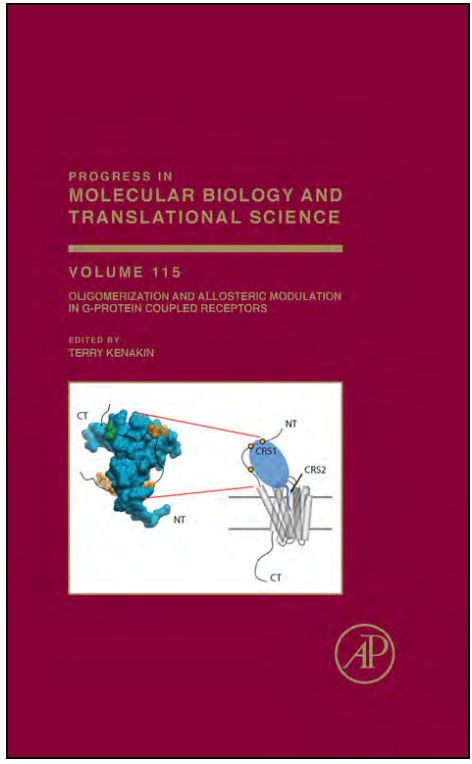

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CHAPTER TEN

Fine-Tuning of GPCR Signals by Intracellular G Protein Modulators

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Contents

Abstract

Heterotrimeric G proteins convey receptor signals to intracellular effectors. Superimposed over the basic GPCR–G protein–effector scheme are three types of auxiliary proteins that also modulate Gα. Regulator of G protein signaling proteins and G protein signaling modifier proteins respectively promote GTPase activity and hinder GTP/GDP exchange to limit Gα activation. There are also diverse proteins that, like GPCRs, can promote nucleotide exchange and thus activation. Here we review the impact of these auxiliary proteins on GPCR signaling. Although their precise physiological functions are not yet clear, all of them can produce significant effects in experimental systems. These signaling changes are generally consistent with established effects on isolated Gα; however, the activation state of Gα is seldom verified and many such changes appear also to reflect the physical disruption of or indirect effects on interactions between Gα and its associated GPCR, Gβγ, and/or effector.

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1. INTRODUCTION: HETEROTRIMERIC G PROTEINS AND THEIR BINDING PARTNERS

G protein-coupled receptors (GPCRs) play a major role in physiological functions and also serve as essential therapeutic targets. In spite of their fundamental importance, some key aspects of how GPCRs convey extracellular signals into cells remain poorly understood. The basic unit of G protein-mediated signaling comprises five gene products: a seven transmembrane-spanning receptor that recognizes extracellular signals, an effector that produces a change in cellular homeostasis, and the Gα, Gβ, and Gγ subunits of the heterotrimeric G protein that work together to carry signals from the receptor to the effector. Over the years numerous other proteins have been identified that interact with one or more of these components and thereby have an impact on signaling. This review will deal with auxiliary proteins that bind to Gα and alter its ability to bind or hydrolyze the activating nucleotide GTP. Specifically we will focus on how such proteins affect G protein-mediated signaling, and in some cases also interface with GPCRs themselves.

G protein Gα subunits belong to a superfamily of GTPases that function as molecular switches to control a wide array of cellular processes.[1,2] Other such GTPases include the smaller Ras-like signaling proteins, initiation and elongation factors such as eIF2 and eEF1a, and specialized proteins such as tubulin and dynamin. Generally speaking, GTPases are activated by the binding of GTP, and self-deactivate by hydrolyzing this to GDP plus inorganic phosphate. The rates at which these biochemical steps take place can be altered by modulatory proteins: guanine nucleotide exchange factors (GEFs) promote GDP dissociation and thus facilitate the binding of GTP, guanine nucleotide dissociation inhibitors (GDIs) impede GDP dissociation and thus delay GTP binding, and GTPase-accelerating proteins (GAPs) facilitate GTP hydrolysis.

Gα proteins have multiple-binding partners that can act as GEFs, GDIs, and GAPs. Of primary importance among these are the other members of the basic receptor–G protein–effector (R–G–E) signaling unit. GPCRs activate Gα proteins by stimulating GDP dissociation and consequently facilitate GTP binding, thus they act as GEFs. Also, the rate of GDP dissociation from heterotrimeric G proteins is slower than from free Gα, which indicates that the stable Gβγ heterodimer (Gβ and Gγ tend to come apart only under denaturing conditions) actually functions as a GDI.[3] In spite of this inhibitory effect, Gβγ is still required for a GPCR to productively

couple to and activate Gα.[4] Whereas receptors and Gβγ have effects on nucleotide exchange, some effector proteins have been found to increase the rate at which their Gα-binding partners hydrolyze GTP. Specifically, phospholipase Cβ acts as a GAP for Gαq,[5] while the Rho-activating factors p115RhoGEF and leukemia-associated RhoGEF (LARG) are GAPs for Gα12/13.[6]

Besides their immediate signaling partners, Gα proteins are also governed by a variety of other proteins. Perhaps the best known among these are the regulator of G protein signaling (RGS) proteins, which act as GAPs for members of the Gαi/o and Gαq subfamilies of Gα proteins. By promoting G protein deactivation, RGS proteins can limit the duration of GPCR signals, and also serve to decrease agonist efficacy and/or potency.[7] The RGS protein family is encoded by 20 different genes, some of which yield multiple splice variants. The RGS proteins ranges in size from about 200 to 1400 amino acid residues and can be subdivided into four distinct subfamilies (A/RZ, B/R4, C/R7, and D/R12).[7] Common to all of these is a conserved 120-amino acid-residue RGS domain that binds preferentially to activated Gα and facilitates GTP hydrolysis.[8] Additionally there are a number of structurally diverse RGS-like (RL) proteins that contain an RGS homology domain with either minimal (e.g., G protein-coupled receptor kinases)[9] or atypical GAP activity (e.g., p115RhoGEF, LARG).[6]

In contrast to RGS proteins, which affect GTP hydrolysis, there are also auxiliary Gα-binding proteins that can alter the rate of nucleotide exchange on Gα. G protein signaling modifier (GPSM) proteins contain one to four conserved 20–15 amino acid residue motifs that bind to and stabilize the inactive GDP-bound forms of their target Gαi/o proteins.[10] These interactions produce a GDI effect on Gα and also appear to promote Gβγ dissociation from the heterotrimer, both of which are changes that may impact GPCR signaling. In addition, a handful of proteins with GEF activity have been identified that, like GPCRs, can promote Gα activation. Most of these novel GEFs bear little structural resemblance to one another.

