGS, regulator of G protein signaling; RTK, receptor tyrosine kinase; shRNA, short hairpin RNA; siRNA, small interfering RNA; SKF-81297, 6-chloro-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol; TGN, *trans*-Golgi network; WD, tryptophan-aspartic acid; WIN 55212-2, (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone.

Assuming a broader role for G$\beta\gamma$ beyond their roles in cellular signaling per se, different receptor complexes may also have used the diversification of Gβ and Gγ subunits, or vice versa, resulting in Gβ and Gγ sequence diversity and varying cellular function. GPCR complexes are organized sets of signaling-specific proteins [reviewed in Bockaert et al. (2003, 2004) and Rebois and Hébert (2003)]. Unique G$\beta\gamma$ pairs, involved in the specificity of cellular signaling, may also be involved in the assembly of particular GPCR complexes. Given the potential involvement of G$\beta\gamma$ subunits in GPCR signaling complex formation [discussed later, and reviewed in Dupré et al. (2009)], it is possible that the pool of G$\beta\gamma$ dimers in a particular cell may drive and/or dictate which receptor complexes can form in that cell. In this section, we discuss the evolutionary expansion of G$\beta\gamma$ function and the implications of G$\beta\gamma$ subunit diversity.

With the exception of Gβ_5, Gβ subunits share high amino acid sequence conservation (Table 1). G$\beta_{1\text{-}4}$ subtypes share between 79 and 90% sequence similarity, whereas Gβ_5 is approximately 52% similar to the other Gβ subunits. Compared with Gβ subunits, Gγ subunits are more diverse, and their protein sequences are between 26 and 76% similar (Table 2). Given their evolutionary divergence, the question of the different roles these homologous Gβ subunits play in signal transduction becomes important. It is now generally recognized that we cannot consider an eponymous G$\beta\gamma$ subunit. Thus, evolution has played a large and largely unappreciated role in a plethora of G$\beta\gamma$ functions. This may reflect a limited understanding of what G$\beta\gamma$ subunits do in cells beyond their roles in GPCR signaling.

We performed a phylogenetic analysis of Gβ and Gγ subunit protein sequences from various species, including invertebrates in which G$\beta\gamma$ function has been characterized, plant species, and a wide variety of mammalian species. Upon analysis of the Gβ phylogenetic tree in Fig. 1, it can be seen that Gβ subunits from various species cluster into five groups—that is, five clusters around each mammalian Gβ subunit (nodes A-D in Fig. 1). It is evident that Gβ diverged from a common ancestor into two superfamilies very early on in their evolution, giving rise to one superfamily consisting of the G$\beta_{1–4}$ subtypes and another consisting of Gβ_5 subtypes. This observation is not surprising, given that, as mentioned earlier, mammalian Gβ_5 is least similar to the other Gβ subtypes.

The phylogenetic tree for Gγ subunits in Fig. 2 paints a more complex picture. Gγ subunits diverged from each other into five classes, which can be grouped as follows: class I: Gγ_7 and Gγ_{12}; class II: Gγ_2, Gγ_3, Gγ_4, and Gγ_8; class III: Gγ_5 and Gγ_{10}; class IV: Gγ_1, Gγ_9, and Gγ_{11}; and class V: Gγ_{13}. Interestingly, Gγ_7 and Gγ_{12} diverged from the rest of the group early in the evolutionary process, with Gγ_{12} representing a more ancestral Gγ subunit than Gγ_7. It is also interesting to note that these two subunits are most similar to each other, as they exhibit 76% sequence similarity (Table 2).

TABLE 1
Sequence similarities of human Gβ subunits

Amino acid sequences for human Gβ subunits were obtained from the National Center for Biotechnology Information protein database. Sequence alignments of the different Gβ subunits were performed using EMBL-EBI's ClustalW2 via a slow pairwise alignment. All known subtypes of human Gβ subunits were included in the alignment, including the long- and short-length forms of Gβ_5. Values indicated in each cell represent the percentage sequence similarity between Gβ subtypes.

	Gβ_1	Gβ_2	Gβ_3	Gβ_4	Gβ_5-5	Gβ-L
Gβ_1	100	—	—	—	—	—
Gβ_2	90	100	—	—	—	—
Gβ_3	83	80	100	—	—	—
Gβ_4	90	90	79	100	—	—
Gβ_5-S	52	51	52	52	100	—
Gβ_5-L	52	51	52	52	100	100

*A. G*β *and G*γ *Subunits in Lower Eukaryotes*

We did not include many fungal or *Dictyostelium* G$\beta\gamma$ subunits in our comparative analysis of protein sequences, with the exception of the budding yeast, *Saccharomyces cerevisiae*. In *S. cerevisiae,* G$\beta\gamma$ has been shown to play a role in the pheromone response pathway (Whiteway et al., 1989; Grishin et al., 1994; Hirschman et al., 1997). Gβ subunits in *S. cerevisiae* reflect a different evolutionary pattern with respect to invertebrates: both Gpb1 (guanine nucleotide-binding protein, beta subunit 1) and Gpb2 (guanine nucleotide-binding, beta subunit 2) are homologous with vertebrate G$\beta_{1–4}$ counterparts, with Gpb1 being most similar to vertebrate Gβ_1 and Gpb2 being most similar to Gβ_3 (Fig. 1). It is interesting to note that the Gγ subunit from *S. cerevisiae* displays a tight codivergence pattern with eukaryotic Gγ_5/Gγ_{10} class counterparts, suggesting a basic, yet absolute functional requirement for this class of Gγ subunits in cellular processes. Interestingly, a noncanonical Gβ subunit, a large, tryptophan-aspartic acid (WD) repeat containing protein distinct even from Gβ_5, Vps15, has also been identified in *S. cerevisiae*, and is coupled to a phosphoinositide 3-kinase (PI3K) pathway that does not seem to involve a Gγ subunit (Slessareva and Dohlman, 2006). The fission yeast, *Schizosaccharomyces pombe*, expresses a single G$\beta\gamma$ pair [git5 (Kim et al., 1996) and git11 (Landry and Hoffman, 2001), respectively] that is also involved in pheromone signaling. Another Gγ-independent Gβ subunit, Gnr1, which in this case is actually homologous to mammalian Gβ subunits, as G$\beta_{1–4}$ subunits can complement its loss, likely negatively regulates pheromone signaling in *S. pombe* (Goddard et al., 2006).

Most filamentous fungi and *Dictyostelium* also express single Gβ and Gγ subunits [reviewed in Shpakov and Pertseva (2008)]. Within the filamentous fungi, the sequence of Gβ subunits is fairly similar

TABLE 2

Sequence similarities of human Gγ subunits

Amino acid sequences for human Gγ subunits were obtained from the National Center for Biotechnology Information protein database. Sequence alignments of the different Gγ subunits were performed using EMBL-EBI's ClustalW2 via a slow pairwise alignment. All known subtypes of human Gγ subunits were included in the alignment. Values indicated in each cell represent the percentage sequence similarity between Gγ subtypes.

	Gγ_1	Gγ_2	Gγ_3	Gγ_4	Gγ_5	Gγ_7	Gγ_8	Gγ_9	Gγ_{10}	Gγ_{11}	Gγ_{12}	Gγ_{13}
Gγ_1	100	—	—	—	—	—	—	—	—	—	—	—
Gγ_2	32	100	—	—	—	—	—	—	—	—	—	—
Gγ_3	29	76	100	—	—	—	—	—	—	—	—	—
Gγ_4	31	77	69	100	—	—	—	—	—	—	—	—
Gγ_5	25	45	45	42	100	—	—	—	—	—	—	—
Gγ_7	30	66	58	55	51	100	—	—	—	—	—	—
Gγ_8	31	70	55	60	42	51	100	—	—	—	—	—
Gγ_9	63	34	31	28	26	38	30	100	—	—	—	—
Gγ_{10}	29	48	45	45	52	52	45	33	100	—	—	—
Gγ_{11}	76	29	28	27	27	35	28	63	30	100	—	—
Gγ_{12}	31	56	54	50	45	76	47	36	44	36	100	—
Gγ_{13}	28	28	25	26	23	28	26	32	23	31	25	100

[between 70 and 90%; see Li et al. (2007)], whereas their Gγ subunits show more sequence diversity [between 40 and 90%; see Li et al. (2007)]. Functional Gβ (GNB-1; guanine nucleotide-binding protein, beta polypeptide-1) and Gγ (GNG-1; guanine nucleotide-binding protein, gamma polypeptide-1) subunits have been identified in *Neurospora crassa*, where they play a role in regulating female fertility and asexual development (Krystofova and Borkovich, 2005). Interestingly, G$\beta\gamma$ in *Neurospora crassa* stabilizes Gα

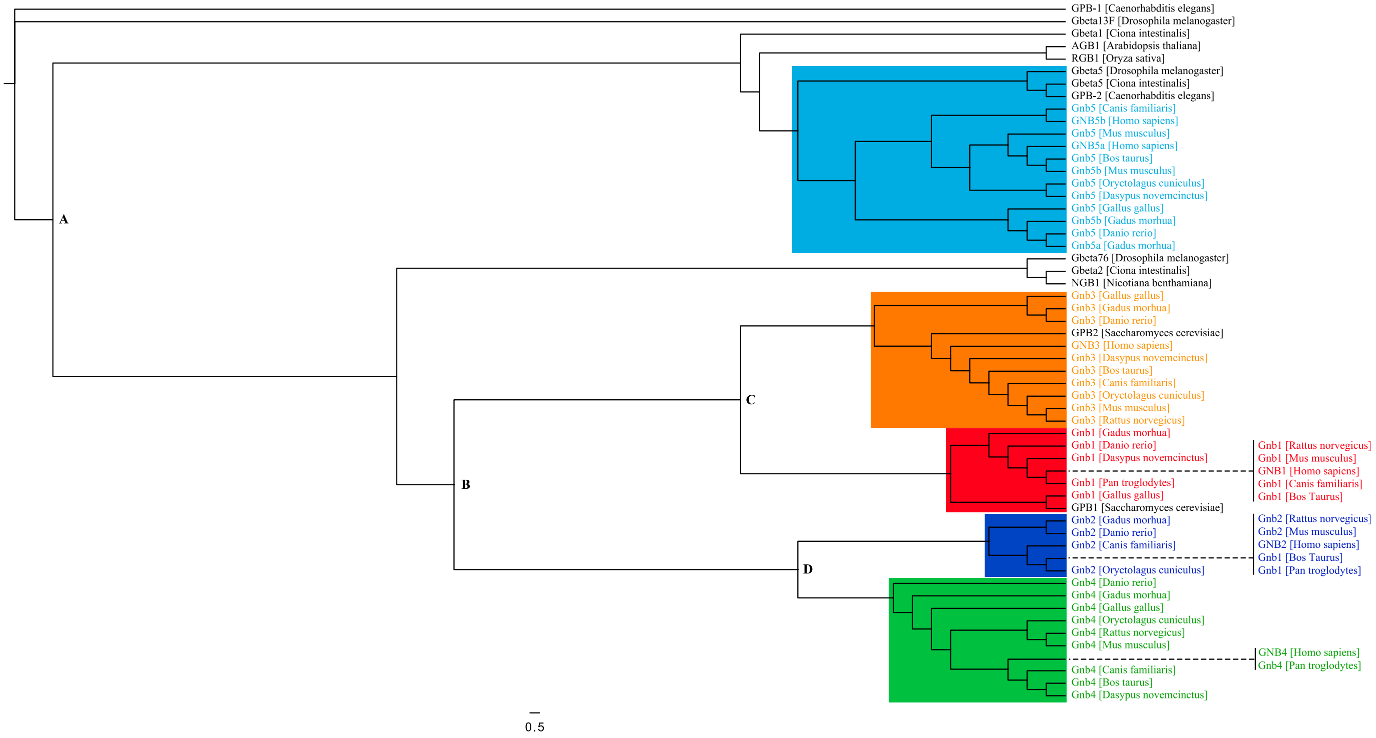

Fig. 1. Phylogenetic relationships of Gβ subunits across various species. To construct the trees, amino acid sequences for known Gβ homologs were collected from the National Center for Biotechnology Information PubMed and Ensembl databases. Redundancy of amino acid sequences was eliminated to conserve only one copy of each distinct amino acid sequence. Multiple sequence alignments were produced for each family using T-Coffee (Notredame et al., 2000), and quality of the alignments was ascertained using T-Coffee's alignment scoring mechanism. Adequate parameters for inference of the phylogeny were obtained by submitting the produced alignments to the ProtTest evolutionary model selection software (Abascal et al., 2005). Maximum likelihood-based phylogeny was then inferred using PhyML (Guindon et al., 2010), using the parameters suggested by ProtTest based on the Akaike information criterion framework score, namely, the LG (Le and Gascuel) model along with gamma correction. Tree topology optimization was accomplished through the subtree pruning and regrafting technique, and a total of five random starting trees were used in each inference. Robustness of the obtained tree was evaluated by running 1000 bootstrap iterations of the inference process. To preserve the integrity of the tree, redundant Gβ sequences from different species that aligned at the same node are represented as groups, linked to the tree by dashed lines. Clusters, clades, and nodes are identified by their different color backgrounds and text color. A distance bar scale is shown under the tree. The five Gβ subunit subtypes from different species form clusters, and these are depicted by the following color scheme: Gβ_1 (red), Gβ_2 (dark blue), Gβ_3 (orange), Gβ_4 (green), and Gβ_5 (light blue).

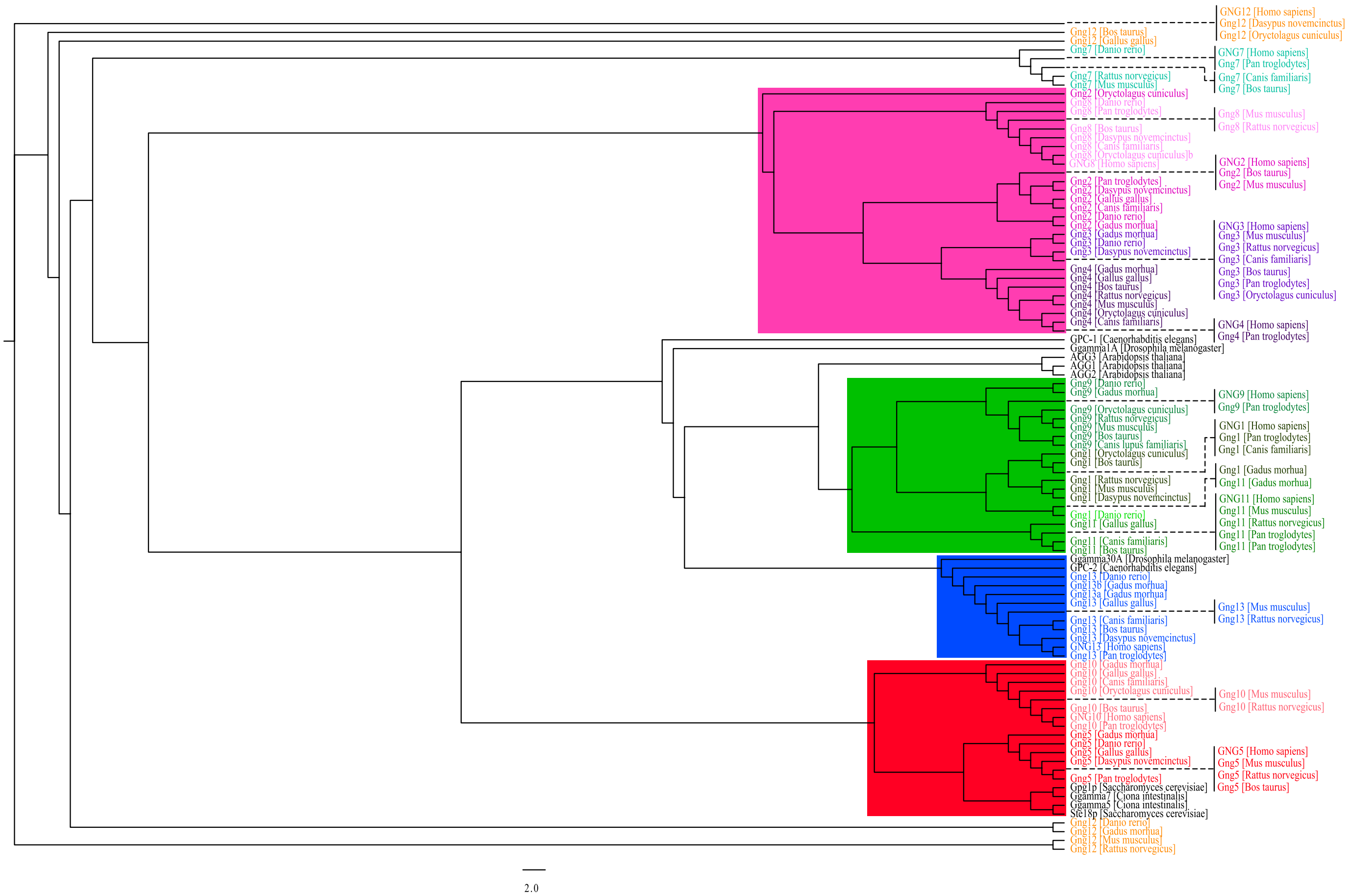

Fig. 2. Phylogenetic relationship of Gγ subunits across various species. The phylogenetic tree for Gγ subunits was constructed by a similar method as described in Fig. 1. In this case, the parameter used for this maximum-likelihood phylogenetic tree was the JTT (Jones Taylor Thornton) evolutionary model along with gamma correction. As in Fig. 1, redundant sequences are grouped and linked to the tree by dashed lines, and a distance bar scale is shown under the tree. Gγ subunits group into five main clusters, and consist of cluster 1 (pink)—Gγ_2, Gγ_3, Gγ_4, and Gγ_8; cluster 2 (green)—Gγ_1, Gγ_9, and Gγ_{11}; cluster 3 (red)—Gγ_5 and Gγ_{10}; cluster 4 (blue)—Gγ_{13}; and cluster 5—Gγ_7 and Gγ_{12}. Gγ_7 and Gγ_{12} appear to be the least divergent Gγ subunit, being most similar to the initial ancestral Gγ subunit.

subunits, suggesting that the heterotrimer is the functional unit. Although GNG-1 is highly similar to a number of other fungal Gγ isoforms, it is only 40% similar to Gγ in *S. cerevisiae* and 9% similar to Gγ in *S. pombe* (Krystofova and Borkovich, 2005), already suggesting a potential divergence of function. However, all of these Gγ subunits possess a conserved CAAX box, which allows farnesylation and thus facilitates membrane association, suggesting that their activity consistently depends on membrane anchoring.

B. Invertebrate G$\beta\gamma$

From our analysis, invertebrate Gβ isoforms from the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster* share a common pattern of subunit evolution. Intriguingly, Fig. 1 demonstrates that each of these species contains a Gβ subtype highly divergent from the rest of the Gβ subunits analyzed (GPB-1 and Gβ13F from *C. elegans* and *D. melanogaster*, respectively) and one Gβ that is homologous to vertebrate Gβ5 (GPB-2 and Gβ5 from *C. elegans* and *D. melanogaster*, respectively). *D. melanogaster* contains one additional Gβ (Gβ76C) that is homologous to vertebrate G$\beta_{1–4}$. With regard to invertebrate Gγ subtypes, a similar divergence pattern as with Gβ subtypes is also observed for *C. elegans* and *D. melanogaster*: GPC-1 (guanine nucleotide-binding protein, gamma polypeptide-1) and Gγ_1 are most similar to the vertebrate G$\gamma_{1/9/11}$ superfamily, whereas GPC-2 and Gγ_{30} are most similar to vertebrate Gγ_{13} subunits, respectively.

In *C. elegans,* Gβ and Gγ are required for embryonic development, as GPB-1 and GPC-2 control spindle orientation and positioning events during early embryonic stages (Zwaal et al., 1996; Gotta and Ahringer, 2001; Tsou et al., 2003). Accordingly, *gpb-1* mutant embryos fail to hatch, and have a highly disorganized tissue distribution. In this system, GPB-1 and GPC-2 are likely to function as negative regulators of the activity of two Gα subunits (i.e., as a classic G$\beta\gamma$), GOA-1 (guanine nucleotide-binding protein, alpha subunit Go) and GPA-16 (guanine nucleotide-binding protein, alpha subunit), as the spindle-positioning defect observed in *gpb-1*–depleted embryos can be suppressed by codepletion of these two Gα subunits (Tsou et al., 2003). Regarding GPB-2, this vertebrate Gβ_5 homolog has been shown to bind Gγ–like (GGL) domains containing regulator of G protein signaling (RGS) proteins, much like its vertebrate counterparts, suggesting it may also regulate the GTPase activity of Gα subunits (Chase et al., 2001; Robatzek et al., 2001; van der Linden et al., 2001). GPC-2 shows ubiquitous expression in *C. elegans*, and is most related to the vertebrate Gγ_{13}, which is least similar to all of the other Gγ subunits in humans (see Table 2). On the other hand, *C. elegans* GPC-1 is only expressed in sensory neurons, and has been shown to be involved in chemosensation (Jansen et al., 2002). Figure 2 shows that this subunit is more closely related to vertebrate Gγ_1 and Gγ_9. Interestingly, these two vertebrate subunits have been shown to be expressed in the rods and cones of the human eye (Hurley et al., 1984; Ong et al., 1997), suggesting that GPC-1 and the two vertebrate homologs are specifically required in the nervous system.

In *D. melanogaster*, G protein subunits are encoded by three Gβ and two Gγ genes. As in *C. elegans*, G$\beta\gamma$ dimers are involved in the control of asymmetric cell division in neuroblasts and sensory organs, gastrulation, heart function (Schaefer et al., 2001; Izumi et al., 2004; Yi et al., 2006, 2008), and the visual system (Dolph et al., 1994; Schulz et al., 1999). One study suggested that free G$\beta\gamma$ subunits are involved in wing expansion accompanied by epithelial-mesenchymal transition (Katanayeva et al., 2010). Beyond this role in early development, G$\beta\gamma$ subunits also serve other canonical roles in GPCR signaling in the adult fly, as in *C. elegans*.

Interestingly, the sea squirt, *Ciona intestinalis*, and *Drosophila* display a very similar evolutionary pattern: *C. intestinalis* also expresses 3 Gβ subunits that are divergent from the rest of the Gβ subunits of the species analyzed (Gβ_1), similar to vertebrate Gβ_5, or similar to vertebrate G$\beta_{1\text{–}4}$ (Gβ_2-like-1). However, the *C. intestinalis* Gγ homologs cluster with the vertebrate G$\gamma_{5/10}$ superfamily. *C. intestinalis* has the smallest genome of manipulable chordates, making it an excellent candidate to study evolutionary and developmental biology, and in particular, its similarity to the vertebrate G$\gamma_{5/10}$ superfamily makes it a suitable model to understand G protein subunit diversity (Prasobh and Manoj, 2009).

C. Plant G$\beta\gamma$

We analyzed the sequence of Gβ subunits in three different plant species: AGB1 (arabidopsis GTP binding protein beta subunit) in *Arabidopsis thaliana* (thale cress), NGB1 (nicotiana heterotrimeric G protein beta subunit) in *Nicotiana benthamiana* (similar to tobacco plant), and RGB1 in *Oryza sativa* (rice). Whereas *A. thaliana* and *O. sativa* Gβ subunits are similar to each other and diverge from a common ancestor in the Gβ5 cluster, Gβ from *N. benthamiana* is more similar to vertebrate Gβ_2 counterparts (Fig. 1). This finding is intriguing, as this suggests that, perhaps during evolution, plants have been able to retain certain classes of Gβ subtypes according to particular cellular requirements. *A. thaliana* AGB1 has been shown to be involved in the negative regulation of auxin-induced cell division, gene transcription regulation, and pathogen resistance pathways (Ullah et al., 2003; Trusov et al., 2006).

O. sativa expresses two different types of Gγ [RGG1 (rice heterotrimeric G protein gamma subunit) and RGG2] (Kato et al., 2004), but unfortunately had to be removed from our phylogeny analysis, as their sequence divergence proved too disruptive to the inference process. Gγ subtypes from *N. benthamiana* are yet to be characterized, and were also not included in the present analysis. However, phylogenetic analysis of three *A. thaliana* Gγ subunits, including the recently characterized third Gγ subunit (Thung et al., 2012), reveals that all three subunits, AGG1 (arabidopsis GTP binding protein gamma subunit), AGG2, and AGG3, share a common ancestor with the vertebrate Gγ1/9/11 class. Since this common ancestor was the most divergent ancestral Gγ from the initial Gγ subunit, this suggests that Gγ subunits have evolved to become highly specialized in *A. thaliana*. Despite their tight co-divergence? AGG1 and AGG2 have been shown to exhibit functional selectivity within this species, playing different roles in pathogen resistance, germination, lateral root development, and gravitropism (Trusov et al., 2007). It remains to be determined if species such as *Arabidopsis,* which have Gβ_5-like proteins, actually require Gγ subunits in vivo.

D. Fish and Mammalian G$\beta\gamma$

Throughout evolution, fish and mammals have acquired a larger and more diverse set of Gβ and Gγ subunits. Whether these sets of G$\beta\gamma$ subunits are redundant or serve specific cellular roles remains unknown. We analyzed the Gβ sequences from two different types of fish, *Danio rerio* (zebra fish) and *Gadus morhua* (cod), and observed that all Gβ subunits (with the exception of Gβ_4) from both of these species seemed to have evolved from the same common ancestor that yielded Gβ in mammalian species (Fig. 1). The same can be concluded regarding Gγ subunits in both of these fish species; however, in *Gadus morhua*, it is interesting to note that Gγ_1 and Gγ_{11} were found to be redundant for our analysis. Whether these subunits are functionally redundant remains to be determined.

Genomic analysis of humans and mice Gγ genes revealed a general, but not absolute conservation, with differences appearing primarily at the 5′ ends of these genes (Cook et al., 2001; Yang and Hildebrandt, 2006).

Certain Gγ genes were found to be less than 10 kb in length, whereas others were greater than 100 kb in length, which is remarkable given that Gγ protein sequences typically contain 65–75 amino acids (Yang and Hildebrandt, 2006). Our phylogenetic analysis of Gβ and Gγ from various species indicates a modest level of sequence conservation between both fish and mammalian species.

Mammalian Gβ and Gγ subunits display a subunit-specific clustering pattern, as described earlier. The roles that these specific G protein subunits play in cellular signaling are also described in more detail later. Our analysis reveals that, regardless of differences in species types, mammalian Gβ and Gγ subunits display tight conservation of protein sequence within each subtype of Gβ or Gγ, with each subtype in each species coming from a particular common ancestral Gβ and Gγ. The observation that different Gβ and Gγ subunit subtypes are similar/conserved across species has been used as sound reasoning to use certain mammalian species such as mice (*Mus musculus*) and rats (*Rattus norvegicus*) as experimental models to study G protein function, and has yielded great insight into the roles that these Gβ and Gγ subunits play. However, the crucial question that remains to be answered is what the evolutionary diversity of Gβ and Gγ subunits implies for broader G protein function. Although it is quite possible that some of these subunits may serve redundant roles, it is highly probable that these differences in Gβ and Gγ protein sequences within a certain species impart essential structural differences to these subunits, conferring them specificity and selectivity in their function.

E. Structural Features of G$\beta\gamma$ Subunits

The number of Gβ and Gγ genes is strikingly higher in mammals compared with *C. elegans* and other simpler organisms. As discussed earlier, humans express five distinct Gβ subunits along with their variants (β_1, β_2, β_3, β_{3S}, β_4, β_5, β_{5L}) and 12 Gγ proteins (G$\gamma_{1\text{-}5,7\text{-}13}$) (Hurowitz et al., 2000), whereas *C. elegans* possesses only two genes for each subunit (Jansen et al., 2002; Bastiani and Mendel, 2006). Thus, over evolution, a number of new and distinct functions for G$\beta\gamma$ may have come into play through gene duplication and subsequent selection, and they may not be limited to the open reading frames of the various genes. However, our current understanding of the basis of mammalian G$\beta\gamma$ diversity is rudimentary, and has been mostly focused on canonical signaling functions. Almost nothing is known about how this diversity affects either the organizing or the transcriptional regulatory functions of different G$\beta\gamma$ subunits discussed later.

A number of crystal structures have been generated for G$\beta\gamma$ subunits, alone and in complexes with known effectors [reviewed in Lin and Smrcka (2011)]. The β-propeller structure of the WD repeats in Gβ and its association with Gγ (Sondek et al., 1996) and Gα (Wall et al., 1995; Lambright et al., 1996) have become iconic. All of these have used either G$\beta_1\gamma_1$ or G$\beta_1\gamma_2$, and have addressed how subunit diversity might impact function. The Gγ subunits are where this diversity is most obvious, a somewhat curious notion given that they are among the smallest proteins involved in G protein signaling. Molecular modeling of the human Gβ and Gγ subunits give some indication as to why this is important (Fig. 3). Here, we can see that the three nonconserved regions of Gγ subunits, the N and C termini, as well as the central hinge, all face outward, away from the Gβ subunit (which is generally much more conserved), where they can interact differentially with a number of different and possibly unique effectors. Thus, the Gγ subunits have evolved to provide a great deal of the structural diversity or "granularity" necessary for serving the diverse roles of G$\beta\gamma$ in cellular function.

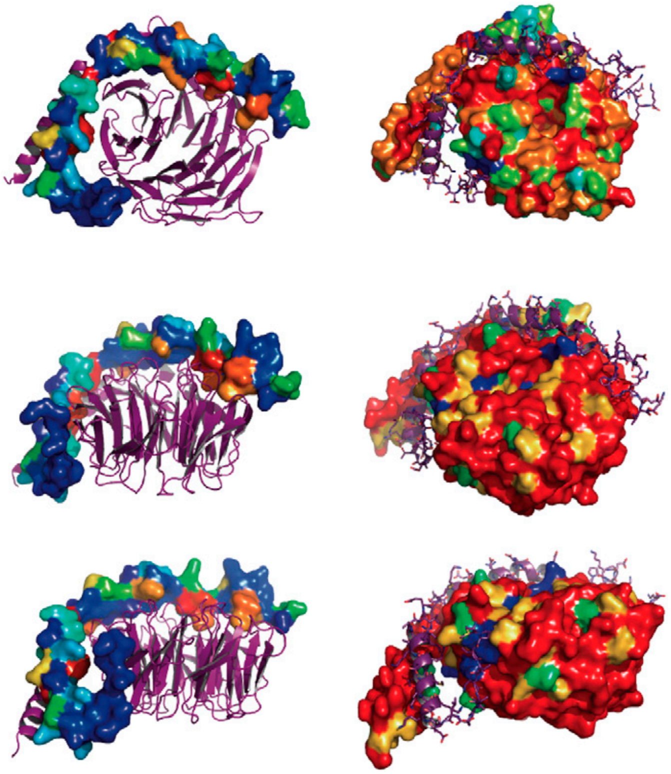

Fig. 3. Structural mapping of G$\beta\gamma$ subunits. Modeling of conserved and nonconserved regions of G$\beta_{1\text{-}5}$ and G$\gamma_{1\text{-}13}$, based on the published structures of G$\beta_1\gamma_1$ and G$\beta_1\gamma_2$. SWISS MODEL was used to generate structures. The align feature (clustal) in Pymol was used to align the different subunits. Protskin (Deprez et al., 2005) was used to color the gradient of conservation (red: conserved, blue: nonconserved). Views from three different vantage points are presented with either the Gγ (left) or Gβ (right) colored using Protskin. Nonconserved N termini, hinge, and C termini of Gγ subunits are located on the external face of the G$\beta\gamma$ subunit. Gβ subunits show greater conservation (especially on the face which contacts Gα).

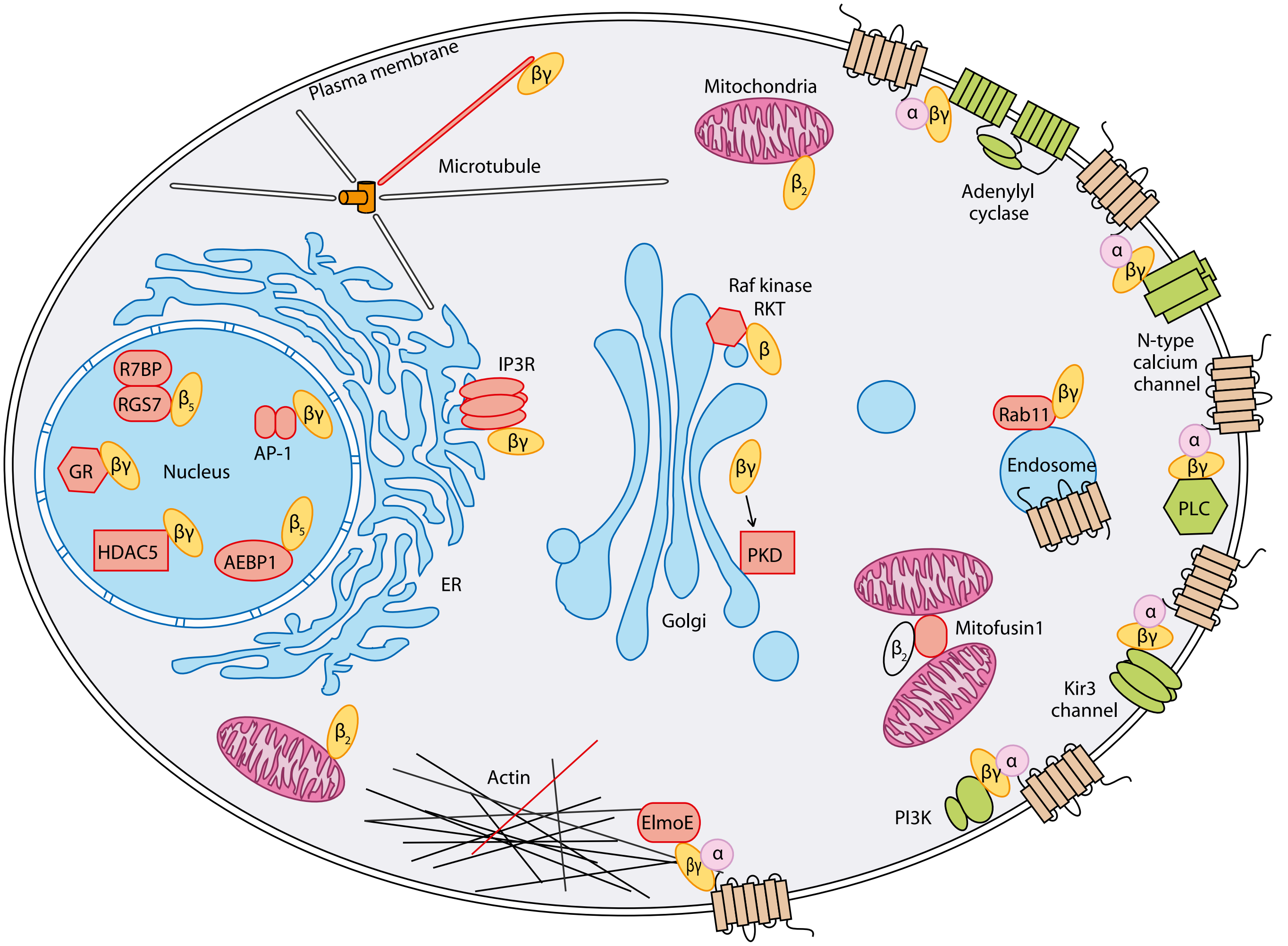

Fig. 4. Canonical and noncanonical $G\beta\gamma$ effectors. $G\beta\gamma$ subunits regulate a number of effectors at the cell surface, including adenylyl cyclase isoforms, Kir3 and voltage-gated calcium channels, phospholipase $C\beta$ isoforms, and PI3K isoforms, among others. More recently, a number of novel interacting proteins have been identified which transduce $G\beta\gamma$-dependent signals in other subcellular compartments such as endosomes, mitochondria, ER (IP_3 receptors), Golgi apparatus (Raf kinase, PKD), cytosol (HDAC5), nucleus [AP-1, R7BP, AEPB1 (adipocyte enhancer-binding protein), GR, and possibly HDAC], and cytoskeleton (tubulin, ElmoE). Whether all of these intracellular events require GPCRs or $G\alpha$ subunits remains to be determined. The examples presented here are representative and do not include all of either classic or novel effectors. See text for details.