Mounting evidence indicates that proteins that alter Gα nucleotide exchange rates can affect G protein-mediated receptor signals. However unlike RGS proteins, which clearly act as negative regulators of GPCR signals, how novel GEFs and GDIs contribute to signaling has been difficult to ascertain, as their biochemical properties overlap respectively with those of Gβγ and GPCRs. Both GPSM-type proteins and the nonreceptor GEF Ric-8a are also known to be important in intracellular Gα-dependent processes such as asymmetric cell division, a topic that has been covered in several recent reviews.[11–13]

2. ORGANIZATION OF G PROTEIN-MEDIATED SIGNALS

Most or all of the proteins involved in G protein-mediated signaling presumably have been identified, but the interplay among these is complex and not fully understood. Early studies showed that the basic components (namely the GPCR, Gα, Gβγ, and the effector) could be physically separated from one another and recombined experimentally, while other functional data seemed to suggest that the depletion of cellular effectors and G proteins respectively could have limiting effects on receptor signaling and high-affinity agonist binding.[14] Such findings led to the widespread acceptance of the idea that Gα and Gβγ could dissociate from each other and from their activating GPCR, after which they could either converge on or interact independently with their effector targets.[14]

A mechanism based on random collisions between G proteins and their receptors and effectors would imply that all compatible signaling partners can interact within the plasma membrane of a cell; however, signaling among the dozens of GPCRs within a typical cell tends to be discrete and many biochemically possible pathways evidently are not utilized.[15–18] An alternate view is that G proteins do not shuttle freely between GPCR and effector proteins,[19] which in turn suggests that signaling complexes containing receptor, G protein and effector can exist.[14] The latter idea is supported by a considerable body of physical and functional evidence,[20] and in some cases GPCRs, G proteins, and effectors are found to form stable complexes through which signaling can occur.[20,21] In contrast, other findings show that in some cases G proteins can be dissociated from their GPCRs or effectors, and also that trimeric G proteins can dissociate into their constituent Gα and Gβγ subunits in living cells. Thus it remains a topic of discussion which signaling systems dissociate, and whether this occurs as part of the process of signal transduction.[21,22] On a longer time scale, it is clear that molecular rearrangements do occur, such as the substitution of arrestins for G proteins that occurs during receptor desensitization or activation of some MAP kinase-signaling pathways.[23]

There are a number of cellular-organizing factors, such as scaffolding proteins and lipid microdomains, that can facilitate the existence of signaling complexes and thereby enhance signaling specificity. Scaffolding (or anchoring) proteins are multidomain structures that bind to two or more proteins to bring them into proximity with one another, for example, a receptor and its effector.[24–26] Scaffolding proteins may have additional

binding partners that turn off signaling or localize them to particular cellular structures, and thus respectively can temporally or spatially focus signaling events within the cell.[23,27] Furthermore, scaffolds can also connect to downstream signaling components or other signaling systems such as receptor tyrosine kinases or ligand-gated ion channels.[25] Scaffolded signaling complexes are not necessarily stable entities, as these may be assembled or disassembled as part of the signaling process.[23]

A further layer of complexity comes from the fact that some proteins may be present in multiple copies within signaling complexes. It is well documented that many GPCRs can form homo- and hetero-oligomers,[28] and cooperative interactions within such complexes may profoundly affect observed dose–response relationships[29] and agonist-binding profiles.[30] GPCRs are typically thought to function as dimers, but emerging data suggest that at least some exist in nature as larger oligomers.[31] In some cases, more than one receptor protomer is required for the attainment of proper function, particularly with hetero-oligomeric GPCRs[32]; however, as has been reported with receptor tyrosine kinases,[33,34] at least some GPCRs when in monomeric form still exhibit the ability to signal.[35]

In addition to GPCRs, at least some effector proteins appear to oligomerize, including phospholipase Cβ[36] and adenylyl cyclase.[37] G protein heterotrimers are generally assumed to act as single units, although it is clear that some distantly related GTPases function as requisite dimers,[2] and also there is some evidence to suggest that individual Gα subunits may be able to associate[19] and/or communicate with one another,[38] perhaps via GPCR oligomers. Gα monomers may also be brought into proximity with one another by proteins with multiple Gα-binding domains such as RGS14 and GPSM3. In addition, scaffolding proteins that bind to GPCRs and/or effectors, such as the PDZ-containing protein INAD and the protein kinase A anchoring protein AKAP79,[39] can also self-associate, possibly leading to the formation of extended signaling complexes.

The arrangement and stability of signaling complexes is important from the point of view of understanding the effects of auxiliary GAPs, GDIs, and GEFs, as such proteins are likely to target these as well as free G proteins. In some cases, the presence of these proteins may impede G protein-mediated signaling due, for example, to steric effects,[40] but conversely in other cases it is clear that they are required for normal signaling to take place (e.g., GRK channel regulation in multiple tissues and rapid Gαt deactivation in the retina[7]). RGS and GPSM proteins can interact with free Gα proteins,[41,42] which in the context of the shuttling model would be consistent with the view

that such proteins "intercept" free G proteins in transit between GPCRs and effectors. In contrast to this idea, there is ample evidence to show that G protein modulators additionally can interact with GPCRs, effectors, Gβγ, and scaffolding proteins.[7,43] While it is clear that such arrangements can contribute to the specificity and temporal focusing of GPCR signals, the overall impact of RGS proteins, GPSM proteins, and nonreceptor GEFs on G protein-mediated signals remains an area of active study.

3. RGS PROTEINS

RGS proteins were first discovered to be Gα GAPs in 1995,[41] and since then they have been shown to play an integral role in GPCR signaling. Early studies focused on the effects of RGS proteins on isolated Gα proteins in solution, and revealed that an RGS protein molecule could act catalytically to deactivate multiple equivalents of Gα–GTP.[7] In addition to their ability to deactivate G proteins by promoting GTP hydrolysis, RGS proteins may also prevent activated Gα proteins from productively interacting with their effectors through steric or competitive mechanisms.[40] This is particularly true in the case of Gαs-mediated signaling, where clear inhibitory effects of RGS proteins have been observed[44,45] in spite of the inability of RGS proteins to promote GTP hydrolysis by this Gα protein.[46–48]

While bimolecular RGS–Gα interactions presumably do occur in cells, it may be overly simplistic to view free Gα proteins as the sole or perhaps even the primary target of RGS proteins *in vivo*. For reasons noted above, one might not expect Gα–GTP to routinely venture from its site of activation into the cytosol, and correspondingly the ability of RGS proteins to limit GPCR signaling to some extent correlates with their ability to associate with the plasma membrane.[49,50] Whereas RGS–Gα interactions in solution-based assays would occur randomly in three-dimensional space, interactions with other proteins and with the plasma membrane would tend to restrict the movement, orientation, and available surface area of receptor-activated G proteins. Depending on the exact situation, this might either impede or enhance RGS–Gα interactions.

Gβγ subunits, which are required for GPCRs to activate Gα proteins[4], strongly inhibit RGS GAP effects on isolated Gα–GTP in solution,[5,51,52]. This effect does not preclude the ability of RGS proteins to promote GTPase activity over multiple cycles of nucleotide binding and hydrolysis in the presence of an activated receptor,[53–55] but it again becomes manifest with superstoichiometric Gβγ, which apparently competes with RGS

proteins for Gα.[52] The targeting of RGS proteins to receptor-activated Gα in contrast may be facilitated by other membrane proteins. As previously noted, the inhibitory effects of RGS proteins on signaling can be promoted or otherwise regulated by associating with affiliated GPCRs, effectors, or scaffolding proteins.[7] Such protein–protein interactions may go beyond mere scaffolding effects. For example, the GAP effects of PLCβ on isolated Gαq[5] appear to be weaker than with M1 muscarinic receptor-activated G protein.[56,57] Similarly the maximal GTPase-accelerating effects of PLCβ and RGS4 on Gq co-reconstituted with M1 receptor can exceed three orders of magnitude[53] whereas maximal GAP effects on free Gα proteins are typically found to be about two orders of magnitude.[5,41,51] Thus the ability of RGS proteins (or other GAPs) to stimulate G protein hydrolytic activity may be sensitive to interactions between the GPCR and the G protein and/or RGS protein.

By targeting RGS proteins to G proteins that they have activated, receptors seem to play a role in assisting RGS proteins to achieve selectivity. The underlying basis of this selectivity toward certain receptors but not others, however, remains unclear but may involve particular structural elements. For example, the interaction between the N-terminus of RGS4 and the D2 and D3 Dopaminergic receptors leads to a potent negative effect on receptor-mediated inhibition of cAMP production. This activity is abolished upon deletion of the RGS4-N-terminus. Interestingly, other RGS proteins coexpressed with these receptors such as RGS2 and RGS9 have little or no effect on cAMP production.[58] In an earlier paper, Zeng *et al.* reported a similar observation where deletion of the RGS4-N-terminus led to a 10-fold decrease in RGS4 potency, and this also eliminated RGS4 receptor selectivity.[59] It thus seems like association between receptor and RGS4 may be critical in regulating RGS4 activity. The importance of RGS–receptor interactions is also demonstrated by the relationship between RGS2 and the AT1 angiotensin II receptor. A recent study has shown that the inhibitory effect of RGS2 on AT1 receptor stimulated Ca^{2+} activity is regulated via its N-terminus, possibly by directly interacting with the receptor. Deletion of this region or replacement with the N-terminus from other RGS proteins greatly decreased the ability of RGS2 to inhibit AT1 receptor signaling.[60] Similarly, the N-terminal domain of RGS2 has also been shown to affect M1 muscarinic receptor signaling.[50] Besides regulating RGS protein activity by changing their binding selectivity, the interaction between RGS protein and receptor may also alter RGS protein conformation and thus influence its activity. For example, the association between M3

receptor and RGS7 can induce the DEP domain of RGS7 to separate from Gβ and bind to the C-tail of the receptor, thereby inhibiting M3 receptor-mediated signaling.[61]

Besides direct association between RGS proteins and the receptor, another potential mechanism for RGS protein selectivity in regulating receptor signaling is that other factors such as scaffolding proteins may direct the RGS protein to the receptor and its associated G protein. Neurabin, a multidomain-scaffolding protein expressed in neural tissues, has been shown to assemble a complex between AT1R and RGS4, which allows the latter to attenuate AT1R signaling.[62] In addition, Spinophilin, a scaffolding protein known to regulate many GPCR signaling, is able to interact with multiple RGS proteins, such as RGS1, RGS2, RGS4, RGS16, and GAIP.[63] Recently, this protein has also been reported to inhibit α1-adrenergic receptor signaling and M3 receptor signaling by recruiting either RGS2 or RGS4 to the receptor in a ligand-dependent fashion.[63,64]

Reported effects of GPCRs and scaffolding proteins on RGS proteins have typically been ascertained indirectly, for example, through second messenger or electrophysiological assays.[65–69] To be sure that receptor-dependent differences in RGS protein inhibitory effects on signaling are indeed due to G protein deactivation, one would need to compare actual GAP activities; however, such experiments are technically challenging due to high background GTPase activities in cell-derived systems (multiple purified, reconstituted receptor–G protein preparations would not be practical for most labs). Membranes from baculovirus-infected insect cells, where high levels of protein expression are obtainable, do allow for the reliable measurement of RGS GAP activities on GPCR-activated G proteins,[54,70] and we have used this system to coexpress various GPCRs together with heterotrimeric G proteins. Membranes derived from Sf9 cells were used to compare different purified RGS proteins with different receptors (Fig. 10.1), anticipating that some GPCR–RGS combinations might work better than others. Contrary to this expectation we found no obvious selectivity between RGS proteins and GPCRs. Somewhat surprisingly a number of receptors tested did not appear to support RGS protein GAP activity at all. For some of these (δ-opioid, D2 dopaminergic, and 5HT1B receptors) there was little measurable agonist-stimulated GTPase activity (data not shown) so we could not be completely certain that there was no RGS GAP activity; however, the D4.4 dopaminergic receptor yielded an agonist signal comparable to those seen with the RGS protein-sensitive M2 muscarinic and α2a-adrenergic receptors coexpressed with

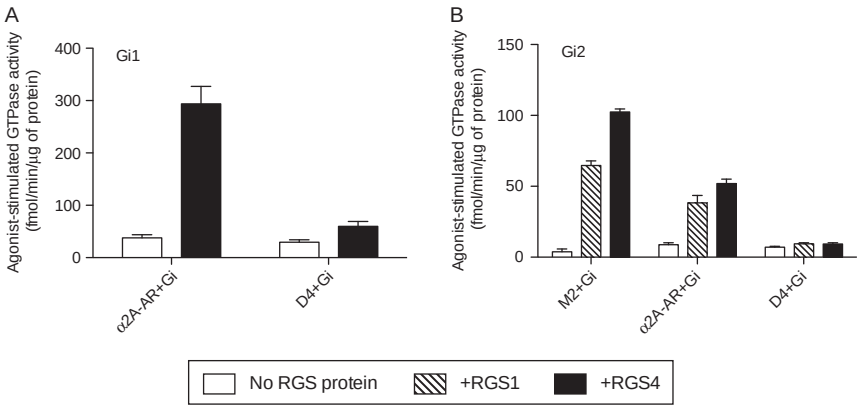

Figure 10.1 Sf9 insect cells were infected with baculoviruses encoding Gβ1, Gγ2, Gαi1 (A) or Gαi2 (B) and GPCRs as indicated and membranes from these were prepared as described by Cladman and Chidiac.[54] Agonist-stimulated GTPase activities were assayed essentially as described previously.[54] Agonists used were Epinephrine (α2a-adrenergic receptor), carbachol (M2 muscarinic receptor), and Dopamine (D4.4 dopaminergic receptor). The steady-state hydrolysis of [γ^{32}P]GTP by Sf9 membranes was measured in the absence and presence of purified RGS proteins. 50 μl reaction mixtures containing 20 mM HEPES, pH 7.5, 1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 10 μg/ml leupeptin, 1 μg/ml aprotinin, plus 10–50 mM NaCl, and 10 mM $MgCl_2$ were incubated for 5 min at 30 °C. The assay was stopped by adding 90 μl of ice-cold 5% Norit in 0.05 M NaH_2PO_4, the mix was centrifuged and the level of ^{32}Pi in the supernatant was determined by liquid-scintillation counting. Agonist-stimulated GTP hydrolysis was determined by subtracting the value from un-stimulated or antagonist-treated samples. Graphs are mean ± SEM values of 2 (A) or 3 (B) experiments with triplicate samples.