III. Canonical Signaling Regulated by $G\beta\gamma$ Subunits

An independent role in signaling for $G\beta\gamma$ subunits, distinct from $G\alpha$, has come to light in the last 25 years, and several canonical effector molecules have been identified. Here, we review the most important examples of these signaling molecules modulated by $G\beta\gamma$ subunits (Fig. 4). With respect to the subunit dissociation model of G protein activation, the $G\alpha$ subunit is believed to dissociate from its cognate $G\beta\gamma$ partner, allowing effector binding surfaces to be exposed and subsequent downstream signaling by the $G\beta\gamma$ subunit (Smrcka, 2008). This implies that different $G\alpha$ subunits might share a pool of diverse $G\beta_x\gamma_y$ dimers, and that heterotrimerization at the cell surface (especially after receptor stimulation) would occur via collisional coupling. However, an alternate "clamshell" model has been described wherein GPCR activation induces conformational changes in the G protein subunits and exposes otherwise hidden surfaces at the $G\alpha$ and $G\beta\gamma$ interface. This leads to interaction with effectors, without the $G\alpha$ and $G\beta\gamma$ unhinging completely from one another, effectively remaining associated throughout activation (Robishaw and Berlot, 2004). This is supported by a number of studies using resonance energy transfer techniques (Bünemann et al., 2003; Frank et al., 2005) but may not be applicable to all G protein heterotrimers (Digby et al., 2006, 2008), suggesting a spectrum of organizational paradigms [reviewed in Hebert et al. (2006) and Lambert (2008)]. This makes sense in light of a later discussion, which describes a number of canonical $G\alpha$ effectors that are also regulated by $G\beta\gamma$ subunits. This model implies that $G\beta\gamma$ dimers must exhibit selectivity for association with $G\alpha$ subunits and for other components of a receptor complex. The role of $G\beta\gamma$ subunits in interacting with other components of GPCR-based signaling complexes was summarized in more detail in a previous review (Dupré et al., 2009). In brief, it is clear that $G\beta\gamma$ subunits interact with receptors, $G\alpha$ subunits, effectors, and regulatory enzymes such as G protein–coupled receptor kinases (GRKs), highlighting the central role they play in all aspects of GPCR signaling. This notion calls to mind again the idea that all $G\beta\gamma$ dimer combinations are not equal, may have distinct functions, and that different cellular pools of $G\beta\gamma$ control a great deal of the architecture of cellular signaling.

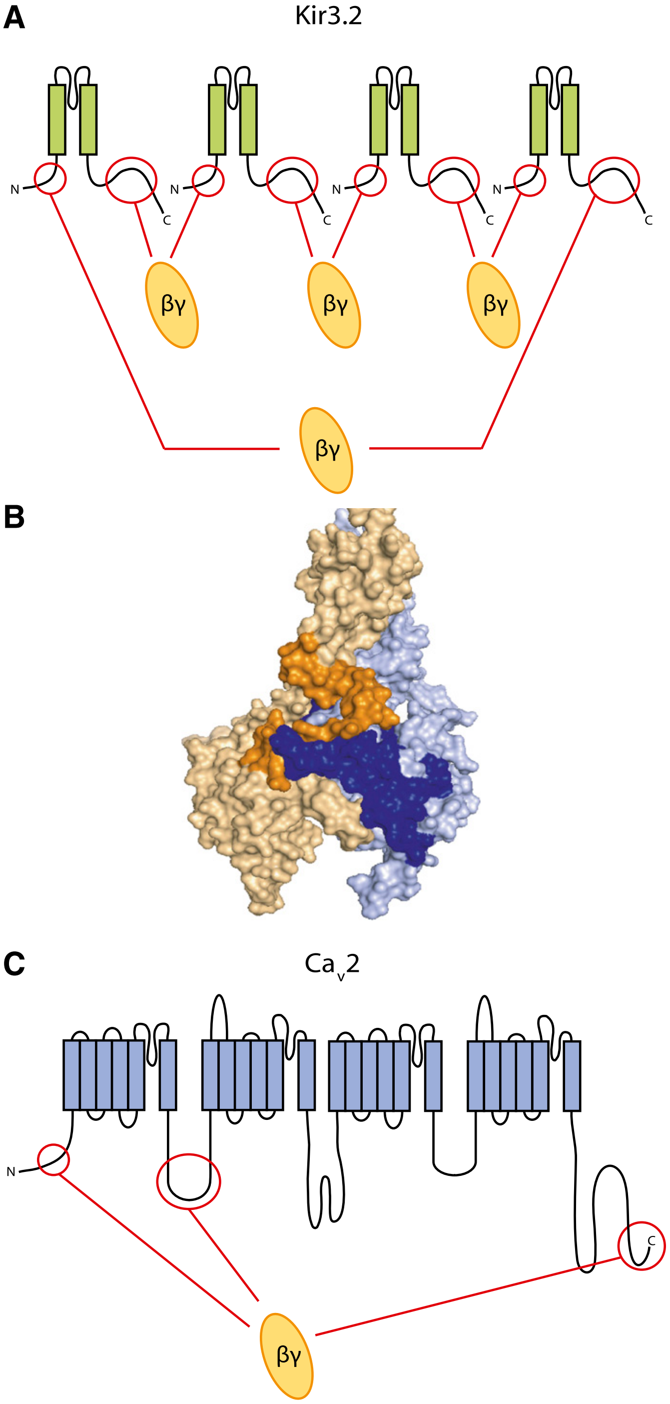

Fig. 5. $G\beta\gamma$ binding sites on Kir3 and Ca$_v$ channels. (A) $G\beta\gamma$ sites identified using biochemical approaches mapped onto the structure of Kir3 channels. $G\beta\gamma$ "holo" binding sites are formed by fragments coming from adjacent subunits. (B) Structure of Kir3.2 (Protein Data Bank access number 3SYA) (Whorton and MacKinnon, 2011). Pale orange and pale blue indicate adjacent subunits (two of four shown). Dark orange represents N-terminal $G\beta\gamma$-interacting fragments, whereas dark blue represents C-terminal $G\beta\gamma$-interacting fragments, according to Ivanina et al. (2003), showing that multiple channel subunits form each $G\beta\gamma$ binding site such that there are four per channel. (C) $G\beta\gamma$ sites identified using biochemical approaches mapped onto the structure of Ca$_v$ channels. $G\beta\gamma$ "holo" binding sites are formed by fragments coming from adjacent subunits. See text for more details.

A. Kir3 Channels

As noted earlier, the first direct effector identified for $G\beta\gamma$ was the cardiac muscarinic-gated inwardly rectifying K$^+$ channel. The activating effect of $G\beta\gamma$ on the channel was demonstrated by perfusing excised atrial membrane patches with $G\beta\gamma$ purified from bovine brain (Logothetis et al., 1987). This atrial channel is now known to comprise a heterotetramer of Kir3.1 and Kir3.4 subunits, which are members of the larger family of Kir3 channels. The Kir3 family consists of four distinct subunits encoded on separate genes, Kir3.1 through Kir3.4, and they can all be regulated, in principle, by direct binding of $G\beta\gamma$ subunits (Schoots et al., 1999). Activation of Kir3 channels leads to the hyperpolarization of electrically excitable cells via an outward flux of potassium ions under physiologic conditions. They have been shown to be expressed in various peripheral tissues, such as the heart and pancreas, and are widely expressed in the brain, serving roles as diverse as reduction of heart rate to hormone secretion. For review, see Luscher and Slesinger (2010) and Yamada et al. (1998).

Channel gating involves two restrictions within the channel pore. The inner helix gate is located within the plane of the plasma membrane, and is composed of the inner helices of the transmembrane domain, whereas the G loop gate is situated at the interface of the transmembrane and cytoplasmic region, and is formed by the proximal C-terminal domain (Whorton and MacKinnon, 2011). As with most ion channels, Kir3 channels require the presence of phosphatidylinositol (4,5)-bisphosphate (PIP$_2$) for activation, where binding of four PIP$_2$ molecules to the channel couples the opening of the inner helix gate and the G loop gate upon $G\beta\gamma$ binding (Huang et al., 1998). In the absence of PIP$_2$, $G\beta\gamma$ binding only opens the G loop gate, and no ions pass through the channel.

Potentially distinct $G\beta\gamma$ binding sites have being localized to both the N and C termini of the Kir3 channel subunits (He et al., 2002). Specifically, Kir3.1 subunits contain one and two binding sites in their N and C termini, respectively, whereas the other three family members contain one binding site in each of their N- and C-terminal domains, as identified using biochemical approaches with purified proteins (Huang et al., 1995, 1997). The structure of a Kir3.2

homotetramer has been solved, and evidence from the structure implies that the N terminus of one subunit folds with the C terminus of the adjacent subunit in the holochannel, putting the N- and C-terminal G$\beta\gamma$ binding sites in close proximity (Fig. 5, A and B). The G$\beta\gamma$ binding sites on Kir3 channels have also been investigated by NMR (Yokogawa et al., 2011; Mase et al., 2012). By mapping the residues affected by G$\beta\gamma$ binding to the structure, the authors proposed that G$\beta\gamma$ binds at the interface between adjacent subunits allowing for contacts with the N terminus of one subunit and the C terminus of an adjacent subunit. This would allow the Kir3- G$\beta\gamma$ binding pocket to involve both the N- and C-terminal binding sites, thus explaining the distinct G$\beta\gamma$ binding sites on single subunits detected in biochemical studies. With this arrangement, the holochannel would contain four G$\beta\gamma$ binding sites. This idea of multiple G$\beta\gamma$ binding sites is further solidified, as channel activation through G$\beta\gamma$ binding has been shown to be a graded and possibly cooperative mechanism where increasing the number of G$\beta\gamma$ dimers occupying the binding sites on the channel leads to an increase in channel current (Ivanova-Nikolova et al., 1998; Sadja et al., 2002). Some studies have suggested that Gβ_1-containing G$\beta\gamma$ dimers activate Kir3 channels, whereas those containing Gβ_5 result in channel inhibition (Mirshahi et al., 2002). As detailed later, most differences between Gβ subunits have resulted in grouping G$\beta_{1–4}$ against Gβ_5. More work is required to tease out the specific roles of individual Gβ subunits. Some evidence suggests that different receptor/Kir3 combinations are preferentially favored by different G$\beta\gamma$ combinations as well (Robillard et al., 2000). However, as discussed later, it is not clear whether this specificity occurs at the level of channel activation or in the control of assembly of particular receptor-based complexes [also reviewed in Dupré et al. (2009)].

B. Voltage-Dependent Ca^{2+} Channels

Voltage-dependent Ca^{2+} channels mediate calcium ion flux across the plasma membrane. At resting membrane potentials, these channels are typically closed, but they open upon membrane depolarization. The main pore-forming protein of these channels, the α_1 subunit, is classified into three groups: Ca_v1, Ca_v2, and Ca_v3 [for review, see Minor and Findeisen (2010)]. Although all three classes of channel are regulated by G$\beta\gamma$ to some extent, the best understood at present are the Ca_v2 channels.

Ca_v2 channels can be classified into three subtypes: $Ca_v2.1$ (P/Q-type channels), $Ca_v2.2$ (N-type channels), and $Ca_v2.3$ (R-type channels). The N-type calcium channel is widely expressed in the brain and controls neurotransmitter release along with P/Q- and R-type channels. These channels are localized to presynaptic terminals, where their voltage-dependent activation leads to an influx of calcium ions, which in turn initiates exocytosis of synaptic vesicles containing various neurotransmitters (Wheeler et al., 1994).

Multiple types of voltage-dependent calcium channels possess binding sites for G$\beta\gamma$ subunits. The first demonstration of a modulatory effect of G proteins on calcium channels was the inhibitory effect of norepinephrine on Ca^{2+} current amplitudes in chick sensory neurons (Dunlap and Fischbach, 1981). It is now appreciated that GPCRs use multiple mechanisms for regulating calcium channels. The best-studied mechanism involves direct interaction of G$\beta\gamma$ subunits with the channel, which underlies a phenomenon called voltage-dependent inhibition. This manifests as a reduction in peak amplitude of the whole-cell calcium current, although the reduction is minimal at depolarized membrane potentials. Channel activation kinetics are also slower, and a depolarizing prepulse can relieve the inhibition, reduce G$\beta\gamma$ binding, and restore channel kinetics. This inhibitory effect of G$\beta\gamma$ has been seen in multiple types of calcium channels, but has been best characterized in Ca_v2 channels [for review, see Currie (2010)].

Ca_v channels are hetero-oligomeric protein complexes composed of a pore-forming α_1 subunit, a cytoplasmic $Ca_v\beta$ subunit, and a membrane-associated $\alpha_2\delta$ subunit (Catterall, 2000). The α_1 subunit has been found to harbor G$\beta\gamma$ binding sites (Bourinet et al., 1996), and different isoforms of this subunit determine the distinct properties of these different channels (Gray et al., 2007). G$\beta\gamma$ binding sites have been localized to the N terminus, the C-terminal domain, and the intracellular loop connecting transmembrane domains I and II (De Waard et al., 2005). Although there are multiple potential sites of interaction within the α_1 subunit, it has been proposed that only a single G$\beta\gamma$ binding surface exists per channel, with the multiple interaction sites folding into close proximity in the native conformation of the channel (Zamponi and Snutch, 1998). Studies using glutathione S-transferase fusion proteins have identified that the I-II loop also contains two distinct binding sites (Fig. 5C), as seen in the case of Kir3 channels. One binding site overlaps with the protein-protein interaction domain between α_1 and the $Ca_v\beta$ subunit. The $Ca_v\beta$ subunit binding exerts a modulatory role on the inhibitory effect of G$\beta\gamma$ on the channel α subunit. This site also contains a putative G$\beta\gamma$ binding consensus sequence (QXXER) found in other G$\beta\gamma$ binding partners such as phospholipase Cβ_2 and type 2 adenylyl cyclase (De Waard et al., 1997). The second binding site contains the G protein interaction domain (Zamponi et al., 1997). The N-terminal domain has also been shown to form intramolecular interactions with the I-II loop that are impaired upon G$\beta\gamma$ binding (Geib et al., 2002). The C-terminal binding site has been demonstrated to be essential for the inhibitory action of G$\beta\gamma$ subunits on the R-type channel, but has also been found to serve more of a modulatory role on N-type calcium channels (Li et al., 2004). The C terminus is also known to contain binding sites for other signaling proteins, i.e., PKCε for Ca$_v$2.2 channels and calmodulin, Ca^{2+}-binding protein 1, and visin-like protein 2 for Ca$_v$2.1 channels, which could potentially allow for cross-talk and fine-tuning of the G$\beta\gamma$-mediated inhibition (Evans and Zamponi, 2006).

Other calcium channels are also regulated by G$\beta\gamma$ subunits, although their characterization is less advanced than in Ca$_v$2 channels. G$\beta\gamma$ regulates certain T-type calcium channels (Ca$_v$3-containing channels)—Ca$_v$3.2, but not Ca$_v$3.1 are inhibited by G$\beta\gamma$ binding to their α_1 subunit. This inhibition was shown to be mediated solely by G$\beta_2\gamma_2$ dimers interacting with the intracellular loop connecting transmembrane domains II and III. Reduced channel activity was independent of voltage, and was thus different from the regulation of Ca$_v$2 channels by G$\beta\gamma$ (Wolfe et al., 2003). L-type calcium channels, i.e., Ca$_v$1 channels, are modulated both directly and indirectly by G$\beta\gamma$. Neuronal and cardiac L-type calcium channels composed of α_{1C} subunits have been shown to be inhibited by G$\beta\gamma$. These channels possess G$\beta\gamma$ and calmodulin binding sites in their N and C termini, and the inhibitory effect requires the presence of calmodulin (Ivanina et al., 2000). On the other hand, vascular L-type calcium channels have been demonstrated to be regulated indirectly by G$\beta\gamma$, specifically via a G$\beta\gamma$-sensitive PI3K activated by the GPCRs. It was also observed that a PKC inhibitor reduced G$\beta\gamma$- and PI3Kγ-induced Ca^{2+} currents, implying a role for PKC in the pathway (Viard et al., 1999).

C. Adenylyl Cyclase Isoforms

Adenylyl cyclase (AC) is the canonical G protein–signaling cascade effector. This enzyme was characterized based on its activation by Gα_s-coupled GPCRs and its inhibition by Gα_i-coupled GPCRs. AC is the enzyme responsible for catalysis of ATP conversion into cyclic AMP; currently, there are multiple mammalian isoforms of adenylyl cyclase, but only a subset of them are known to be directly regulated by G$\beta\gamma$ (Sunahara et al., 1996). All AC isoforms have a similar structure consisting of two membrane-integrated domains of six transmembrane helices each as well as two catalytic domains (C1 and C2) localized on the cytoplasmic side of the membrane (Krupinski et al., 1989). Catalytic activity is regulated by a wide variety of mechanisms—beyond Gα, AC isoforms are regulated by multiple protein kinases such as PKA (protein kinase A) and PKC, phosphatases such as calcineurin, calcium, and most important to this review, G$\beta\gamma$ (Sunahara and Taussig, 2002). Certain isoforms of AC are regulated by direct interaction with G$\beta\gamma$ subunits. The effect of G$\beta\gamma$ on AC activity is dependent on the particular AC isoform, with increased production of cAMP for some, whereas reduction of activity is seen for others.

The mechanism of G$\beta\gamma$ stimulation of AC is through direct interaction with the catalytic domains. The first identified interaction site between G$\beta\gamma$ and AC was via a QEHA sequence located within C2 on AC2 (Chen et al., 1995). It was later determined that this site was of minor importance for the actual stimulatory effect, because chimeric proteins within the QEHA region replaced by corresponding sequences from other isoforms of AC did not alter the stimulatory effect of G$\beta\gamma$ (Weitmann et al., 2001). A second site of interaction was then identified in C1 through deletion analysis. This site of interaction contained a PFAHL motif that was absolutely necessary for the stimulatory action of G$\beta\gamma$ and is present in all AC isoforms that are stimulated by G$\beta\gamma$ (AC2, AC4, and AC7) and absent in those that are not. Therefore, it is proposed that G$\beta\gamma$ stimulation of AC is achieved by two contact points within the two catalytic domains (Diel et al., 2006).

Inhibition of AC by G$\beta\gamma$ is less well understood. The inhibitory effect of G$\beta\gamma$ on AC was first identified for AC1, and is now known to occur for AC5 and AC6 as well. The G$\beta\gamma$ site of interaction on AC1 was identified in the C1 and C2 regions, with the C1a region comprising the minimal required binding region to see G$\beta\gamma$-mediated inhibition of enzyme activity stimulated by Gαs or Ca^{2+}/calmodulin (Wittpoth et al., 1999). It was also observed that AC1 inhibition is mediated by G$\beta\gamma$ released from Gα_s-coupled receptors and not Gα_i-coupled receptors (Nielsen et al., 1996). It has been shown that inhibition is dependent on particular subtypes of Gβ and Gγ (Bayewitch et al., 1998a,b). Overexpression of AC5 or AC6 in COS-7 cells along with Gβ_1 alone or with Gγ_2 demonstrated reduced cAMP accumulation. The same was seen for cotransfection of G$\beta_5\gamma_2$, but when Gβ_5 was overexpressed along with AC5 or AC6, there were similar levels of cAMP accumulation compared with when the AC isoforms were overexpressed alone.