either Gi1 or Gi2. Notwithstanding this, essentially no RGS protein GAP activity could be detected with G proteins activated by the D4.4 dopaminergic receptor (Fig. 10.1).

The findings shown in Fig. 10.1 suggest that some GPCRs may be generally less supportive than others of RGS protein GAP activity. Although the reasons for this are unclear, it is consistent with the requirement for scaffolding proteins to enable certain RGS proteins to target particular signaling pathways.[71] Alternatively, another possible (and not mutually exclusive) explanation is that observed inhibitory effects of RGS proteins on signaling do not always reflect just their ability to promote GTP hydrolysis but may at least partly stem from other inhibitory effects, as noted above. To gain insight into the possible contributions of GAP-independent inhibitory mechanisms of RGS proteins on GPCR signaling, Anger and coworkers compared the effects on PLCβ activity of various RGS proteins coexpressed with M3 muscarinic receptor and either wild-type

or GTPase-deficient Gαq.[72] Based on the different rank orders observed among RGS proteins with the two different Gαq proteins, it was concluded that some RGS proteins were more reliant than others on GAP-independent mechanisms for their inhibitory effects on signaling.[72] While that is a reasonable interpretation, a more stringent test would be to compare the GAP effects of RGS proteins to their inhibitory effects on second messenger regulation. While that apparently has never been done systematically, another study did compare the effects of purified RGS16 and full length and N-terminally truncated RGS2 in membrane-based PLCβ and GTPase assays with activated M1 muscarinic receptor.[73] In the second messenger-based assay, full length RGS2 was the most potent of the three, RGS16 was intermediate, and truncated RGS2 was essentially without effect; in stark contrast to this, all three produced similar maximal GAP effects, with RGS16 the most potent, and full length and truncated RSG2 characterized by EC_{50} values respectively about three- and tenfold greater than RGS16.[73] Based on this finding, it would seem premature to assume that an RGS protein-induced decrease in GPCR signaling could generally be taken as an accurate readout of its GAP activity. Rather, G protein deactivation due to increased GTP hydrolysis may only be a partial contributing factor to such inhibitory effects.

4. GPSM PROTEINS

Similar to RGS proteins that regulate the deactivation of Gα proteins, GEFs and GDI proteins are able to modulate the activation rate of G protein-mediated cell signaling. GDI proteins directly interact with and stabilize the inactive (GDP-bound) Gα proteins and inhibit GDP dissociation, which will in turn slow down the activation of Gα. Being recognized as a GDI, the Gβγ dimer is considered to stabilize Gα in its inactive state, suppressing spontaneous Gα activation while also facilitating Gα coupling to receptors.[52,74]

In recent years, proteins other than Gβγ have been identified as GDIs. Oxidized human neuroglobin, a heme protein that is expressed in the brain binds to Gαi–GDP, competes with Gβγ binding, and inhibits GDP release.[75] Interestingly, a 25–35% homology was found between neuroglobin and the RGS domain of GPCR kinases (GRKs).[76]

Among the GDIs identified for heterotrimeric G proteins, GPSM proteins have been studied extensively over the last decade or so. These proteins all share a highly conserved 15–20 amino acid Gα-binding motif

that impedes GDP dissociation and also promotes Gβγ dissociation.[77–79] Although there is broad agreement regarding the biochemical nature of this domain, there is no consensus on what to call it. The first protein found to contain the domain was loco, an RGS12 homologue, found in Drosophila melanogaster.[80] The observation that RGS12 shared a similar Gα protein-interacting region distinct from the RGS domain led to the name Gαi/o-Loco motif, or GoLoco for short.[81] The same conserved region was named G protein-regulatory (GPR) domain at around the same time by Cismowski and colleagues, who identified it as a receptor-independent activator of Gβγ signaling.[77,78] The domain also was found in the Leu-Gly-Asn-enriched protein LGN and thus has also been referred to as the LGN motif,[82,83] and proteins bearing this domain are sometimes referred to collectively as Group II Activator of G Protein Signaling (AGS) proteins.[10] The term GPR while adequately descriptive is also used for GPCRs (particularly orphans) (www.genenames.org), whereas the remaining terms do not convey functional information. Therefore we prefer the term *GPSM domain*, in accordance with the names proposed by the HUGO Gene Nomenclature Committee for several of the proteins that contain it (GPSM1/AGS3; GPSM2/AGS5/LGN; GPSM3/AGS4/G18; GPSM4/Pcp-2/L7) (www.genenames.org). Although to date only four proteins have been named in this manner, we also suggest the collective term *GPSM proteins*.

Among the Gα subfamilies, most GPSM domains favor binding to Gαi/o proteins with typically higher affinity toward Gαi than Gαo.[84] Some GPSM proteins also interact with G proteins other than Gi or Go. For example, AGS3 interacts with Gαt and blocks rhodopsin-induced dissociation of GDP.[85] The selectivity between GPSM proteins and Gα protein subtypes seems to be influenced by amino acid residues outside of the core domain of the GPSM domain, as well as the all-helical domain of Gα.[86]

The GPSM domain has a higher binding affinity for inactive GDP-bound Gα relative to either nucleotide-free or activated Gα.[86] The association between the GPSM domain and Gα-GDP decreases the rate of nucleotide exchange on Gα, thus this leads to an inhibitory effect on Gα-activation.[87] In addition, binding of the GPSM motif results in a significant conformational change of the switch regions of the Gα subunit and impairs the binding of Gβγ. As a result, Gα–GDP–Gβγ and Gα–GDP–GPSM complexes are mutually exclusive.[42,87,88]

Since the GPSM motif and Gβγ are able to compete for Gα binding,[77] it has been hypothesized that the GPSM–Gα interaction may either promote heterotrimer dissociation or interfere with subunit re-association. A GPSM

domain-based peptide derived from GPSM1 has the ability to inhibit Gα binding to Gβγ 10 times more effectively than the Gβγ hot spot-binding peptide (SIGK) (which also interferes with the binding between Gβγ and Gα) with an IC_{50} of 250 nM. In addition, this GPSM peptide was able to cause a rapid dissociation of Gβγ from Gα about 13-fold higher than the intrinsic K_{off} of Gβγ from Gα.[89] However, on the other hand, full-length RGS14 failed to disrupt pre-formed G protein heterotrimer.[90] Still, a GPSM peptide derived from RGS14 seemed to be able to prevent the reformation of the Gαβγ heterotrimer.[91] Overall, the ability of the GoLoco motif to promote G protein subunit dissociation may depend on the experimental or cellular context and the particular proteins in question.

The original discovery of GPSM motif containing proteins was based on a yeast-based screen for receptor-independent activators of heterotrimeric G protein signaling.[77] In this screen, GPSM1 was identified as being able to "release" Gβγ, and thus promote the selective activation of Gβγ-mediated signaling.[77] In addition, silencing GPSM1 in neuron progenitor cells resulted in a shift in spindle orientation and an abnormal differentiation of the cells into neurons, further suggesting a positive link between GPSM1 and Gβγ function.[92]

Ectopic GPSM3 expression was also found to inhibit $PLC\beta_2$ activation by Gβγ dimer in COS-7 cells.[93] This result is somewhat surprising since one would expect GPSM proteins to activate Gβγ signaling. In this study, it appeared that inhibition was due to an interaction between GPSM3 and the Gβ subunit. However, this interaction was observed in a COS-7 cell overexpression system, thus whether this reflects a direct interaction between GPSM3 and Gβ or an involvement of these two proteins in a complex is unclear.

Full-length GPSM2 (LGN) and constructs expressing only the GPSM domains of this protein were found to activate basal K^+ current, but inhibited dopamine receptor-mediated GIRK channel activation. The molecular mechanisms that control these distinct outcomes may reflect different effects of GPSM motifs on Gα and Gβγ signaling. The elevated basal K^+ current is likely due to a GPSM-promoted dissociation of the heterotrimer and subsequent Gβγ-dependent channel activation. On the other hand, the inhibitory effect on receptor signaling may stem from the GDI activity, which decreases Gα activation.[94]

The effects of GPSM proteins on ion channel activity were also demonstrated in Xenopus oocytes expressing Cav2.1 and κ-opioid receptors. Low levels of GPSM4 (Pcp-2) enhanced the inhibitory effect of opioid receptor

has on Cav2.1 current. This effect was blocked by adding the C-terminus of β-adrenergic receptor kinase (GRK2), indicating a Gβγ-dependent pathway. Interestingly, at a higher expression level, GPSM4 exhibited the opposite effect.[95]

Although GPSM proteins appear to regulate Gα activation, they also may disrupt receptor–G protein coupling, ligand binding and signaling, etc. It has been reported that cytosolic GPSM1 (AGS3), but not membrane-associated GPSM1, inhibits 5HT receptor coupling to Gα subunits in a dose-dependent manner by interfering with the membrane association of Gαi1 subunits.[96] This inhibitory effect may in turn lead to a reduction in receptor-mediated cell signaling, however, this hasn't been tested. BRET assays using HEK293 cells suggest that receptor activation leads to a moderate decrease in GPSM1–G protein interaction, apparently due to Gα activation. In addition, a small Gα-dependent BRET signal is also detected between GPSM1 and cell-surface receptor, which indicates the possible formation of a receptor–G protein–GPSM complex.[97] A GPSM peptide derived from GPSM1 inhibited GDP dissociation subsequent to rhodopsin-dependent activation of transducin. This inhibitory effect may be due to its GDI activity; however, the possibility that the GPSM domain competes with the receptor for binding to G protein again cannot be excluded.[85]

GPSM3 (a.k.a. G18 or AGS4) is another multiple GPSM protein of great interest. Our lab has shown that beside the GPSM motif, which acts as a GDI on Gαi, the N-terminal proline-rich domain of GPSM3 may also serve as a Gα protein interaction partner; moreover, this domain may have varying biochemical activities toward different G proteins.[98] Not many studies have looked at the effects of GPSM3 on receptor signaling. Similar to GPSM1, a G protein–GPSM3 complex may together serve as a substrate for agonist-induced receptor activation. Upon receptor activation the interaction between GPSM3 and G protein is again reduced.[43] The remaining BRET signal between these two proteins may reflect either a membrane or intracellular localization of the complex. We have shown that the GPSM domains of this protein are able to inhibit the M2 receptor and RGS4-stimulated GTPase activity of Gi in isolated Sf9 membranes. However, whether this effect is due to changes in nucleotide exchange rate *per se* is difficult to know. Alternatively, this inhibitory effect may reflect competition between GPSM3 and the receptor for Gα proteins, or disruption of the G protein heterotrimer. It would be interesting to see if binding between GPSM3 and the G protein is able to alter receptor–G protein-coupling

properties in the presence of agonists. In another study, ectopic GPSM3 expression in HEK293 cells inhibited signaling of the agonist-activated lysophophatidic acid receptor to endogenous PLCβ activation and the generation of inositol phosphate.[93]

Taken together, the available data on the effects of GPSM proteins on GPCR signaling would appear to indicate that under basal conditions Gβγ signaling may be stimulated while in the presence of a GPCR agonist signaling both Gα- and Gβγ-mediated signals are decreased due to a reduced nucleotide exchange rate and/or a loss of coupling between the receptor and the G protein heterotrimer. The detailed mechanism of how GPSM proteins affect GPCR signaling still remains largely unknown. It seems like a GPSM–Gα complex may serve as substrate for Ric-8 and together, play an important role in asymmetric cell division.[99] On the other hand, whether a GPSM–Gα protein complex is able to serve as an alternate Gαβγ and mediate receptor signaling also remains apparently untested.

Functional studies of GPSM motif proteins have implied a wide range of physiological roles. Many investigations have focused on the involvement of GPSM proteins in cell division,[100] which can also result in physiological changes. However, the role of GPCR-mediated signaling may also be significant. GPSM proteins, especially GPSM1, may have critical functions in cocaine, heroin, and alcohol addiction. During late withdrawal from repeated cocaine administration, the expression level of GPSM1 was found to be increased. Such changes in turn appear to lead to a decrease in Dopamine and/or opioid receptor-mediated Gi signaling and the activation Gβγ signaling, resulting in behavioral sensitization to cocaine challenge.[101] On the other hand, GPSM1 knock down is associated with the opposite effect.[102,103] The effect of GPSM4 (Pcp-2) on behavioral function has also been studied, and GPSM4 KO mice show a sex-dependent anxiety-like phenotype, with male mice exhibiting an increased anxiety level, similar to 5-HT1AR KO mice.[104] However, whether this is due to an inhibitory effect of GPSM4 on 5HT signaling remains unknown. Although clearly further study is required, the available data are consistent with the notion that GPSM proteins modulate GPCR signals *in vivo*, albeit in ways that are not yet well defined.