D. Phospholipase C

Phospholipase C (PLC) is another well-studied G protein–signaling effector that is regulated by G$\beta\gamma$. PLC activation leads to cleavage of PIP$_2$ into diacylglycerol and inositol 1,4,5-triphosphate (IP$_3$). Diacylglycerol directly activates PKC while IP$_3$ diffuses to the endoplasmic reticulum (ER), binds the IP$_3$ receptor, and mobilizes intracellular calcium stores. There are currently 13 known isoforms of PLC classified into six families, namely, PLC-β, -γ, -δ, -ε, -ζ, and -η. There are conserved core domains within this family, which include a pleckstrin homology (PH) domain in the N-terminal domain, a series of EF-hands, a catalytic TIM (triose phosphate isomerase) barrel, and a C-terminal C2 domain [for review, see Bunney and Katan (2011)].

PLC activity is regulated by a wide range of signaling partners. Some isoforms of PLC are regulated by downstream signaling from receptor tyrosine kinases (PLCγ), whereas others are regulated by GPCRs (PLCβ). The first subfamily of PLC shown to be regulated by G$\beta\gamma$ was PLCβ. PLCβ isoforms are dually regulated by G$\beta\gamma$ and Gα_q, where both G protein subunits increase phospholipase activity, although Gα_q has a higher binding affinity for the enzyme. The sites of G protein interaction on PLCβ have been identified at opposite ends of the protein—the binding site for Gα_q on PLCβ has been located at the C2 domain and the C terminus (Wang et al., 1999b), whereas G$\beta\gamma$ was demonstrated to interact with and activate PLCβ_2 through the PH domain in the N-terminal domain (Wang et al., 2000). PH domains bind phosphatidylinositol lipids, and are thus involved in membrane targeting. PH domains have also been well characterized as G$\beta\gamma$ interaction sites; therefore, it was little surprise that G$\beta\gamma$ interactions were mediated by a PH domain on PLCβ. Other studies have investigated the site of interactions with G$\beta\gamma$, and the Smrcka group has published a number of articles presenting evidence that G$\beta\gamma$ binds to the catalytic domain of PLCβ2 [thoroughly summarized in Smrcka (2008)]. Since there is strong evidence for G$\beta\gamma$ interaction at both sites, it is unlikely that the debate will be resolved without a co-crystal structure of PLCβ in complex with G$\beta\gamma$. There are four isoforms of PLCβ (PLCβ_1–β_4), and it was first observed that G$\beta\gamma$ could increase PLCβ activity using purified G$\beta\gamma$ with PLCβ_1 and PLCβ_2 derived from cell extracts (Camps et al., 1992). These authors also noted that the magnitude of enzyme stimulation was isoform-dependent. It was later demonstrated that the different isoforms of Gβ and Gγ had differential effects on the magnitude of PLCβ activation, depending on both G$\beta\gamma$ composition and PLCβ subtype (Boyer et al., 1994; Poon et al., 2009). However, the G$\beta\gamma$ isoform differences in PLCβ activation are not without controversy—other groups have shown no differential effect of G$\beta\gamma$ isoforms (Ueda et al., 1994).

There are a few points that must be taken into consideration when assessing the claim of functional diversity of G$\beta\gamma$ isoforms. First, the G$\beta\gamma$ isoforms that have demonstrated a functionally distinct profile are typically isoforms with limited tissue expression profiles (G$\beta_1\gamma_1$) or known to play divergent roles (Gβ_5). Thus, it is not overly surprising that they show a difference from other G$\beta\gamma$ combinations when activating PLCβ or other effector molecules. Although these considerations make it more difficult to understand functional diversity among G$\beta\gamma$ isoforms, we may have to consider their roles beyond canonical signaling to understand the need for such diversity.

Apart from PLCβ, two other subfamilies are regulated by G$\beta\gamma$—PLCε and PLCη. PLCε, originally characterized as being activated downstream of a number of GPCR-mediated pathways (Kelley et al., 2001, 2004; reviewed in Smrcka et al., 2012), was also suggested to be activated by G$\beta\gamma$ (Lopez et al., 2001). The identification of a previously unrecognized PH domain within the N terminus of PLCε led to experiments examining a regulatory role for G$\beta\gamma$. As with PLCβ, it was demonstrated that PLCε is differentially stimulated by different isoforms of Gβ and Gγ (Wing et al., 2001). Coexpression of PLCε with Gβ_1 and Gγ_1, Gγ_2, Gγ_3, or Gγ_{13} in COS-7 cells resulted in marked stimulation of PLC activity, although with varying efficiency. The rank order from most to least efficient was Gγ_2 > Gγ_3 > Gγ_{13} > Gγ_1. The authors also examined Gβ_2 and Gβ_4 with the same four Gγ subunits, and similar levels of PLCε activity as the Gβ_1-containing dimers were observed. On the other hand, reduced activity with Gβ_3-containing dimers was observed, and virtually no activity was seen in Gβ_5-containing dimers. However, in this particular case, it is important to note that Gβ_3 is poorly expressed in exogenous conditions, and the expression level of G$\beta\gamma$ isoforms was not assessed. These results once again must be considered with the same caveats as mentioned for PLCβ. PLCη is a recently identified subfamily of PLC which has also been demonstrated to be activated by G$\beta\gamma$. Two isoforms of PLCη have been identified, namely, PLCη_1 and PLCη_2. Purified PLCη_2 was activated by G$\beta\gamma$ when reconstituted with PIP$_2$-containing phospholipid vesicles. However, the site of interaction was not the PH domain of the enzyme, since a purified fragment of PLCη_2 lacking this domain was still activated by G$\beta\gamma$ (Zhou et al., 2008). PLCη isoforms are being further investigated, but have been recently implicated in neuronal Ca^{2+} signal modulation due to their Ca^{2+}-sensitivity and their specific expression in neurons (Popovics and Stewart, 2012).

E. Phosphoinositide 3 Kinases

PI3Ks are responsible for phosphorylation of phosphatidylinositols at the position 3-hydroxyl group of the inositol ring. Their primary product is phosphatidylinositol 3,4,5-trisphosphate, which is an activator of protein kinase B. Different PI3K isoforms are common downstream effectors of survival signals (Vanhaesebroeck et al., 2005; Vadas et al., 2011), and can be divided into three classes based on sequence, regulation, and substrate specificity. PI3K activation by G$\beta\gamma$ was originally identified in neutrophils and U937 cells as immunologically, pharmacologically, and chromatographically distinct from receptor tyrosine kinase (RTK)–activated PI3Ks (Stephens et al., 1994). It is now known that there are two class I PI3Ks which are activated by G$\beta\gamma$, namely, PI3Kβ and PI3Kγ. Of the two, PI3Kγ, a class IB enzyme, is the best-characterized G$\beta\gamma$-stimulated isoform (Fruman et al., 1998). PI3Kγ is a heterodimer consisting of a P110γ

catalytic subunit and a p101 regulatory subunit. It is believed that Gβγ-induced stimulation is achieved by direct binding to the heterodimer at multiple binding sites (Leopoldt et al., 1998; Voigt et al., 2005). It has been demonstrated that the p110γ subunit alone exhibits basal activity, but shows minimal stimulation in the presence of Gβγ subunits (Stephens et al., 1997). With the addition of the p101 regulatory subunit, PI3Kγ becomes significantly more responsive to Gβγ and shows increased specificity for PIP_2 (Maier et al., 1999). As with the other effectors we have discussed, PI3Ks may demonstrate Gβγ subtype specificity (Maier et al., 2000). The authors demonstrated that purified recombinant mammalian $G\beta_{1\text{-}3}\gamma_2$ could stimulate PI3K with similar potencies, whereas transducin Gβγ was less potent. They also showed that the $G\beta_1\gamma_2$ dimer could stimulate PI3K, whereas the $G\beta_5\gamma_2$ dimer could not. Interestingly, these authors also found that both of these dimers could activate $PLC\beta_1$ and $PLC\beta_2$, but only $G\beta_1\gamma_2$ could activate $PLC\beta_3$. Another study demonstrated similar results with respect to dimers containing different Gβ subunits and their ability to activate the p110γ isoform of PI3K (Kerchner et al., 2004). They also examined different combinations of Gγ and found stronger evidence for functional selectivity.

F. Mitogen-Activated Protein Kinases

MAPK pathways involve sequential activation of kinases through phosphorylation by the previous kinase in the pathway. These signaling events are activated by growth factor signals at the cell surface with their downstream effect generally being alterations in gene transcription. MAPK pathways are typically activated through ligand binding to RTKs, with the RTK phosphorylating and activating the first kinase in the pathway. It is now appreciated that GPCRs are capable of initiating the MAPK cascade through signaling via Gβγ.

The extracellular signal-regulated kinase 1/2 (ERK1/2) pathway has long been known to be stimulated by $G\alpha_i$-coupled GPCRs. This ERK1/2 activation was demonstrated to be mediated by Gβγ using multiple approaches (Crespo et al., 1994; Koch et al., 1994a), and similar studies demonstrated Gβγ activation of the c-Jun N-terminal kinase (JNK) and p38 pathways (Coso et al., 1996; Yamauchi et al., 1997). Although multiple MAPK pathways are activated by Gβγ, the mechanisms are often distinct. In particular, Gβγ subunits have been shown to initiate MAPK signaling cascades through three different mechanisms. The first involves activation of intracellular effectors such as PI3K, mediating activation of Src family tyrosine kinases, direct activation of the nonreceptor tyrosine kinases, or activation of PLCβ (Hawes et al., 1996; Luttrell et al., 1997; Barr et al., 2002). A second mechanism involves recruitment and formation of a scaffold for MAPK activation. Gβγ can recruit GRK isoforms to phosphorylate GPCRs, thus promoting recruitment of β-arrestin, which functions as an adaptor protein for Src (Luttrell et al., 1999). Gβγ can also recruit KSR-1 (kinase suppressor of Ras-1), a positive regulator of Ras-mediated signaling, identified as Gγ subunit–interacting proteins in yeast two-hybrid assays (Downward, 1995; Bell et al., 1999). The third mechanism involves transactivation of an RTK, activating a classic MAPK signaling pathway (Della Rocca et al., 1999). This has been shown for D4 dopamine receptor transactivation of platelet-derived growth factor β receptors in CA1 pyramidal neurons, where activation of $G\alpha_i$ leads to autophosphorylation of residues on the platelet-derived growth factor β receptor [mediated by Gβγ since βARK-ct (β-adrenergic receptor kinase – carboxy terminal domain; GRK2-ct) blocked transactivation] and downstream ERK1/2 activation (Kotecha et al., 2002). The reason for so many different mechanisms of Gβγ-mediated MAPK activation may reflect cell-type-, GPCR-, and G protein–specific requirements for each pathway (Rozengurt, 1998).

IV. Noncanonical Effectors of Gβγ Signaling

In addition to the canonical effectors discussed earlier, recent studies have identified a number of new effectors for Gβγ. Some of these effector-mediated events may be independent of the receptor per se [for review, see Dupré et al. (2009)], and a number of these events occur at subcellular sites distinct from and not exclusively at the plasma membrane (Fig. 4). Recent reports suggest that a subpopulation of mammalian Gγ subunits may escape prenylation and thus remain soluble (Cook et al., 2006; Kilpatrick and Hildebrandt, 2007). If this observation pans out, new locations for Gβγ signaling may be imagined. These new effectors greatly expand the scope of Gβγ action in cells, with respect to both interacting proteins and subcellular localization, suggesting its roles in the cell are more complicated than previously envisioned.

A. Gβγ Effects on Cell Division and the Cytoskeleton

Among the myriad of cytoskeletal structural elements, the microtubule network plays roles in many different cellular processes [reviewed in Dave et al. (2009) and Gundersen and Cook (1999)], including cell division, vesicle release, and signal transduction (Dong et al., 2000). Microtubules form a cylinder of heterodimeric tubulin subunits (α and β) that can bind GTP. GTP binding polarizes the microtubule with a plus end, formed by tubulin GTP, and it has been established that this GTP can be transferred to Gα subunits of heterotrimeric G proteins, leading to their activation (Wang et al., 1990). Other studies have demonstrated that heterotrimeric G proteins, in turn, can interact with microtubules and regulate their assembly in vitro. Gα activates tubulin GTPase (Roychowdhury et al., 1999) by interaction at the exchangeable nucleotide-binding site of tubulin (Layden et al., 2008) and increases microtubule turnover, whereas G$\beta\gamma$ subunits promote polymerization and stability of microtubules (Roychowdhury and Rasenick, 1997; Popova and Rasenick, 2003; Roychowdhury et al., 2006). Studies have also shown that, in response to agonist stimulation, G$\beta_1\gamma_1$ could interact with tubulin GDP, preventing membrane association of tubulin and resulting in accumulation of G$\beta\gamma$/tubulin complexes in the cytosol of neuroblastoma cells (Popova and Rasenick, 2003). Thus, it is clear that Gα and G$\beta\gamma$ subunits have opposite effects on microtubule dynamics, and that G$\beta\gamma$ interacts directly with the cytoskeleton, playing a key role in cellular dynamics.

Previous work has found that a defect in G$\beta\gamma$ signaling leads to alterations in cell migration, which requires reorganization of the actin-cytoskeleton network (Peracino et al., 1998; Hwang et al., 2005; Xu et al., 2012). Upon GPCR activation by chemokines, G protein heterotrimers are believed to dissociate and modulate many signaling pathways that control cell migration. In *Dictyostelium*, a G$\beta\gamma$ effector, ElmoE, was shown to relay signals from chemokine receptors to actin polymerization at the leading edge of cells via the activation of small G protein RacB (Yan et al., 2012). There is also evidence that, concerning canonical effectors, there is a specificity associated with G$\beta\gamma$ subunits with noncanonical effectors as well. For example, Gγ_{12} was found to be associated with F-actin in Swiss 3T3 cells and C6 glioma cells. In contrast, Gγ_5 seems to be associated with vinculin in the context of focal adhesions (Ueda et al., 1997) rather than directly associating with actin (Hansen et al., 1994).

*B. G*β*γ Signaling in Cellular Organelles*

1. Endosomal Signaling. Studies regarding the role of G proteins in endosomes demonstrated Gα signaling in pheromone responses in yeast (Slessareva and Dohlman, 2006) as well as localization of Gα to endosomes in mammalian cells (Zheng et al., 2004). More recently, evidence has been presented that G$\beta_1\gamma_2$ interacts with Rab11 and is associated with recycling endosomes following activation of the lysophosphatidic acid receptor in human cells (Garcia-Regalado et al., 2008). In this context, G$\beta\gamma$ recruits PI3Kγ and promotes Akt activation, fostering cell survival and proliferation. Finally, G$\beta_1\gamma_7$ was shown to be associated with recycling endosomes following β_2-adrenergic receptor stimulation (Hynes et al., 2004). These data support a role of G$\beta\gamma$ in intracellular trafficking, although the mechanisms are not clear at present.

2. Mitochondria. Among organelles, there is relatively little information regarding G proteins in mitochondria. Some studies have demonstrated that Gα_{12} and Gα_{i1} subunits are associated with mitochondria (Lyssand and Bajjalieh, 2007; Andreeva et al., 2008). It was also noted that Gβ_2 was localized to the mitochondrial outer membrane (Zhang et al., 2010). These authors also showed that Gβ_2 could directly interact with the mitochondrial GTPase protein mitofusin1 via its WD40 repeat domains. This interaction induced mitochondrial aggregation in HeLa cells. Although this effect seems to be independent of GPCR activation, an interesting finding in itself, further investigation would be required to understand the role of G proteins in mitochondrial dynamics.

3. Endoplasmic Reticulum. A number of studies have shown that G$\beta\gamma$ subunits are associated with the ER, and that they associate with many of the other components of GPCR signaling in this compartment. For example, apart from its role in regulating Kir3 channel activation at the plasma membrane, G$\beta\gamma$ has been proposed to play a potential role in channel trafficking during early biosynthesis. Evidence supporting this notion stems from the observation that Kir3 channels interact with G$\beta\gamma$ while the channel is still in the endoplasmic reticulum (Rebois et al., 2006). The interactions were demonstrated to persist throughout anterograde protein trafficking, and were dependent on the particular subtypes of Gβ and Gγ (Robitaille et al., 2009b). Whether these events depend on G$\beta\gamma$ dimers independently of Gα remains an open question, but it is clear that G$\beta\gamma$ interacts with receptors and effectors before Gα becomes a part of these complexes in the ER (Dupré et al., 2006, 2007a).

As with Kir3 channels, G$\beta\gamma$ has been shown to form stable complexes with both GPCRs and AC during early biosynthesis in the ER (Rebois et al., 2006) (Fig. 6). AC2 was demonstrated to form complexes with both β_2AR and G$\beta\gamma$ subunits in the presence of dominant negative mutant forms of small GTPases involved in anterograde trafficking, before interactions with Gα were observed (Dupré et al., 2006, 2007a). This would suggest an early role for G$\beta\gamma$ subunits in contacting receptors and effector molecules, with Gα incorporation occurring at a later time point. Alternatively, Gα interactions might simply be more labile until later stages of signaling complex trafficking. It remains to be determined what, beyond facilitating these interactions, G$\beta\gamma$ and individual combinations of G$\beta\gamma$ are doing with respect to the organization and assembly of cellular signaling complexes.

In the case of Gα_q-mediated GPCR signaling, calcium release from the ER results from activation of PLCβ leading to IP$_3$ binding on its receptor localized on the ER [reviewed in Berridge (2007)]. Surprisingly, some evidence suggests that G$\beta\gamma$ subunits are able to mediate ER calcium release independently of PLCβ activation by binding directly to the IP$_3$ receptor (Zeng et al., 2003). This interaction provides another means

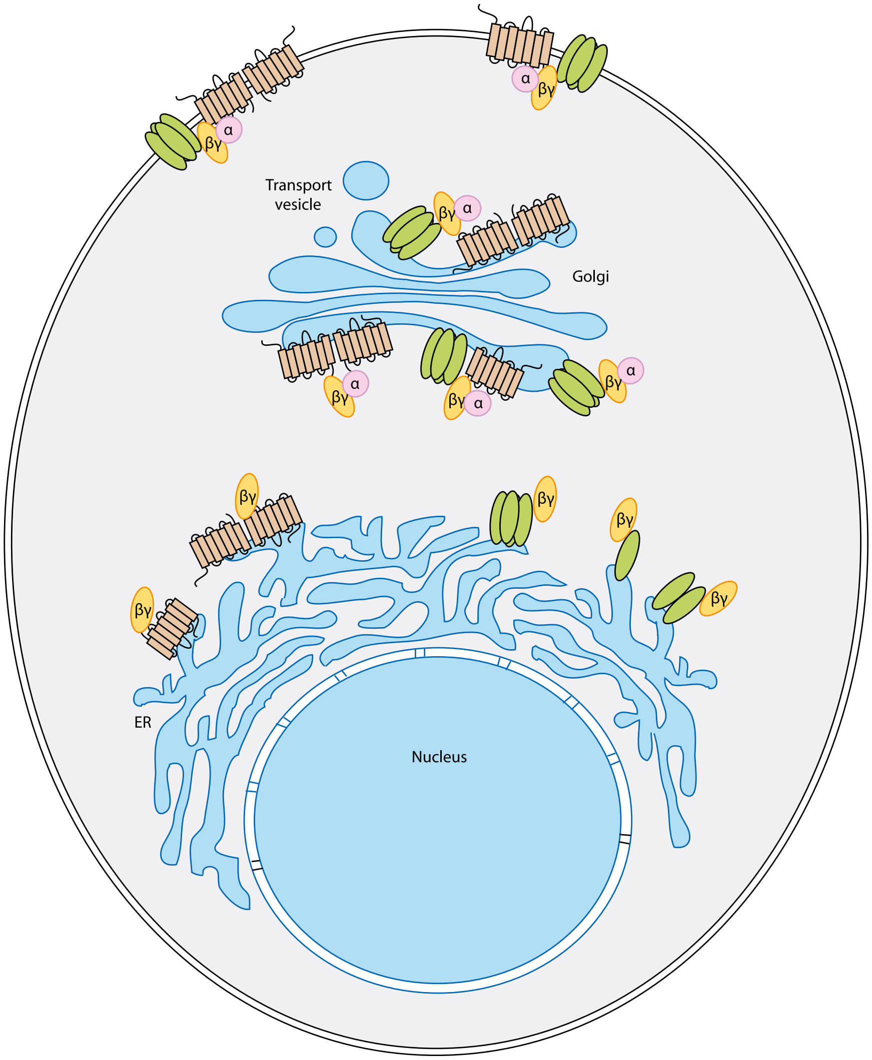

Fig. 6. Putative role of $G\beta\gamma$ subunits in the assembly of GPCR signaling complexes. Data described in the text show that GPCRs and effectors initially associate with $G\beta\gamma$ subunits in the ER. $G\alpha$ subunits join these complexes during transit from the ER to the Golgi apparatus. It is not known yet known whether the initial $G\beta\gamma$ interaction in the ER occurs with channel monomers in the case of Kir3, but some evidence suggests that GPCR dimerization is facilitated by $G\beta\gamma$ in the ER (Dupré et al., 2009).

of direct $G\beta\gamma$-mediated signaling, upon G_i-coupled receptor activation, although the mechanism is unknown at present. As these two mechanisms do not seem to be mutually exclusive, $G\beta\gamma$ could be involved in the local release of calcium near the plasma membrane, or we might also consider a receptor-independent mechanism for ER-resident $G\beta\gamma$ subunits. Alternatively, the two pathways may be mutually inhibitory.

As in mammalian cells, the ER in plants is involved in physiologic processes such as PLC activation and IP_3 production (Andersson et al., 2006). Two studies of G protein signaling in *Arabidopsis* revealed that $G\beta$ subunits that form a stable complex with $G\gamma$ are associated with ER membranes and are involved in the unfolded protein response following tunicamycin treatment (Weiss et al., 1997; Wang et al., 2007). In the absence of functional $G\beta$ subunits, resistance to tunicamycin was increased. Interestingly, $G\beta\gamma$, but not $G\alpha$, subunits are degraded during unfolded protein response, hinting again at potential mutual independence of their effects.

4. Golgi Apparatus. $G\beta\gamma$ dimers have also been functionally associated with the Golgi apparatus, where they may play a role in regulating anterograde protein trafficking. Initial studies have shown that activation of protein kinase D (PKD), a resident protein kinase in the Golgi membrane, by $G\beta\gamma$ is involved in regulation of anterograde trafficking from the trans-Golgi network (TGN) (Jamora et al., 1997, 1999). "Free" $G\beta\gamma$ added to digitonin-permeabilized mammalian cells caused a disruption of Golgi stacks in a $G\alpha$-GTP-independent manner (Jamora et al., 1997). Further, the re-formation of the heterotrimeric G protein inhibited $G\beta\gamma$-mediated Golgi vesiculation. In more recent work, the authors proposed that $G\beta_1\gamma_2$ and $G\beta_3\gamma_2$ heterodimers activate PKD, again in a PKCη-dependent manner, leading to Golgi fragmentation (Diaz Anel and Malhotra, 2005). The authors suggested that G$\beta\gamma$ in the TGN activates PLCβ_3 (Diaz Anel, 2007). PLCβ_3 then activates PKCη, which subsequently activates TGN-bound PKD. Recent work supporting this model comes from studies of vesicle formation at the Golgi membrane by endogenous G$\beta\gamma$ subunits (Irannejad and Wedegaertner, 2010). It was shown that PKD is recruited to the Golgi by G$\beta_1\gamma_2$, and PKD and PLC are required for Golgi fragmentation. Moreover, the sequestration of G$\beta\gamma$ at the Golgi membrane led to Golgi tubulation as a consequence of blocked fission.

Interestingly, GPCR activation at the Golgi membrane is suspected, but has never been convincingly demonstrated, suggesting that these events may ultimately be receptor-independent at this level. Thus, the source of G$\beta\gamma$ in the Golgi (or for that matter, the ER) is unclear. However, an examination of the spatiotemporal dynamics of G$\beta\gamma$ subunits revealed that they shuttle between the plasma membrane and intracellular membranes, a predominant destination being the Golgi apparatus (Chisari et al., 2007). Work from the same group suggested that all G$\beta\gamma$ complexes can translocate from the plasma membrane to the Golgi apparatus upon M3-muscarinic receptor activation (Saini et al., 2010; Karunarathne et al., 2012). This translocation led to vesicle formation and changes in the secretory pathway. The authors also showed recently that upregulation of Gγ_{11} subunits in senescent cells resulted in alterations in Golgi-mediated secretion (Cho et al., 2011). Finally, using a yeast two-hybrid approach, it was shown that G$\beta\gamma$ interacts with the N terminus of RKTG (Raf kinase trapping to Golgi apparatus). This interaction led to the sequestration of G$\beta\gamma$ in the Golgi apparatus (Jiang et al., 2010).

C. G$\beta\gamma$ Effects in the Nucleus and Regulation of Transcriptional Activity

Recently, several studies have indicated a more direct nuclear impact for G$\beta\gamma$ subunits. For example, Gβ_5 subunits interact with a number of RGS proteins, and one RGS class, the R7 subfamily, is enriched in brain and functions in a stable RGS-Gβ_5 complex, localized to both the cytosol and the nucleus (Zhang et al., 2001). A protein known as RGS7-binding protein (R7BP) interacts with the RGS7-Gβ_5 complex and is known to modulate its capacity to regulate Kir3 channels (Drenan et al., 2005). R7BP is normally palmitoylated, which anchors RGS7-Gβ_5 at the plasma membrane. However, palmitoylation is a transient and tightly regulated process (Smotrys and Linder, 2004), and loss of the palmitate moiety on R7BP releases the R7BP-RGS7-Gβ_5 complex from the plasma membrane, allowing it to translocate to the nucleus. Other RGS proteins localized to the nucleus include RGS6, which can regulate transcription in mammalian cells (Liu and Fisher, 2004). The precise role of these proteins in nuclear function remains elusive, as do the requirements for interaction with Gβ_5, and may be part of a mechanism for relaying neurotransmitter signals from receptors at the cell surface directly to the nucleus [for review, see Hepler (2005)]. Interestingly, Gβ_5 mutants that cannot form a complex with RGS7 were not localized to nuclei of either human embryonic kidney 293 (HEK293) or PC12 cells, highlighting the importance of the RGS protein in nuclear localization of Gβ_5 (Rojkova et al., 2003).

As discussed earlier, Gβ_5 is distinct from the other four Gβ subunits. G$\beta\gamma$ subunits containing these other Gβ isoforms can interact with a number of canonical transcription factors. G$\beta_1\gamma_2$ dimers can interact directly with histone deacetylase 5 (HDAC5) and possibly other HDAC isoforms as well (Spiegelberg and Hamm, 2005). In the basal state, HDAC5 interacts with the muscle differentiation factor myocyte enhancer factor 2 (MEF2), resulting in reduced transcriptional activity at MEF2-sensitive promoters. Following stimulation of the α_{2A}-adrenergic receptor at the cell surface, activated G$\beta\gamma$ dimers interacted with HDAC5, disrupting the interaction with the transcription factor MEF2, allowing it to stimulate transcription. Both the G$\alpha_{i/o}$ inhibitor PTX (pertussis toxin) and the G$\beta\gamma$ scavenger βARK-ct (GRK-ct) inhibited MEF2 activity, suggesting a specific G protein heterotrimer was involved (Spiegelberg and Hamm, 2005). Evidence that G protein heterotrimers and even GPCRs are also present in the nucleus [reviewed in Crouch (1991), Tadevosyan et al. (2012), and Vaniotis et al. (2011)] suggests that the signaling pathways concerned might be quite complex, involving cross-talk between surface and nuclear membrane—localized GPCRs, their associated G proteins, and potentially other pools of “free” G proteins. It remains uncertain as to whether cytoplasmic G$\beta\gamma$ dimers sequester HDAC or if these events occur exclusively in the nucleus.

G$\beta\gamma$ subunits also interact with the transcriptional repressor known as the adipocyte enhancer-binding protein (AEBP1) (Park et al., 1999). AEBP1 specifically forms a complex with G$\beta\gamma$ subunits containing Gγ_5 in nuclei of 3T3-L1 cells, but interestingly not NIH 3T3 cells. The G$\beta\gamma_5$/AEBP1 interaction attenuates its transcriptional repression activity, analogous to the role G$\beta\gamma$ plays in attenuating HDAC-mediated gene repression. Another G$\beta\gamma$ effector is the glucocorticoid receptor (GR), which is localized in the cytoplasm and translocates to the nucleus in response to ligand binding, where several target genes are transcriptionally regulated. Both Gβ_1 and Gβ_2 subunits directly interact with the GR and translocate with it to the nucleus following treatment with the agonist dexamethasone (Kino et al., 2005a,b). Once again, the interaction of G$\beta\gamma$ with GR suppresses transcriptional activity most likely by associating with transcriptional complexes formed on GR-responsive promoters. Gβ_2

mutants unable to associate with Gγ subunits cannot suppress GR transcriptional activity.

In contrast to the relief of transcriptional repression described earlier, we demonstrated that Gβγ decreased phorbol 12-myristate 13-acetate (PMA)–stimulated activating protein-1 (AP-1) gene reporter activity in different cell lines (Robitaille et al., 2010). We identified the Fos transcription factors as the relevant interactors of Gβγ subunits in this case. Gβγ did not interfere with dimerization of Fos and Jun or the ability of AP-1 complexes to bind DNA per se. Rather, Gβγ colocalized with the AP-1 complex in the nucleus and recruited HDACs to inhibit AP-1 transcriptional activity as determined using chromatin immunoprecipitation. All five Gβ subunits inhibited AP-1 activity in reporter assays, suggesting that this is a common feature of Gβγ signaling (Robitaille et al., 2010). These results differ from the effect on MEF2-regulated transcriptional events, and highlight the different ways that Gβγ subunits might interact with distinct transcriptional complexes (Spiegelberg and Hamm, 2005).

In fact, under basal conditions, we noted the presence of Gβγ subunits in the nuclei of many cell types, including cardiomyocytes, suggesting they may be resident there (Robitaille et al., 2010). These observations suggest that Gβγ subunits may be more general transcriptional regulators. It is known that cFos transcription is activated by several GPCRs. M2-muscarinic receptor stimulation leads to activation of the cFos promoter, an event that is mediated through Gβγ in addition to being dependent on ERK and JNK (Sun et al., 1999). On one hand, activation of heterotrimeric G proteins leads to activation of cFos transcription, whereas on the other hand, subsequent interaction of Gβγ with AP-1 proteins decreases transcriptional activity, providing, in effect, a negative feedback loop. STAT3 (signal transducer and activator of transcription 3) is another transcription factor that may be a target for dual GPCR and Gβγ regulation (Yuen et al., 2010). The source of Gβγ for these two classes of events may in fact be different, i.e., the formation of Gβγ/transcription factor complexes may not necessarily be receptor-dependent. These proteins may interact directly following their biosynthesis, or there may be a pool (as suggested earlier) of “free” Gβγ in the cell. Intriguingly, modulation of prenylation status has been shown to increase the amount of Gβγ in the nucleus associated with the GR, lending further support to this notion (Kino et al., 2005a,b).

V. Other Effectors

Although we will not discuss them in detail, a number of other canonical and noncanonical Gβγ effectors have been identified, including GRK isoforms (Lodowski et al., 2003a,b), phosducin (Bauer et al., 1998), TRPM1 (transient receptor potential cation channel subfamily M member 1) channels (Shen et al., 2012), $Na_v1.8$ voltage-gated sodium channels (Belkouch et al., 2011), the WD40 repeat protein WDR26 (WD repeat-containing protein 26) (Sun et al., 2011), Radil (Ahmed et al., 2010), ElmoE (Yan et al., 2012), A-type potassium channels (Zhang et al., 2012), and glycine receptors (Yevenes et al., 2006). This list is likely to expand further.

VI. Phenotypes Associated with Knockout and Knockdown of Gβ and Gγ Subunits

Gβγ dimers must, by definition, serve the entire G protein–coupled receptor population, and each receptor system can generate a unique signaling profile depending on the cellular population of G proteins. As discussed earlier, there are 5 Gβ and 12 Gγ subunits in mammals, raising the question of how this diversity manifests with respect to GPCR signaling and function, as well as putative receptor-independent effects. It has been shown that Gβ and Gγ subunits exhibit preferential dimerization patterns among themselves (Lee et al., 1995; Poon et al., 2009; Yan et al., 1996), implicating that a certain level of specificity must be conferred by these dimers onto the “personality” of the complex in terms of identity, formation/assembly, and overall signaling phenotype. Extensive research has been conducted to elucidate the contribution of the different Gα subunits subtypes in GPCR signal transduction. However, much work remains to be done to fully explain the role of specific Gβ and Gγ subunits, and subsequent dimers, in signal transduction. Numerous investigators have used various gene and RNA silencing methods, including shRNA, siRNA, and antisense oligonucleotide approaches, to study the roles of specific Gβγ combinations in GPCR signaling events at the cellular level, in embryonic development, and in behavioral studies. In this section, we review our understanding of Gβ and Gγ subunit diversity uncovered by means of RNA interference and gene deletion strategies.

A. Gβ Subunit Knockout and Knockdown Models

1. $G\beta_{1\text{-}4}$. To date, of the four similar Gβ subunits, only $G\beta_1$ has been knocked out in mice. Interestingly, 40% of $G\beta_1{}^{-/-}$ mice exhibit neural tube defects resulting in embryonic lethality, whereas the knockout mice that do not exhibit this phenotype develop microencephaly and die perinatally (Okae and Iwakura, 2010). Additional defects in $G\beta_1{}^{-/-}$ mice without neural tube defects include abnormal suckling behavior and respiratory defects. Analysis of the brains of these knockout mice revealed a normal morphology of the brain and anterior-posterior length of the cerebral cortex, but interestingly, a reduced cortical thickness and reduced brain volume associated with severe brain malformations was also observed. In addition, abnormal morphologic changes in neural progenitor cells induced by sphingosine-1-phosphate, a known regulator of cell contraction via G$\alpha_{12/13}$-dependent Rho activation (Van Leeuwen et al., 2003; Hurst et al., 2008), was also noted. ERK phosphorylation induced by endothelin-1 and sphingosine-1-phosphate was reduced in G$\beta_1{}^{-/-}$ mice; however, JNK phosphorylation induced by LPA (lysophosphatidic acid) and endothelin-1 was not affected, indicating impaired neural progenitor cell proliferation in these knockout mice. These findings imply that Gβ_1 is critical for embryonic neurogenesis, and that it mediates specific signaling pathways that are not compensated by structurally similar Gβ subunits, Gβ_2, Gβ_3, and Gβ_4. The expression patterns of these other Gβ subunits must be examined in detail before concluding that they cannot, in principle, compensate for Gβ_1. If they are expressed in similar cells during embryonic development but cannot compensate for the loss of Gβ_1, this would imply significant compartmentalization of Gβ_1 signaling, perhaps through specific associations with particular Gγ subunits, or other specific interactors that regulate subcellular localization and function. Conditional, tissue-specific knockouts would be particularly useful, given the embryonic lethality of the global knockout.

The first use of antisense oligonucleotide technology [reviewed in Dias et al. (2002)] to study Gβ functional activity revealed that, by concurrently reducing Gβ_1 and Gβ_2, and to a lesser extent Gβ_3 expression, the inhibition of Ca^{2+} currents mediated by somatostatin and carbachol was abolished in rate pituitary GH3 cells (Kleuss et al., 1992). A more selective approach later revealed that, in fact, these phenotypes were due to suppression of individual Gβ subunits—injection of antisense oligonucleotides specifically targeting Gβ_1 abolished the somatostatin-induced inhibition of Ca^{2+} currents, whereas those targeting Gβ_3 eliminated the Ca^{2+} currents inhibited by carbachol (Kleuss et al., 1992). This suggests that Gβ_1 and Gβ_3 mediate the activity of somatostatin and muscarinic receptors, respectively. During the characterization of Gβ subunits involved in the G$_o$-coupled galanin receptor, antisense oligonucleotide studies showed that knockdown of both Gβ_2 and Gβ_3 in RINm5F (rat insulinoma cell line) cells reduced galanin-induced inhibition of Ba^{2+} currents, with the reduction in inhibition being greater for Gβ_2 silencing than Gβ_3 (Kalkbrenner et al., 1995). In a separate study aimed at identifying the subunit composition of the G protein coupled to M1-muscarinic acetylcholine receptors, it was shown using antisense oligonucleotides in rat basophilic leukemia RBL-2H3-hm1 (rat basophilic leukemia cell line) cells, that the loss of Gβ_1 and Gβ_4 reduced carbachol-mediated Ca^{2+} release (Dippel et al., 1996). Gβ_2 and Gβ_4 knockdown with siRNA demonstrated that these two Gβ subunits mediate coupling of adrenergic and nociceptin receptors to voltage-gated calcium channels in stellate ganglion neurons (Mahmoud et al., 2012).