5. NONRECEPTOR GEFs

The vast majority of known GEFs for Gα proteins are GPCRs, but over the years it has become evident that other proteins exist that can promote the dissociation of GDP from Gα. These include the resistance

to inhibitors of cholinesterase 8 (Ric-8) proteins (Ric-8A, Ric-8B, Ric-8BΔ9),[105] Gα-interacting vesicle-associated protein (GIV),[106] RASD1/AGS1/Dexras1,[107] GAP-43/neuromodulin/B-50,[108] GPSM3/AGS4/G18,[98] cysteine string protein (CSP),[109] human phosphatidylthanolamine-binding protein (hPBP),[110] β-amyloid precursor protein,[111] presenillin-1[112] and the yeast protein Arr4.[113] In addition, nucleobindin 1 and nucleobindin 2 each contain a region of homology to GIV and have been shown to produce measurable increases in Gαi3 steady-state GTPase activity, implying GEF activity.[114] Apart from similarities among the Ric-8 and GIV-like proteins there is no obvious structural resemblance between the noncanonical GEFs, which variously interact with members of all four Gα protein subfamilies. Several of these proteins have been observed to alter GPCR signals, although as discussed below some may have additional properties that might have an indirect impact on signaling. Non-GPCR GEFs could potentially either decrease agonist singling by interfering with receptor–G protein interactions, or alternatively could augment signaling by maintaining receptor-activated G proteins in an activated state, and evidence consistent with both possibilities has been reported. It is expected as well that effects of noncanonical GEFs on GPCR signaling will have physiological and pharmacological consequences, but at present there is little information about such possibilities.

Apart from those noted above, other proteins have been shown to display attributes consistent with GEF activity, but have not been unequivocally shown to promote GDP dissociation from Gα proteins. For example, the human transcription factor E2F8 in *S. cerevisiae*-based reporter assays was found to amplify a-factor GPCR signaling, and to promote signaling in a yeast strain lacking the receptor; positive effects of E2F8 were reduced or lost in strains lacking Gβ or components downstream of the G protein and upstream of the reporter, and reduced in a strain coexpressing RGS4.[115] In addition, E2F8 has been found to co-immunoprecipitate with Gαi (T Baranski, personal communication). These observations suggest that E2F8 can act as a GEF, but this has not yet been demonstrated directly. Another potential nonreceptor GEF, NG-GPA, has been identified in NG108-15 cells; however, even though extracts from these cells have been shown to promote GTPγS binding to purified Gα proteins in a manner that is seemingly distinct from GPCRs, this putative novel GEF has not been successfully isolated.[116] As well, numerous studies have shown that cellular Gα proteins can be turned on in response to activation of receptors that traverse the membrane only once (e.g., growth factor and cytokine receptors),

which was postulated to reflect direct effects on nucleotide exchange and/or changes in G protein activity due to tyrosine residue phosphorylation.[117,118] Evidence supporting such mechanisms may be viewed as less than definitive,[119,120] and it remains uncertain which if any "non-G protein-coupled" receptors are capable of directly activating Gα proteins.[118] Other mechanisms may be possible, and in a growing subset of cases, it is clear that Gα can be turned on indirectly via the transactivation of heptahelical receptors by other receptor types.[119,120]

5.1. Ric-8

The Ric-8 proteins are the most thoroughly studied nonreceptor GEFs. Ric-8 was first identified in *Caenorhabditis elegans* as a gene whose deficiency allowed the organism to survive treatment with cholinesterase inhibitors,[121] inferring that the corresponding protein might somehow promote acetylcholine signaling. Single isoforms of Ric-8 have been found in *C. elegans* and *Drosophila*[122], whereas two *Ric-8* genes have been found in mammals. Ric-8A was identified in two independent yeast two-hybrid screens of rat and mouse brain cDNA libraries respectively using GTPase-deficient mutants of Gαo[123] and Gαq[124] as bait. Ric-8B was found using GTPase-deficient Gαs(long)[123] or Gαs(short)[125] against brain cDNA libraries and also using Gαs homologue Gαolf as bait in a yeast two-hybrid screen of an olfactory epithelium library.[126]

Although Ric-8 proteins primarily target free Gα proteins, they can also bind to Gαβγ heterotrimers and/or free Gβγ albeit with relatively low affinity,[105] and overall Gβγ tends to produce negative effects on Ric-8–Gα interactions.[105,123] As noted above (Section 4), Ric-8 proteins can also bind to some GPSM proteins, and thus such proteins fulfill a role in intracellular Ric-8-stimulated Gα functions analogous to that of Gβγ in GPCR signaling at the plasma membrane. Other signaling proteins identified as Ric-8A binding partners include adenylyl cyclase type 5[127] and neural cell adhesion molecule 180.[128]

The two mammalian isoforms of Ric-8 have distinct effects on Gα proteins. Ric-8A acts as a GEF for free Gαi/o, Gαq, and Gα12 proteins, with no apparent effect on Gαs nucleotide exchange.[123] Full length Ric-8B is primarily a GEF for Gαs and its homologue Gαolf, although in vitro Ric-8B also can have appreciable effects on Gαq and Gα13, but not Gαi1.[105] The shorter splice variant Ric-8BΔ9 appears to be a significant GEF for Gαs only,[105] as it shows little or no effect on other G proteins tested

including Gαolf.[105,126] Full length Ric-8B and Ric-8A also promote GTPγS dissociation (and presumably GTP dissociation) from Gαs and Gαq, respectively, although this effect is not likely to impede G protein activation at normal cellular GTP concentrations.[105] Ric-8 proteins bind preferentially to their Gα targets in the GDP-bound state, and promote GDP dissociation to form a stable nucleotide-free transition state complex.[105,123] The affinity of Ric-8 for Gα tends to be decreased by Gα activation,[105,123] although in some cases, Ric-8 appears to bind to activated Gα.[105,125,129]

Dynamic cellular functions affected by Ric-8 include G protein-mediated signaling, cell division and differentiation/development. In addition, Ric-8 proteins are now recognized to play a major role in G protein processing. The loss of either Ric-8A or Ric-8B results in embryonic death,[130] pointing to a vital role in early development.[131] In cells derived from Ric-8 null blastocysts, the lack of either isoform was associated with greatly decreased G protein levels, consistent with other studies showing that cellular G protein levels tend to track with levels of their functional Ric-8 partners.[130] *Ric-8B*$^{-/-}$ cells were largely deficient in Gαs while *Ric-8A*$^{-/-}$ cells similarly exhibited major decreases in Gαi1/2, Gαq, and Gα13 as well as a moderate decrease in Gβ.[130] This likely reflects the ability of Ric-8 proteins to bind to nascent Gα proteins and act as chaperones that prevent their degradation prior to membrane insertion.[130] In addition, Ric-8 may impede Gα degradation by inhibiting its ubiquitination,[132] and it appears that Ric-8 can target Gα proteins to an endomembrane prior to trafficking to the plasma membrane,[129,130] which might facilitate their interactions with GPCRs. Overall Ric-8 proteins can have profound effects on G protein levels and localization, and it follows that cellular effects associated with changes Ric-8 could potentially be due to altered G protein availability, activity, or both. As summarized below, the data currently available strongly suggest but do not prove that Ric-8 proteins increase G protein signaling by virtue of their GEF activities.