More recently, using lentiviral-shRNA approaches in J774A.1 and RAW264.7 mouse macrophages, it was shown that knockdown of Gβ_2 decreased C5a-receptor-mediated Ca^{2+} release, whereas only concurrent knockdown of both Gβ_1 and Gβ_2 abolished P2Y-receptor-mediated Ca^{2+} release and β_2-adrenergic receptor-mediated cAMP production, providing insight into distinct Gβ specificities for the P2Y receptor and β_2-adrenergic receptor (Hwang et al., 2005; Shin et al., 2006). In addition, a recent study demonstrated that siRNA-mediated knockdown of Gβ_1 in HeLa cells caused a 70% reduction in prostaglandin E2-dependent cAMP synthesis through EP2 (prostaglandin E2 receptor) or EP4 (prostaglandin E4 receptor) receptors, that individual and concurrent knockdown of Gβ_1 and Gβ_2 reduced isoproterenol-stimulated or UK-14,304-mediated inhibition of cAMP formation, and that concurrent knockdown of G$\beta_{1/2}$ reduced prostaglandin E2- and isoproterenol-stimulated or UK-14,304-mediated inhibition of cAMP production (Krumins and Gilman, 2006). Other functional consequences of simultaneous knockdown included a loss of the ability of C5a to increase actin polymerization, as well as impaired C5a-induced migration of the aforementioned RAW264.7 cells (Hwang et al., 2005). It was also observed that knockdown of Gβ_1 reduced expression of Gγ_5, knockdown of Gβ_2 reduced expression of Gγ_5 and Gγ_{12}, and that simultaneous knockdown of both Gβ_1 and Gβ_2 reduced expression of all three of these Gγ subunits. A thorough investigation of the effects of knockdown of different Gβ subunits on expression of other Gβ subunits has also been performed, revealing that knockdown of Gβ_1 resulted in an increase in Gβ_4 expression levels, and vice versa (Krumins and Gilman, 2006). These latter effects suggest that either compensatory systems exists in cells where these key signaling proteins are knocked down or, alternatively, that G$\beta\gamma$ subunits play a broader role (discussed earlier), perhaps in regulating transcriptional events in the cell.

2. Gβ_5. As mentioned previously, Gβ_5 displays lower sequence similarity to the other Gβ subunits than G$\beta_{1\text{-}4}$ (Table 1). Mammalian Gβ_5 exists as two isoforms, a long splice variant (Gβ_5-L) and a short splice variant (Gβ_5-S), the former being expressed exclusively in retinal photoreceptor cells. Gβ_5 is expressed primarily in brain and neuronal tissues and, unlike the other Gβ subunits, is known to interact not only with Gγ but also with the GGL domain containing the RGS R7 family of proteins (Watson et al., 1994, 1996). Given the divergence from the rest of the Gβ subunits, Gβ_5 signaling has been an active area of study, and extensive knockout studies have been carried out to understand its roles.

In retinal photoreceptor cells, Gβ_5-L has been shown to interact with RGS9-1, and is more abundant in cones (Cowan et al., 1998; Zhang et al., 2003). Knockout of RGS9 in mice results in reduced Gβ_5-L protein expression, although Gβ_5-L mRNA levels remain normal, leading to the notion that RGS9 is required for the maintenance of normal levels of Gβ_5-L protein in vivo (Chen et al., 2000b). Conversely, the retina and striatum of G$\beta_5^{-/-}$ mice exhibit abrogated levels of GGL domain-containing RGS proteins, while their respective mRNA levels remain unchanged. Interestingly, levels of Gγ_2 remained unchanged following knockout of Gβ_5, which is perhaps not surprising given their relatively low affinity for one another (Chen et al., 2003). This suggests that the levels of Gβ_5 and RGS proteins are not regulated by the level of their respective transcripts, but instead confer stability upon each other at the protein level. In addition, the finding that it is possible to generate a G$\beta_5^{-/-}$ mice line suggests that, unlike Gβ_1, Gβ_5 is perhaps not an absolute requirement for embryonic neurogenesis or early development (Chen et al., 2003). Loss of Gβ_5 also leads to increased canonical signaling through G$_s$-coupled receptors, as the absence of a Gβ_5/RGS9 complex removes a negative regulator of G$\beta\gamma$ signaling to AC5 (Xie et al., 2012).

Termination of light responses in retinal rods requires GTP hydrolysis by transducin, which is composed of Gα_t, Gβ_5-L, and RGS9-1. Loss of Gβ_5-L did not alter the activation of its cognate G protein cascade, but rather slowed its deactivation and altered the rate of incremental dim flashes during light adaptation, implying that Gβ_5-L is essential for normal G protein deactivation and rod function (Krispel et al., 2003). With regard to the Gβ_5-S isoform, it was shown that Gβ_5-S and RGS11 colocalize with Gα_o at the tips of ON (as opposed to OFF)-bipolar cell dendrites, and morphologic analysis of rod bipolar cells revealed that the retinal outer plexiform layer (OPL) of G$\beta_5^{-/-}$ mice was disorganized with shorter dendrites (Rao et al., 2007). A decrease in the number of synaptic triads in the OPL in these mice was also observed, suggesting a role for Gβ_5-S in OPL synaptic development.

Phenotypically, G$\beta_5^{-/-}$ pups were found to have smaller body size, demonstrated by an increase of up to 80% in normal body weight within two months of birth that remained significantly smaller than wild-type mice throughout their lifetimes (Chen et al., 2003; Wang et al., 2011). These mice also showed a 66% mortality rate before weaning (Chen et al., 2003). Surprisingly, analysis of Gβ_5 heterozygous mice showed that, instead of exhibiting partial reduction in body weight, these animals became heavier than wild-type mice and displayed higher adiposity, exhibiting larger adipocytes. Upon comparison of the effects of a high-fat diet on wild-type, heterozygous, and knockout mice, it was observed that Gβ_5 knockout mice were resistant to a high-fat diet and gained less weight on this diet. Interestingly, comparison of heterozygous mice to wild-type mice revealed that the former showed a 2-fold higher rate of body mass increase than the latter (Wang et al., 2011). Reduction of body weight in knockout mice was also associated with a 5-fold increase in locomotor activity and overall hyperactivity, whereas heterozygous mice displayed a 2-fold increase in comparison with wild-type mice. In addition, since alterations of adiposity are often associated with changes in glucose and lipid metabolism, serum levels of glucose, insulin, leptin, adiponectin, triglycerides, and free fatty acids were assessed. Heterozygous mice displayed a slight increase in leptin levels compared with wild-type mice. In knockout mice, concentrations of triglycerides, free fatty acids, and glucose were decreased, whereas concentrations of insulin were increased, and these mice also had impaired glucose clearance (Wang et al., 2011). These phenotypes suggest that heterozygous mice show characteristics reminiscent of obesity in humans, which in turn is associated with type 2 diabetes and metabolic syndrome. Thus, Gβ_5 may play a role in the progression of these disease phenotypes. In addition, in regions outside the retina, knockout of Gβ_5 leads to impaired neurobehavioral development as knockout mice displayed tiptoe walking with motor learning and coordination deficiencies (Zhang et al., 2011). These mice also exhibited impaired neuronal development in the cerebellum and hippocampus. Interestingly, it was also noted that loss of Gβ_5 led to the dysregulation of multiple genes in the brains of these mice—expression of 150 genes in the cerebellum and 228 genes from noncerebellar regions was altered (Zhang et al., 2011). Based on these findings, it might be speculated that alterations in the overall development of G$\beta_5^{-/-}$ mice may, in part, be a result of the subsequent changes in gene expression due to the loss of Gβ_5. These changes might also point to a direct role for Gβ_5 and other G$\beta\gamma$ subunits in transcriptional regulation.

B. Gγ Subunit Knockdown and Knockout Models

1. Gγ_1. Although Gγ_1 displays a broad tissue expression pattern (it has been detected in the placenta, muscle, liver, kidney, pineal gland, and uterus), the majority of research on this subunit has been focused on its roles in the eye. Indeed, Gγ_1 was initially identified as a member of the transducin heterotrimer in rods (Peng et al., 1992; Scherer et al., 1996; Arshavsky et al., 2002). It was demonstrated that the knockout of Gγ_1 in mice causes significant downregulation of both Gα_t and Gβ_1 protein expression in the retina, Gβ_1 being downregulated the most in the rods (Lobanova et al., 2008). Surprisingly, as seen with Gβ_5 and RGS9-1, knockout of Gγ_1 did not alter mRNA expression in rods, suggesting post-translational alterations in overall transducin protein stability. Compensatory increases in other Gβ or Gγ subunits were not observed. This further strengthens the notion of high-level specificity in the composition of particular heterotrimeric G proteins, and that subunits in each complex individually stabilize the entire complex. It was observed that, in Gγ_1 knockout mice, Gα_t and Gβ_1 were distributed throughout the length of the dark- and light-adapted rods; however, light-dependent translocation of these subunits from outer to inner rod segments was diminished. In addition, the absence of Gγ_1 led to photoreceptor degeneration. However, despite the reduced expression of heterotrimeric transducin, the small amount remaining was still able to support light signaling with decreased sensitivity.

2. Gγ_2. Gγ_2 has been a common choice for a great variety of overexpression studies aimed at examining GPCR and G protein function—as is common knowledge, the eponymous G$\beta\gamma$ designation usually applies to the G$\beta_1\gamma_2$ pair. However, there are a limited number of gene-silencing studies directed at elucidating its specific function. Gγ_2 exhibits a ubiquitous tissue expression pattern, and has been shown to be the most abundant Gγ subunit in the brain (Betty et al., 1998; Wettschureck and Offermanns, 2005). Using antisense oligonucleotides to silence Gγ_2, a reduction in galanin-induced inhibition of voltage-gated Ca^{2+} channels was observed in rat pituitary-derived GH cells and RIN5mF rat insulinoma cells, suggesting a heterotrimeric G protein composed of G$\alpha_o\beta_2\gamma_2$ was coupled to galanin receptors (Kalkbrenner et al., 1995). Interestingly, an even more pronounced reduction of inhibition of the channel was seen when both Gγ_2 and Gγ_4 were silenced, suggesting a role for Gγ_4 in galanin receptor G protein coupling as well.

Gγ_2 has also been implicated in nociception. Injection of antisense oligonucleotides against Gγ_2 into the right lateral ventricle of mice followed by administration of morphine, the δ-opioid receptor agonist DPDPE ([D-Pen2,D-Pen5]-Enkephalin), and the non-opioid receptor agonists WIN 55212-2 ((R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-napthalenylmethanone; CB1 cannabinoid receptor) and clonidine (α_2-adrenoreceptor) all showed a marked decrease in analgesia compared with control mice (Hosohata et al., 2000; Varga et al., 2005).

Gγ_2 has also been implicated in angiogenesis during embryonic development. Given the fact that their developing embryos are transparent (Zon and Peterson, 2005), and that their G protein subunits share high sequence homology with humans, zebrafish provide an excellent model to study the role of G proteins in angiogenesis, and embryonic development in general. It was shown that the Gγ_2 transcript can be detected in the central nervous system as early as 18 hours postfertilization, and that between 18 and 24 hours postfertilization, it is detectable in axial vasculature, including the dorsal aorta from which intersomitic vessels sprout (Leung et al., 2006). Using morpholino antisense oligonucleotides, it was found that, 2.5 days postfertilization, 87% of *gng2*-silenced animals displayed abnormal angiogenesis, exhibiting reduced or abolished formation of intersomitic vessels, and RNA rescue experiments restored normal angiogenesis in 58% of fish studied (Leung et al., 2006). In addition, loss of Gγ_2 in zebrafish reduced levels of vascular endothelial growth factor–mediated phosphorylation of PLCγ_1 and Akt, suggesting a mechanism by which Gγ_2 regulates angiogenesis by acting on downstream signaling components of the vascular endothelial growth factor signaling pathway in vivo (Leung et al., 2006). Interestingly, Gγ_2 levels are decreased in malignant melanomas, although the cause or consequences of this remain unknown (Yajima et al., 2012).

3. Gγ_3. In a study which was perhaps the first attempt to silence Gγ to study its function and selectivity, it was found that antisense-mediated knockdown of Gγ_3 led to the somatostatin-induced (but not carbachol-induced) inhibition of Ca^{2+} influx via voltage-gated calcium channels in rat pituitary GH3 cells. This provided the first line of evidence that Gγ subunits also contribute to selective receptor effector coupling (Kleuss et al., 1993). Predominantly expressed in the brain (Cali et al., 1992), Gγ_3 knockout in mice has been linked to increased susceptibility to seizures, reduced body weight, decreased adiposity, and resistance to a high-fat diet (Schwindinger et al., 2004, 2009). These findings suggest a role for Gγ_3 in neuronal excitability and regulation of appetite and/or metabolism. Loss of Gγ_3 leads to significant reductions in the levels of Gβ_2 in the striatum, cortex, and cerebellum of mice; a decrease in expression of Gβ_1 in the cerebellum; and slight decreases in Gα_{i3} in the cortex, hippocampus, and cerebellum (Schwindinger et al., 2004). These coordinate reductions in G$\alpha_{i3,}$ Gβ_1, and Gβ_2 in similar brain regions suggest that Gγ_3 stabilizes a heterotrimer of $\alpha_{i3}\beta_{1/2}\gamma_3$. The observation that G$\gamma_3$-null mice are resistant to morphine treatment points to the possibility of this particular G protein heterotrimer associating with the μ-opioid receptor (Schwindinger et al., 2009).

4. Gγ_7. Of all the Gγ subunits that have been knocked out, Gγ_7 was the first Gγ subunit studied using a gene-targeting strategy in mice. Gγ_7 is expressed almost exclusively in the striatum of the brain, although significant expression is also observed in the neocortex and hippocampus (Betty et al., 1998). Behavioral analysis of Gγ_7 knockout mice revealed an increased startle response, but normal prepulse inhibition, a phenotype observed in mice with mutations in the glycine binding site of the *N*-methyl-D-aspartate receptor (Ballard et al., 2002; Schwindinger et al., 2003).

This finding led to the suggestion that Gγ_7 may be involved in the signal transduction pathways of GABAergic neurons, which are implicated in the pathology of schizophrenia. It was also demonstrated that loss of Gγ_7 abolished adenylyl cyclase activity upon dopamine and 6-chloro-PB (a selective dopamine D_1 receptor agonist) treatment, as well as reduced activity upon activation of adenosine A_{2A} receptors with CGS-21680 (3-[4-[2-[[6-amino-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]purin-2-yl] amino]ethyl]phenyl]propanoic acid) (Schwindinger et al., 2003, 2010). Reduction in Gα_{olf} and Gβ_2 levels, by >85 and 30% compared with wild-type animals, respectively, was found in the striatum of G$\gamma_7{}^{-/-}$ mice (Schwindinger et al., 2003, 2010). A decrease in SKF-81297 (6-chloro-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol)–stimulated adenylyl cyclase activity was measured in HEK293 cells stably expressing the dopamine D_1 receptor but not in cells expressing the dopamine D_5 receptor (Wang et al., 2001). These results implicate Gγ_7 in both dopamine D_1 receptor and adenosine A_{2A} receptor signaling, and provide evidence that all members of the same receptor family do not necessarily couple to the same Gγ subunit. Silencing of Gγ_7 revealed a coordinate reduction in Gβ_1 expression, as well as a 30% reduction in cAMP accumulation in response to isoproterenol-stimulated β_2-adrenergic receptors (Wang et al., 1997, 1999a, 2001). The observed reductions in levels of Gα_{olf}, Gβ_1, and Gβ_2 in these different models provide further evidence of cell-type-specific post-translational mechanisms where Gγ subunits stabilize partner subunits of the heterotrimeric G protein. This is interesting in the context of esophageal cancer, where levels of Gγ_7 are reduced (Ohta et al., 2008), suggesting that the effects again go beyond a simple signaling phenotype. Furthermore, it was reported that, in wild-type mice, there is a 4-fold molar excess of Gγ_2 yet equimolar amounts of Gγ_3 compared with Gγ_7 in the striatum, and surprisingly, a 40% increase in Gγ_3 and slight decrease in Gγ_2 was noted upon loss of Gγ_7, a possible reflection of an adaptive mechanism in which G$\alpha_{i/o}$ and Gα_{olf} signaling are involved in a common neurologic pathway (Schwindinger et al., 2010). Changes in levels of Gγ_2 and Gγ_3 also suggest compensatory mechanisms triggered to maintain or rescue Gα_{olf}-mediated signaling pathways. Interestingly, phylogenetic analysis indicates that Gγ_3 and Gγ_7 belong to different subfamilies of Gγ subunits (Fig. 2), although they share 58% sequence similarity (Table 2). In an attempt to explain the observed phenotypes, these authors also produced double knockout Gγ_3/Gγ_7 mice (Schwindinger et al., 2012). Phenotypes observed in the double knockout animals included high mortality, with mice dying before the age of 1 year (median survival of 75 days), and development of severe seizures. It is thought that the severe seizures were the cause of the deaths, a notion further solidified by the observation that a ketogenic diet prolonged their life span. Analysis of dopamine D_1 and adenosine A_{2A} receptor signaling revealed that the loss of both Gγ_3 and Gγ_7 did not affect adenylyl cyclase activity more than that observed in single Gγ_7 knockout mice. With regard to expression of the different Gα and Gβ subunits previously studied in single knockout mice, a modestly higher decrease in Gα_{olf} and Gβ_2 was seen in double knockout mice, whereas other G protein subunits, such as Gα_s or Gβ_1, were not affected. Furthermore, it was shown that there was a loss of baclofen-induced Kir3 activation in hippocampal neurons of Gγ_3 knockout mice, suggesting that Gγ_3 is a requirement in $GABA_B$ signaling, providing a possible explanation for the increased seizure susceptibility in these mice.

Using RNA interference techniques, functions for individual Gβ and Gγ subunits have begun to be attributed. The roles they play in heterotrimeric G protein assembly and composition, receptor signaling pathways, and embryonic development have been highlighted in the aforementioned animal knockout models. Indeed, it is evident that, not only do these subunits couple to specific receptor signaling events and confer a degree of overall stability to the G protein heterotrimer, but do so in a subtype-specific manner. The data summarized here support the notion that even closely related Gβ and Gγ subunits play distinct roles in signaling and confer a level of specificity to their cognate receptor signaling complexes. Given that compensatory mechanisms are invoked upon loss of particular Gβ or Gγ subunits, multiple and conditional knockdown studies may provide further insights into the mechanisms of signaling complex function. Furthermore, the possibility exists that Gβ and Gγ subunits play roles beyond signal transduction and G protein stability. However, the precise roles of the other Gβ and Gγ subunits remain to be determined.

C. Phenotypic Changes Due to G$\beta\gamma$ Polymorphisms and Mutations

In addition to the effects of knockdowns and knockouts, there is significant information regarding the consequences of Gβ and Gγ mutants on their function and potential roles in disease. In particular, polymorphisms in Gβ3 have been linked to outcomes in atrial fibrillation [C825T; homozygotes for the T allele are less prone to develop this condition (Schreieck et al., 2004)], coronary artery disease, hypertension [the T allele is associated with hypertension (Benjafield et al., 1998; Siffert et al., 1998; Siffert, 2005)], gastrointestinal disease [the T allele is associated with increased symptoms in the face of treatment with proton pump inhibitors (Holtmann et al., 2011)], mood disorders (Zill et al., 2000), depression [the T allele confers a higher risk for major depression (Zill et al., 2000; Bondy et al., 2002)], and obesity [the T allele is associated with obesity (Benjafield et al., 2001)]. They have also been linked to changes in responses to chemotherapy and relapse rates in chronic lymphocytic leukemia [the C allele is associated with higher relapse rates (Nuckel et al., 2003)] and in responses to anticoagulants [the T allele leads to increased bleeding in patients treated with orbofiban (Maree et al., 2010)]. Interestingly, C825T produces a truncated splice variant of Gβ_3 that lacks the ability to modulate either calcium or Kir3 channels (Ruiz-Velasco and Ikeda, 2003), although this finding, at least with respect to Kir3 channels, is controversial (Dobrev et al., 2000). Furthermore, the T allele is associated with enhanced receptor-stimulated chemotaxis in human neutrophils, suggesting that it alters signaling patterns in a cell-specific or effector-specific manner (Virchow et al., 1998, 1999; Rosskopf et al., 2003). Somatic mutations have also been noted in GNG10 coding for Gγ_{10} in human melanoma isolates, although the consequences remain unexplored (Cardenas-Navia et al., 2010). Undoubtedly, deep sequencing will identify other G$\beta\gamma$ mutations and polymorphic variants.

VII. Assembly of G$\beta\gamma$ Subunits

Given the notion that individual G$\beta\gamma$ pairs are distinct with respect to a wide range of functions, it is perhaps not surprising that their assembly is tightly controlled. Over the past two decades, significant research has been dedicated to elucidating the mechanisms by which Gβ and Gγ subunits assemble with one another. It is generally believed that the individual subunits are unstable, and therefore require dimerization to effectively perform their physiologic functions (Pronin and Gautam, 1993). Interestingly, a recent study has demonstrated that a regulator of cytoplasmic dynein, called Nudel, interacts with misfolded Gβ subunits and promotes their polyubiquitilation and degradation by the proteasome (Wan et al., 2012). This notion of obligatory dimerization, where G$\beta\gamma$ essentially functions as a single protein, is further substantiated by the fact that G$\beta\gamma$ dimers can only be separated under denaturing conditions, and that Gβ subunits expressed in the absence of Gγ form misfolded oligomeric aggregates that do not localize to the plasma membrane (Schmidt and Neer, 1991; Simonds et al., 1991; Pronin and Gautam, 1993). Gβ knockout in *Dictyostelium* also leads to reduced levels of Gγ subunits that are prenylation-deficient (i.e., not membrane-bound), implicating G$\beta\gamma$ assembly in the proper posttranslational processing of Gγ subunits (Knol et al., 2005).

Initially, it was hypothesized the α-helical coiled-coil domains located on the N termini of both subunits mediated the assembly of G$\beta\gamma$, as removal of the coiled-coil domain of Gβ abolished interactions with Gγ (Garritsen et al., 1993). Yet when synthetic peptides containing the coiled-coil domains of Gβ_2 and Gγ_2 were combined in solution, no dimerization was observed as measured by CD (circular dichroism) spectroscopy and cross-linking experiments (Marin and Neubig, 1995), suggesting that the coiled-coil domains alone were insufficient for G$\beta\gamma$ assembly. This led investigators to begin searching for molecular chaperones that may play a role in facilitating the dimerization of G$\beta\gamma$.

A. Cytosolic Chaperonin Complex/Phosducin-Like Protein 1 and Its Role in G$\beta\gamma$ Assembly

The phosducin family of proteins has long been implicated in negative modulation of G$\beta\gamma$ signaling through their ability to tightly bind and sequester G$\beta\gamma$, preventing the latter from relaying activated GPCR signals to canonical effectors (Bauer et al., 1992; Hawes et al., 1994; Hekman et al., 1994; Xu et al., 1995; Savage et al., 2000). A related member of the phosducin family, phosducin-like protein 1 (PhLP1), initially thought to have a similar inhibitory effect on G$\beta\gamma$ signaling (Miles et al., 1993; Schroder and Lohse, 1996; Thibault et al., 1997; Savage et al., 2000; McLaughlin et al., 2002a), has the opposite effect compared with phosducin, whereby it serves as a positive regulator of G$\beta\gamma$-dependent signaling (Kasahara et al., 2000; Garzon et al., 2002; Blaauw et al., 2003).

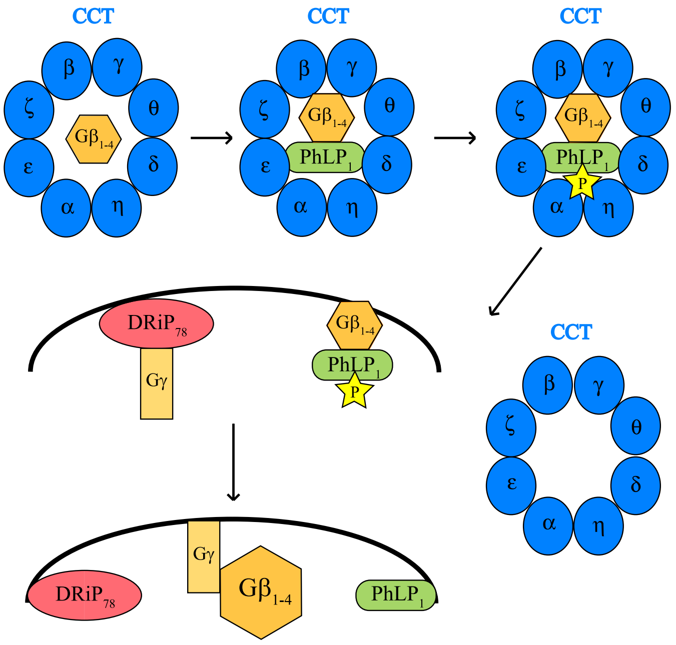

Fig. 7. Assembly of the G$\beta\gamma$ dimer. To achieve properly folded G$\beta\gamma$ dimers, individual Gβ subunits must first interact with the CCT, a protein essential for the correct folding of the seven-bladed propeller conformation of Gβ. PhLP1 accelerates the folding process by facilitating and stabilizing the interaction between CCT and Gβ, whereas DRiP78 ensures the appropriate folding of individual Gγ subunits. Once Gβ has achieved proper conformation, CK2 phosphorylates PhLP1 at serine 20, which permits the Gβ-PhLP1 complex to dissociate from CCT, thereby allowing space for Gγ to assemble with Gβ. It is hypothesized that the stability of the DRiP78-Gγ complex is susceptible to competition by correctly folded Gβ subunits. It is unknown how PhLP1 dissociates from Gβ, yielding the mature G$\beta\gamma$ dimer.

Since PhLP1 has been shown to interact with the cytosolic chaperonin complex (CCT) (McLaughlin et al., 2002b; Martin-Benito et al., 2004), an essential component in the proper folding of newly synthesized β-propeller–containing proteins, such as actin and tubulin (Martin-Benito et al., 2002), it was hypothesized that PhLP1 may have a role in the folding or assembly of G$\beta\gamma$ subunits. The localization of PhLP1 is restricted to the cytosol, in contrast to the membrane-localized phosducin. When PhLP1 was knocked out in *D. discoideum* AX3 cells, there was a 20-fold reduction in overexpressed green fluorescent protein (GFP)-Gβ and GFP-Gγ protein levels, and these subunits could no longer be coimmunoprecipitated, as compared with control AX3 cells (Knol et al., 2005). The localization of overexpressed G$\beta\gamma$ was also altered in PhLP1 knockout cells, as noted by a marked absence of G$\beta\gamma$ localization at the plasma membrane (Knol et al., 2005). It is known that G$\beta\gamma$ surface localization is dependent on post-translational prenylation of Gγ (Muntz et al., 1992; Higgins and Casey, 1996), leading these authors to assess the hydrophobicity of G$\beta\gamma$ using Triton X-114 partitioning. In control AX3 cells, they observed a substantial amount of G$\beta\gamma$ in the detergent phase, indicative of membrane-bound G$\beta\gamma$. In contrast, when the same experiment was conducted with AX3 PhLP1 knockout cells, all of the G$\beta\gamma$ was found in the aqueous phase, demonstrating a lack of isoprenylation of Gγ and implicating PhLP1 in the proper processing of the G protein dimer (Knol et al., 2005). Finally, when endogenous levels of Gβ were quantified in the PhLP1 knockout cells, a similar decrease in Gβ expression levels was noted, although no changes in Gβ mRNA levels were observed, suggesting a role for PhLP1 in the stability or assembly of Gβ protein (Knol et al., 2005) (Fig. 7).

A similar study in HeLa cells showed a 40% reduction in Gβ_1 protein levels, with no alterations in Gβ_1 mRNA levels, following PhLP1 knockdown (Lukov et al., 2005). Using pulse-chase experiments in HEK293 cells, it was shown that the rate of assembly of G$\beta\gamma$ was significantly reduced, with a 300-minute half-life of assembly for G$\beta\gamma$ in PhLP1-depleted cells, compared with a 60-minute half-life of assembly in control cells (Lukov et al., 2005). In concordance with this, overexpression of PhLP1 significantly accelerated the rate of assembly of G$\beta\gamma$ (Lukov et al., 2005).

Further evidence implicating PhLP1 in the assembly of G$\beta\gamma$ dimers comes from studies using a mutant form of PhLP1, containing the amino acid substitutions S18-20A, precluding phosphorylation by casein kinase 2 (CK2). When PhLP1 S18-20A was overexpressed in HEK293 cells, a decrease in G$\beta\gamma$ expression levels and a 15-fold reduction in the rate of G$\beta\gamma$ assembly were observed, in addition to a loss of PLCβ signaling (Humrich et al., 2003; Lukov et al., 2005). Interestingly, PhLP1 S18-20A was still able to form a complex with Gβ, but did not require the presence of Gγ, suggesting that phosphorylation of PhLP1 is somehow essential in facilitating the assembly of G$\beta\gamma$. Similarly, when PhLP1, Gβ_1, and Gγ_2 were coexpressed and radiolabeled in HEK293 cells, only Gβ_1 could be detected following PhLP1 immunoprecipitation. When Gγ_2 was immunoprecipitated under the same transfection conditions, Gβ_1 was present as expected, but PhLP1 was notably absent (Lukov et al., 2005). The authors hypothesized that PhLP1 S18-20A formed a stable ternary complex with CCT and Gβ that could not interact with Gγ due to steric hindrance by CCT. Evidence corroborating this came from the finding that, when CCT was immunoprecipitated, PhLP1 and Gβ, but not Gγ, were present in the precipitate (Lukov et al., 2006). Moreover, the interaction between CCT and Gβ following CCT immunoprecipitation was further strengthened when PhLP1 S18-20A or an N-terminal truncation mutant of PhLP1 was expressed (Lukov et al., 2006). When Gγ_2 was overexpressed and CCT was immunoprecipitated, a reduction in the CCT-Gβ complex was noted, likely due to Gγ_2 competing with CCT for interactions with Gβ (Lukov et al., 2006). Finally, when Gγ_2 was overexpressed and CCT-Gβ dissociation rates were measured via radiolabeling and CCT immunoprecipitation, a substantial increase in CCT-Gβ dissociation was observed, whereas overexpression of PhLP1 S18-20A greatly prolonged the association between CCT and Gβ subunits (Lukov et al., 2006). The authors concluded that PhLP1 must be phosphorylated by CK2 for PhLP1-Gβ to be released from the CCT-PhLP1-Gβ ternary complex, which would then allow Gγ to interact with Gβ. Prolonged association of G$\beta\gamma$ with PhLP1 and its splice variants might be a quality-control mechanism leading to degradation via the proteasome as well (Humrich et al., 2005).

Similar results were obtained in *Cryphonectria parasitica*, a pathogenic fungus that is the main cause of chestnut blight, where Gβ stability was found to be dependent on the presence of beta disruption mimic factor-1 (a PhLP1 homolog) and its phosphorylation by CK2 (Salamon et al., 2010). Another study confirmed the finding that Gβ interacts with the CCT complex in the absence of Gγ, and that Gγ competed with CCT for binding to Gβ (Wells et al., 2006). Further, they showed that there was selectivity within the Gβ family for binding to CCT. Immunoprecipitation of various Gβ subunits in rabbit reticulocyte lysates showed that the relative amounts of Gβ associated with CCT were $G\beta_5 < G\beta_{3\text{-}S} < G\beta_3 < G\beta_2 < G\beta_1 < G\beta_4$, suggesting specificity for the role of CCT in mediating specific G$\beta\gamma$ dimer assembly (Wells et al., 2006). This was indirectly corroborated in that Gβ_2, but not Gβ_3, could compete for the interaction between Rluc-Gβ_1 and GFP-PhLP1, as measured by bioluminescence resonance energy transfer (BRET) in HEK293 cells

(Dupré et al., 2007b). Last, siRNA knockdown of TCP-1α (T-complex protein 1 alpha), a component of CCT, resulted in decreased G$\beta\gamma$ levels in HEK293 cells, lending further credence for a role of CCT in G$\beta\gamma$ formation (Humrich et al., 2005).

B. Specificity of G$\beta\gamma$ Assembly

Another intriguing question in G$\beta\gamma$ assembly concerns the specificity with which Gβ subunits form dimers with distinct Gγ subunits, and whether the CCT-PhLP1 complex and/or other as yet unidentified cellular chaperones (general or cell-specific) play a role in this specificity. With respect to specificity of G$\beta\gamma$ dimer formation, much research has been conducted to determine whether certain Gβ subunits preferentially interact with different Gγ subunits. Using an assortment of techniques, including gel filtration, tryptic digestion, chemical cross-linking, immunoprecipitation, PLC signaling assays, and yeast two-hybrid screening, it was shown that Gγ_1 only interacts with Gβ_1, whereas Gγ_2 and Gγ_3 can readily interact with G$\beta_{1/2/4}$ subunits (Pronin and Gautam, 1992; Schmidt et al., 1992). The Gβ_3 subunit does not efficiently interact with any of the Gγ subunits [although there is some debate regarding this (Poon et al., 2009)], whereas Gβ_1 and Gβ_4, being similar to one another, can interact with almost all Gγ subunits (Schmidt et al., 1992; Dingus et al., 2005). Gβ_2 seems to show the most selectivity in terms of Gγ subunit binding, as it did not dimerize with G$\gamma_{1/11/13}$ and dimerized only weakly with Gγ_8 (Dingus et al., 2005). Gβ_5, being the most divergent of all the Gβ subunits (~52% sequence similarity compared with G$\beta_{1\text{-}4}$; Table 1), weakly interacts with Gγ subunits, and only in the absence of RGS proteins that contain a Gγ-like domain (discussed further later) (Dingus et al., 2005). Using chimeras of different Gβ and Gγ subunits, it was determined that a stretch of the N-terminal 14 amino acids of Gγ conferred selectivity for assembly with Gβ subunits (Spring and Neer, 1994; Lee et al., 1995), whereas regions in both the N- and C-terminal domains of Gβ contained elements to provide selectivity for distinct Gγ subunits (Garritsen and Simonds, 1994; Katz and Simon, 1995). The region on Gγ_1 that is critical for assembly with Gβ_1 was narrowed down to a 3–amino acid stretch (amino acids 36–38), as mutation of these residues allowed Gγ_1 to interact with Gβ_2 (Meister et al., 1995). Note that, although Gβ_5 does not interact with Gγ subunits as detected in vivo, Gβ_5 and various Gγ subunits were able to stimulate PLCβ activity in vitro (Dingus et al., 2005), adding another layer of complexity to the dichotomy between specificity in G$\beta\gamma$ assembly and functionality.

Whether PhLP1 serves a function in determining which Gβ subunits can assemble with different Gγ subunits was elegantly addressed by a series of immunoprecipitation experiments in HEK293 cells. When PhLP1 was knocked down and HA (hemagglutinin)-tagged Gγ_2 and any of the four FLAG-Gβ isoforms were expressed, there was a similar attenuation (65–85%) in this set of G$\beta\gamma$ interactions, regardless of which specific dimer was analyzed [Gβ_5 does not form a dimer with Gγ—see section VII.D below (Howlett et al., 2009)]. When PhLP1 was overexpressed, the opposite result was obtained: HA-Gγ_2 interactions with all four Gβs were proportionally increased (Howlett et al., 2009). A complementary experiment was performed where FLAG-Gβ_2 was coexpressed with the 12 known Gγ subunits, and PhLP1 was knocked down. Once again, depending on which class of Gγ subunits was examined (Gγ can be phylogenetically separated in five distinct classes as shown earlier; Fig. 2), there was a proportional decrease in the amount of Gγ coimmunoprecipitated with Gβ_2 (Howlett et al., 2009). Taken together, these results indicate that PhLP1 plays a general role in the assembly of G$\beta\gamma$ dimers and does not affect the specificity of subunit interactions.

C. Dopamine Receptor Interacting Protein 78 and Its Role in G$\beta\gamma$ Assembly

Although the mechanisms involved in mediating Gβ assembly with Gγ have been fairly well described, the events regulating Gγ stability and assembly with Gβ are less well understood. Progress in this regard was made when an ER-residing, GPCR-interacting protein, termed dopamine receptor interacting protein 78 (DRiP78), was shown to serve as a molecular chaperone for Gγ subunits, akin to the role that PhLP1 plays for the Gβ subunit. Using immunoprecipitation and resonance energy transfer techniques in HEK293 cells, it was demonstrated that DRiP78 interacted with Gγ_2 but not G$\beta_{1\text{-}5}$, and that this interaction could be competed by overexpressed Gβ_1 (Dupré et al., 2007b). The interaction between DRiP78 and Gγ_2 was shown to be specific using BRET, as untagged DRiP78 was able to compete with Rluc-DRiP78 for interactions with GFP-Gγ_2. Additionally, when DRiP78 was knocked down by shRNA treatment, Gγ protein levels decreased, whereas Gβ levels remained unaltered, and immunoprecipitation between FLAG-Gβ_1 and HA-Gγ_2 was significantly reduced (Dupré et al., 2007b). DRiP78 expression increased the amount of Gγ_2-GFP in the cell, even without overexpression of Gβ_1. In contrast, Gβ_1-GFP fluorescence was enhanced by Gγ_2 expression but unaffected by DRiP78 expression (Dupré et al., 2007b). Interestingly, PhLP1 was also able to interfere with the interaction between Gγ and DRiP78, perhaps highlighting a role for PhLP1 in dissociating the Gγ-DRiP78 complex to facilitate assembly of Gγ with Gβ (Fig. 7).