Ric-8 proteins have been found to enhance G protein-mediated receptor signals in a number of systems. Evidence suggests an important role in the function of sensory GPCRs, where heightened sensitivity to external stimuli might convey a survival advantage. For example, Ric-8B exhibits a similar expression pattern to the olfactory G protein Gαolf in the nasal cavity and brain.[126] The mouse-odorant receptor mOR-EG when transiently expressed by itself or together with either Gαolf and Ric-8B in HEK-293 T cells failed to respond to an activating ligand (eugenol), but was able to stimulate cAMP production when all three proteins were expressed

simultaneously.[133] This may be partly due to the increased presence of Gαolf at the cell periphery[133] and/or its upregulation by Ric-8B.[134] However, in other experiments the stimulation of adenylyl cyclase via β2-adrenergic or D1-dopaminergic receptors was measureable in cells transfected with receptor only, and these signals were greatly enhanced by the coexpression of both Gαolf and Ric-8B not by the coexpression of only one of these.[126] The enhancement of G protein activation in each case could only be observed in the presence of a receptor agonist,[126,133] which suggests the possibility that Ric-8B acts as an amplifier for these GPCR signals. Similar functions have been suggested for Ric-8 in other sensory systems, for example in *C. elegans* Ric-8 appears to work in conjunction with AGS-3 to fine-tune responses to aversive by amplifying and/or prolonging GPCR-initiated Gαo signaling via GPCRs coupled to Gαo.[135]

There is indirect evidence that Ric-8A may potentiate Gαq-mediated GPCR signals, as Ric-8A siRNA treatment of HEK293 T cells decreased signals mediated via endogenous Gq-coupled P2Y purinergic and endothelin receptors.[124] While such changes again could reflect a decrease in endogenous G protein levels associated with the loss of Ric-8A, it was also noted in that study that Ric-8A became translocated to the plasma membrane in an agonist-dependent manner.[124] Although the additional expression of exogenous wild-type Ric-8A did not enhance agonist signaling in this study, a myristoylation sequence-tagged form of the protein that spontaneously localized to the plasma membrane was associated with a significant increase in agonist-stimulated signal.[124] These observations reinforce the findings of an earlier study where Ric-8A colocalized at the plasma membrane with Gαq and Gαs subsequent to agonist activation of Gq- and Gs-coupled GPCRs, respectively.[125] It thus appears that Ric-8 may be recruited, through an undefined mechanism, to GPCR-activated Gα proteins in the plasma membrane to strengthen existing signals.

The mechanistic details of how Ric-8 may enhance GPCR signals are not yet known. Ric-8 proteins appear not to be able to interact productively with Gβγ-associated Gα subunits.[105,123] Gα and Gβγ may remain attached to one another[136] and also to the activating GPCR[21] during signal transduction. Therefore it is unclear how Ric-8 might usurp Gα–effector interactions, but it has been proposed that this may occur in conjunction with GPSM proteins, which are known to promote the dissociation of G protein heterotrimers.[135] This idea is supported by observations in *C. elegans* that the ability of Ric-8 to promote Gαo-mediated responses to aversive stimuli is completely dependent upon GPSM 1 (AGS3).[135] Alternatively, it is

conceivable that Ric-8 could promote signaling by docking onto the effector protein, and indeed adenylyl cyclase type V (AC5) is able to bind to Ric-8A, an interaction that appears to facilitate the ability of Gαi to inhibit both forskolin-stimulated and receptor-stimulated cAMP production.[127] An anchoring site for Ric-8 might not always be required for post-GPCR stimulation of Gα signals, as G protein heterotrimer dissociation may be more complete in some cases than in others.[136]

5.2. Gα-interacting vesicle-associated protein (GIV)/Girdin

GIV is a large (1870 amino acid residue) multidomain protein that contains a G protein-binding domain that was identified by its ability to interact with inactive, GDP-bound Gαi3.[137] Interestingly, Gαi3 binding can occur through two separate domains on GIV, one insensitive and the other sensitive to the activation state of the Gα protein.[106] A truncated form of GIV made up of the last ~250 amino acid residues containing the activation-sensitive domain but lacking the activation-insensitive one was found to promote guanine nucleotide exchange on Gαi3; a single point mutation (F1685A) in the G protein-binding domain of this construct was associated with profound decreases in both GEF activity and Gαi3 binding.[138] In addition to its Gα-binding regions, GIV also contains a hook domain that interacts with microtubules, a coiled-coil domain that mediates homodimerization, a phosphatidylinositol 4-monophosphate lipid-binding domain, which interacts with plasma membrane and Golgi, and an Akt/actin/epidermal growth factor-binding domain, which modulates growth factor signaling.[139] GIV has been shown to have important but incompletely understood effects in cell migration, wound healing, and cancer metastasis.[139]

Wild-type GIV transiently expressed in cells promotes both Akt (protein kinase B) activation and cell migration, effects that are not observed with a full-length construct bearing the F1685A substitution.[106] GIV-dependent Akt activation and chemotaxis appear to occur through the Gαi-dependent, Gβγ-mediated stimulation of PI3 kinase, which can be initiated via either GPCR (fMLP, LPA) or receptor tyrosine kinase (insulin, EGF) signaling.[106,137] When heterotrimeric GSH–Gαi3–Gβγ was isolated on glutathione beads and exposed to increasing amounts of the C-terminal region of GIV or the corresponding F1685A mutant, a dose-dependent decrease in the amount of heterotrimer was observed with the former but not the latter.[106] Since an activating nucleotide apparently was not included in this experiment, it would appear that the GEF effect of GIV and consequent G protein activation may

not be required for Gβγ-dependent PI3 kinase activation, but rather this could reflect competition between GIV and Gβγ for Gα.

Regardless of the activation state of Gα, it is not clear how either GPCR or RTK activation might lead to the promotion of Gβγ signaling by GIV. The direct binding of RTKs to GIV[139] suggests the intriguing possibility that these receptors could induce a conformational change in GIV that affects its ability to bind to and/or promote nucleotide exchange on Gαi; however, evidence for such a mechanism is lacking. For GPCRs, it is conceivable that GIV could maintain free Gβγ levels by re-activating Gα, but again there are no data to either support or refute this notion.