One difference between PhLP1 and DRiP78 is that, whereas PhLP1 does not appear to discriminate between different Gβ subunits in their assembly with Gγ (Howlett et al., 2009), DRiP78, in contrast, seems to regulate only a subset of Gγ subunits with respect to their assembly with Gβ. When BRET competition assays were performed using $G\gamma_2$-GFP, DRiP78-Rluc, and various untagged Gγ subunits, selectivity was noted in the ability of different Gγ subunits to compete for the interaction between $G\gamma_2$ and DRiP78 (Dupré et al., 2007b). The more closely related the Gγ subunit was in terms of structure and sequence to $G\gamma_2$, the larger the attenuation of the interaction between $G\gamma_2$ and DRiP78. $G\gamma_3$ was most efficient at reducing the BRET interaction, whereas the structurally unrelated $G\gamma_1$ was least effective in this assay. $G\gamma_{7/11}$, being intermediate in terms of their sequence similarity to $G\gamma_2$, were moderately effective in reducing the BRET interaction between $G\gamma_2$ and DRiP78 (Dupré et al., 2007b). This suggests that DRiP78 may serve a specific chaperone role for a subset of Gγ subunits, and that perhaps other, as of yet unidentified chaperones regulate the assembly of other Gγ subunits with Gβ.

D. Assembly of $G\beta_5$ with Regulator of G Protein Signaling Proteins

Although it has been shown that $G\beta_{1\text{-}4}$ subunits can form dimers with many Gγ subunits, the $G\beta_5$ subunit is unique, insofar as it forms dimers with Gγ subunits in a manner distinct from other Gβ subunits, and rather associates with RGS proteins (Chen et al., 2000a; Witherow and Slepak, 2003). Although $G\beta_5$ can form dimers with Gγ subunits in vitro and in cellulo, they are distinct with respect to their detergent lability compared with other Gβγ combinations (Lindorfer et al., 1998; Yoshikawa et al., 2000; Yost et al., 2007). The RGS family of proteins plays an essential role in the termination of GPCR signaling by accelerating GTP hydrolysis upon receptor activation (Ross and Wilkie, 2000; Willars, 2006). As the CCT-PhLP1 complex is involved in the dimerization of various Gβγ combinations (Lukov et al., 2005, 2006), it was of interest to see if this chaperone complex was involved in the formation of $G\beta_5$-RGS dimers as well. Importantly, $G\beta_5$ forms dimers with only select members of the RGS family, namely, the R7 family, which contain RGS 6, 7, 9, and 11 (Witherow and Slepak, 2003).

$G\beta_5$ was initially observed to immunoprecipitate with both CCT and PhLP1, albeit to a much lesser degree compared with $G\beta_1$, suggesting that the CCT-PhLP1 complex might also be involved in $G\beta_5$-RGS assembly (Howlett et al., 2009). When PhLP1 was knocked down or overexpressed as a dominant-negative N-terminal truncation mutant, a decrease in the total protein levels of both $G\beta_5$ and RGS7 was noted, as in the case of Gβγ assembly, although a less severe reduction in the amount of RGS7 coimmunoprecipitating with $G\beta_5$ compared with other Gβ subunits and Gγ was also observed (Howlett et al., 2009). When the conditions were reversed and PhLP1 was overexpressed, the authors saw an increase in $G\beta_5$ protein levels, comparable to the increases observed with other Gβ subunits, yet they saw no proportional increase in the amount of RGS7 coimmunoprecipitating with $G\beta_5$ compared with other Gβγ dimers (Howlett et al., 2009). Other RGS proteins that interact with $G\beta_5$, such as RGS6, RGS9, or RGS11, were not specifically examined. These authors then examined whether overexpression of PhLP1 or RGS7 would weaken the interaction of $G\beta_5$ with CCT, as was the case with $G\beta_1$-CCT interactions after PhLP1 or $G\gamma_2$ overexpression. When PhLP1 was overexpressed, the authors noted an increase in the $G\beta_5$-CCT interaction, and RGS7 had no effect on this interaction. Another surprising difference was that RGS7 was able to coimmunoprecipitate with CCT in the presence of $G\beta_5$, and that PhLP1 overexpression had no effect on this interaction. Moreover, PhLP1 and RGS7 were never able to be coimmunoprecipitated, regardless of whether $G\beta_5$ was coexpressed or not (Howlett et al., 2009; Fig. 8).

When the rate of dimerization was assessed using pulse-chase experiments, differences between Gβγ and Gβ-RGS dimerization were further highlighted by the observation that overexpressed PhLP1 in fact hindered assembly of Gβ-RGS7 (Howlett et al., 2009). Based on available structural information, the authors surmised that PhLP1 and RGS7 bind to similar regions on $G\beta_5$, and therefore compete with one another for $G\beta_5$ binding. To test this, either $G\beta_5$-RGS9 or $G\beta_1\gamma_2$ was immobilized on FLAG-agarose beads, and the binding of radiolabeled PhLP1 to each complex was examined. Whereas PhLP1 binding was readily detected with the

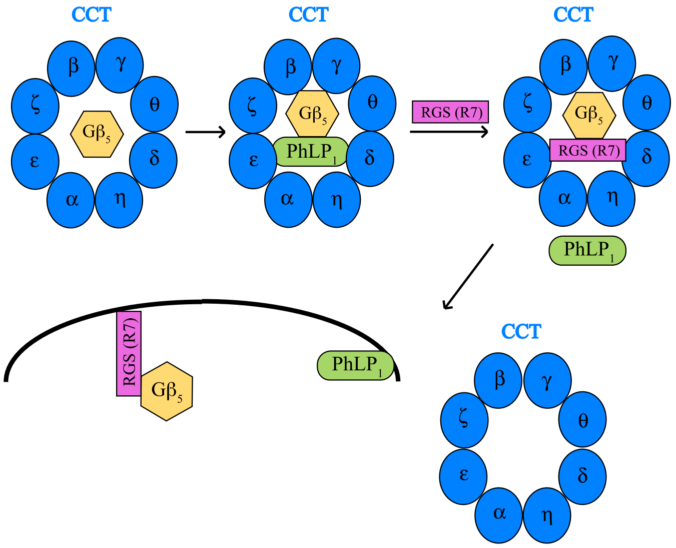

Fig. 8. Assembly of the $G\beta_5$-RGS dimer. To achieve properly folded $G\beta_5$-RGS dimers, individual Gβ subunits must first interact with CCT. PhLP1 accelerates the folding process by facilitating and stabilizing the interaction between CCT and $G\beta_5$. Once $G\beta_5$ is correctly folded, RGS proteins containing the Gγ-like domain compete with PhLP1 for binding to the $G\beta_5$-CCT complex, resulting in the dissociation of PhLP1 from the complex. $G\beta_5$-RGS dimers are then released from CCT through an unknown mechanism, permitting proper post-translational modifications of the dimer.

G$\beta_1\gamma_2$ dimer, virtually no PhLP1 was detected in the Gβ_5-RGS9 complex (Howlett et al., 2009). It was concluded that, although PhLP1 was essential for the dimerization of G$\beta\gamma$ as indicated earlier, it served a less essential role in the assembly of Gβ_5-RGS, as its primary function was to stabilize the interaction between Gβ_5 and CCT, yet it impeded dimerization of Gβ_5-RGS, if it did not disassociate from Gβ_5-CCT. How PhLP1 favors the interaction between Gβ_5-CCT, and yet reduces the association of other Gβ subunits with CCT, is a question that remains unanswered.

VIII. Pharmacological Targeting of G$\beta\gamma$ Subunits

Given the vast array of physiologic functions regulated by G$\beta\gamma$ subunits, and further, the ever-expanding roles that they play in different subcellular compartments, it is not surprising that alterations in G$\beta\gamma$ signaling are associated with a number of pathologies. This notion originated from the finding that the C terminus of GRK2 (GRK2-ct) was able to inhibit receptor-stimulated PLC and ACII activation in HEK293 and COS7 cells by tightly binding to the G$\beta\gamma$ dimer (Inglese et al., 1994; Koch et al., 1994b). The inhibition of PLC and ACII was specific to the isoforms modulated by G$\beta\gamma$, as Gα-activated isoforms of PLC and AC were unaffected by GRK2-ct expression. When inhibition of G$\beta\gamma$ was studied in a more physiologic setting using recombinant GRK2-ct adenoviruses in rabbits undergoing carotid artery grafts with the jugular vein, GRK2-ct was able to significantly reduce the intimal hyperplastic abnormalities, a condition often seen after such procedures (Davies et al., 1998; Huynh et al., 1998). G$\beta\gamma$ inhibition was further demonstrated to be effective in preventing restenosis and vascular smooth muscle intimal hyperplasia after injury to rat carotid arteries, emphasizing G$\beta\gamma$ as a key signaling component in vasculature homeostasis (Iaccarino et al., 1999).

Alterations in G$\beta\gamma$ subunit expression and signaling have also been implicated in the progression of various types of cancer (Kirui et al., 2010; Yajima et al., 2012), and much work has been done to determine the benefits of interfering with G$\beta\gamma$ signaling in cancer growth. GRK2-ct–mediated inhibition of G$\beta\gamma$ signaling in a human prostate cancer cell line, PC3, reduced cancer cell proliferation, and impeded formation of prostate tumors in a mouse model (Bookout et al., 2003). Similar findings were observed in a breast cancer cell line and a mouse xenograft breast cancer model, whereby GRK2-ct blocked both tumor cell proliferation and migration, resulting in a diminished manifestation of lung metastasis from primary tumors (Tang et al., 2011).

A. G$\beta\gamma$ and the Emergence of the “Hot Spot”

As discussed in previous sections, G$\beta\gamma$ serves as a signal transducer to a wide array of effectors in multiple subcellular locations. Although intriguing results have been obtained using G$\beta\gamma$ sequestrants such as GRK2-ct and phosducin in addressing which effectors are regulated by G$\beta\gamma$, it will be critical to design more selective, small-molecule inhibitors of G$\beta\gamma$ interactions with individual effectors, if G$\beta\gamma$ is to ever become a viable therapeutic target. In addition, the ability to target individual G$\beta\gamma$ combinations will likely be required as well. The first attempt to determine selective G$\beta\gamma$ binding domains for its effectors came from studies examining AC2 activation by G$\beta\gamma$. Using molecular docking, Chen et al. (1997) demonstrated that peptides corresponding to residues 86–105 and 115–135 on Gβ were essential for the activation of AC2. It was shown that a peptide sequence corresponding to residues 956–984, termed QEHA, in AC2 was essential for activation by G$\beta\gamma$, and interestingly, this synthetic peptide was able to prevent G$\beta\gamma$ activation of numerous effectors such as AC2, PLCβ3, Kir3 channels, and GRK2 (Chen et al., 1995). This effector-interacting site on G$\beta\gamma$ was shown to overlap with the Gα binding site on G$\beta\gamma$ (Weng et al., 1996). Based on these findings, Smrcka et al. (2012) took a more unbiased approach to characterizing the putative hot spot by screening 16 random peptide phage-display libraries for binding to biotinylated G$\beta_1\gamma_2$ on immobilized streptavidin (Scott et al., 2001). The notion of a G$\beta\gamma$ “hot spot” that was masked in the G$\alpha\beta\gamma$ heterotrimer, essential in the activation of many of its effectors, was developed to explain the fact that all of the identified hits in a random unbiased phage-display screen targeted a single site in G$\beta\gamma$. This suggested that this protein surface on G$\beta\gamma$ had intrinsic physicochemical properties of an optimal protein-interaction surface. Four different peptide sequences were obtained based on binding to G$\beta\gamma$, and one of these, termed the SIRK peptide, was used in competition assays to confirm the binding of the other candidates. The SIRK peptide was shown to have significant sequence overlap with a known effector of G$\beta\gamma$, PLCβ_2, and the authors demonstrated that SIRK was able to prevent G$\beta\gamma$ activation of not only PLCβ_2 but also PLCβ_3 and PI3Kγ, but not AC1 or Ca$_v$ channels (Scott et al., 2001). Importantly, when the crystal structure of G$\beta_1\gamma_2$, in complex with SIGK (a peptide highly related to SIRK), was obtained, it revealed that SIGK targets the Gα_{i1} switch II binding surface of G$\beta_1\gamma_2$, corroborating the observation that the hot-spot region of G$\beta\gamma$ is masked when associated with Gα (Davis et al., 2005). Of note, it was also shown that SIRK was able to dissociate G$\beta\gamma$ from Gα by directly interfering with their interactions (Goubaeva et al., 2003). A virtual screening assay was then used to evaluate the ability of 1990 compounds to bind the G$\beta\gamma$ hot spot and two additional peptide sequences. One of these competed with SIRK for binding to G$\beta\gamma$ and bound to distinct surfaces on the hot-spot region

(Bonacci et al., 2006). Termed M119 and M201, these small-molecule ligands were both able to prevent G$\beta\gamma$ binding to GRK2, whereas only M119 was able to additionally interfere with G$\beta\gamma$ binding to PLC$\beta_{2/3}$ and PI3Kγ (similar to SIRK). Surprisingly, M201 enhanced G$\beta\gamma$ binding and activation of PLCβ_3 and PI3Kγ, but not PLCβ_2 (Bonacci et al., 2006). Following are a few examples of G$\beta\gamma$ effectors whose activity has been inhibited by administration of these novel small-molecule inhibitors.

*B. Small-Molecule Interference of G*β*γ Signaling*

Using the novel G$\beta\gamma$ inhibitor, gallein, identified through the aforementioned phage-display screen, Lehmann et al. (2008) demonstrated a reduction in chemoattractant-dependent neutrophil migration and paw swelling in a carrageenan-induced paw edema mouse model. This gallein-mediated anti-inflammatory action was shown to be caused by the abolition of the interaction between G$\beta\gamma$ and PI3Kγ, an essential protein in the initiation of events leading to chemotaxis (Lehmann et al., 2008; Li et al., 2000).

Although work has been done assessing the efficiency of small-molecule disruption in G$\beta\gamma$-effector interactions in vitro, these G$\beta\gamma$ inhibitory peptides are also able to affect signaling in vivo. It has been shown that PLC$\beta3^{-/-}$ mice experience a 10-fold increase in antinociception compared with their wild-type controls following morphine administration (Xie et al., 1999). As M119, but not M201, was found to inhibit PLCβ3-G$\beta\gamma$ interactions, it was of interest to see whether this compound could enhance antinociception in mice treated with morphine. As expected, M119 led to an 11-fold increase in morphine-induced analgesia in mice, and importantly, had no effect on nociception in the absence of morphine (Bonacci et al., 2006). This morphine-dependent antinociceptive property of M119 was later shown to be selective for μ-opioid receptor stimulation, and reduced attenuating antinociceptive dependence and tolerance following morphine administration in mice (Mathews et al., 2008). Promising studies have also appeared suggesting that such compounds might be of use in treating heart failure (Casey et al., 2010), morphine-induced hyperalgesia (Bianchi et al., 2009), and inflammation (Lehmann et al., 2008).

Perhaps the most conclusive evidence supporting the notion of G$\beta\gamma$ as a therapeutic target stems from studies investigating G$\beta\gamma$ signaling in heart failure. One of the hallmarks of heart failure is an overabundance of catecholamines, which chronically stimulate the βAR, leading to its desensitization and gradual downregulation via phosphorylation by GRK2 (Bristow et al., 1982; Ungerer et al., 1993). As G$\beta\gamma$ is directly involved in the recruitment of GRK2, which is upregulated in heart failure, to the plasma membrane (Pitcher et al., 1995), it was hypothesized that a small-molecule inhibitor of G$\beta\gamma$-GRK2 interactions could be of benefit to the onset and progression of heart failure. Evidence corroborating this originates from the observation that overexpression of GRK2 potentiates heart failure (Koch et al., 1995), whereas genetic ablation of GRK2 yields cardioprotective phenotypes (Matkovich et al., 2006; Raake et al., 2008). Interestingly, both M119 and the structurally related gallein were able to enhance cardiac contractility upon βAR stimulation in human cardiomyocytes, in addition to reducing GRK2 expression levels, demonstrating that M119 and gallein prevent GRK2-mediated desensitization of βAR (Casey et al., 2010). When these same two inhibitors were used in an isoproterenol-induced mouse model of heart failure, cardiac contractility, hypertrophy, and left ventricular volumes and wall size, which were significantly perturbed in mice treated with isoproterenol only, were maintained at normal levels (Casey et al., 2010). Similar results were obtained in a mouse model that already had established heart failure for a persistent period of time, suggesting that these inhibitors could be beneficial for both the prevention and management of heart failure (Casey et al., 2010). The use and development of G$\beta\gamma$ inhibitors has been reviewed, but it remains to be seen if inhibitors for specific combinations might be developed (Smrcka, 2008; Dessal et al., 2011).

IX. Conclusion and Future Directions

Our discussion suggests an exciting future for our understanding of G$\beta\gamma$ function, with much to learn about canonical and noncanonical effectors, receptor- and Gα-dependent and -independent actions, and an expanding role as a transcriptional regulator. As a therapeutic target, there are many concerns that still need to be addressed. G$\beta\gamma$ dimers are a ubiquitously expressed family of proteins whose functions cover nearly every aspect of cellular, tissue, and organ physiology. Disruption of G$\beta\gamma$ signaling events, even if restricted to the inhibition of specific G$\beta\gamma$ pairs, of which our understanding remains rudimentary, will likely lead to an assortment of off-target effects, rendering them difficult to use in a clinical setting. Targeted drug delivery of G$\beta\gamma$ inhibitors is certainly one way of circumventing this issue, but further strategies are needed if modulating G$\beta\gamma$ is to expand beyond being a useful research tool. One interesting development in GPCR signaling is the emerging concept of biased or ligand-dependent signaling. In this approach, distinct ligands of a GPCR can selectively stimulate one arm of a signaling pathway without affecting the other effectors coupled to the same activated GPCR. Combining small peptide interference of G$\beta\gamma$ interactions with the knowledge obtained from studies of biased signaling [reviewed in

Kenakin (2011)] might potentially result in more selective activation/inhibition of distinct GPCR signaling pathways, especially in circumstances where a biased ligand activates only a subset, yet still multiple, downstream effectors.

Authorship Contributions

Performed data analysis: Khan, Sleno, Laverdure, Labbé, Miller, Hébert.

Wrote or contributed to the writing of the manuscript: Gora, Khan, Sleno, Zylbergold, Laverdure, Labbé, Miller, Hébert.

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