5.3. RASD1/AGS1/Dexras1

RASD1 (also called AGS1 or Dexras1) is an atypical monomeric G protein that along with its homologue RASD2 (Rhes) belongs to a distinct subclass in the Ras superfamily.[140] The architecture of these two proteins closely resembles that of other Ras proteins, although they are larger due to the presence of two cationic insert regions.[107] Both RASD1[107] and RASD2[141] display atypical guanine nucleotide-binding characteristics, with relatively low affinity for GDP and increased GTP binding under basal conditions compared to other Ras-like G proteins.

RASD1 can activate Erk1/2 in a pertussis toxin-sensitive,[107] Gβγ-dependent[142] manner when transfected into cells and it can act as a guanine nucleotide exchange factor for Gαi/o proteins,[107,143] but it does not appear to interact appreciably with either Gαs or Gα16.[78] Mutations in highly conserved amino acid residues in the predicted guanine nucleotide-binding site of RASD1 do not appear to destabilize the protein, but do prevent it from signaling through Gαi in cells,[78] suggesting that the binding of either GDP or GTP to RASD1 itself may be necessary for its activating effects. RASD2 is not known to act as a GEF but it has been reported to interact with Gα proteins.[144] To complicate matters further, both RASD1 and RASD2 can apparently bind to Gβγ dimers.[140,145] In addition to their interactions with heterotrimeric G proteins or subunits thereof, RASD1 and RASD2 can bind to several other signaling proteins. For example, RASD1 binds to the scaffolding protein CAPON (carboxyl-terminal PDZ ligand of neuronal nitric oxide synthase) through which it can form a ternary complex with nNOS, and this juxtaposition facilitates *S*-nitrosylation-mediated increases in the binding of GTP to and the GEF activity of RASD1.[146] Like other Ras proteins, RASD2 can activate p85-dependent PI3 kinase.[141,147]

Multiple effects of RASD1 and RASD2 on GPCR-dependent and GPCR-independent heterotrimeric G protein signaling have been described, but in general the underlying mechanisms are not well understood. In contrast to its ability to independently stimulate Gαi/Gβγ-dependent ERK activation,[107,142] RASD1 has been found to inhibit D2 dopaminergic-[148] or *N*-formyl peptide receptor-activated ERK signaling.[142] RASD1 also decreased the ability of the *N*-formyl peptide receptor agonist f-MLF to promote GTPγS binding to membranes containing this receptor.[142] RASD1 was also found to inhibit the ability of the Gαi-coupled M2 muscarinic receptor to activate Gβγ-dependent inwardly rectifying potassium (GIRK) currents, whereas a mutant RASD1 containing an inactivating point substitution in its nucleotide-binding site failed to inhibit signaling.[149] In another study, RASD1 and RASD2 both were able to attenuate M2 muscarinic receptor and Gβγ-dependent inhibitory effects on N-type calcium channels, but had no effect on the ability of β2-adrenergic receptors to inhibit this channel via Gαs and Gβγ.[150] The strong similarity between homologues in the latter study suggests that either RASD2 may also be able to act as a GEF on Gαi, or alternatively that GEF activity *per se* does not play an important role in the inhibition of this Gαi- and Gβγ-mediated signaling pathway. Several studies have examined the potential effects on adenylyl cyclase regulation of RASD1 and RASD2, some of which have yielded findings consistent with the general idea that these proteins promote inhibitory Gαi–Gβγ signaling in the absence of GPCR activation.[142,144] Overall, it seems that both RASD1 and RASD2 stimulate signaling via Gi heterotrimers under basal conditions but that this in turn can impede the activating effects of GPCRs; however, the details of the interplay among the various signaling proteins involved in these changes it is not yet clear.

5.4. Other nonreceptor GEFs

While the impact of Ric-8, GIV and RASD1 on GPCR signaling is not well understood, even less is known in this regard about the other nonreceptor GEFs that have been identified to date. GAP-43 is a 43-kDa neuronal protein that was found to promote GDP dissociation from both isolated Gαo protein and Go heterotrimer, with comparable effects on Gαi but not Gαs.[108] The addition of purified GAP-43 to reconstituted vesicles containing purified M2 muscarinic receptors plus heterotrimeric Go increased agonist-stimulated GTPase activity in a greater-than-additive

manner, and correspondingly the injection of purified GAP-43 into *Xenopus* oocytes expressing either M2 muscarinic or 5HT1C serotonergic receptors exacerbated agonist effects on chloride channel opening.[151] Similarly, the μ-opioid receptor-mediated activation of GIRK channels in oocytes was augmented by the intracellular injection of the novel Gαi1 GEF hPBP,[110] and the nonreceptor yeast GEF Arr4 was found to increase GPCR signaling stimulated by α-factor.[113] In contrast to these effects, the N-terminal GEF function of GPSM3/AGS4/G18 was not found to increase M2 muscarinic receptor-stimulated Gαi1, but rather decreased this activity, suggesting that the receptor and the nonreceptor GEF may be competing for the Gα protein.[98] Taken together, the available data suggest that nonreceptor GEFs can have direct (and possibly also indirect) effects on GPCR-stimulated G protein signaling, and that this can either augment or impede GPCR signaling.

6. INTERACTIONS BETWEEN G PROTEIN MODULATORY DOMAINS

Although we have largely focused on individual effects of nonreceptor GEFs and RGS and GPSM domains, there are two broad areas that merit further attention, namely interactions *between* different Gα modulators, and *within* the various proteins that contain multiple Gα interaction sites. For example, there are now multiple studies demonstrating coordinated interactions between Ric-8 and GPSM proteins,[99,152–156] and in one case this may have an impact on GPCR signaling.[135] It seems likely that GPSM may serve as an anchoring domain in such cases, analogous to the role played by Gβγ in GPCR signaling.

About half of the GPSM proteins contain multiple GPSM domains,[10] but differences between the roles of the individual domains have not been identified nor is it known how they might function in a coordinated fashion. In a few cases G protein-interacting domains with contrasting activities are found in the same protein. Whether these act on G proteins in a coordinated, sequential, or mutually exclusive manner remains to be investigated. GPSM3 in addition to its three GPSM domains contains a proline-rich N-terminal region that acts as a GEF for Gαi,[98] and it is conceivable that these might function in a coordinated manner analogous to Ric-8–GPSM complexes.

The two largest RGS proteins, RGS12 and RGS14, each contain a GPSM domain in addition to an RGS domain. At first blush this pairing might seem odd, as the GDI function of the GPSM domain would presumably leave little for the RGS domain to do. This does not seem to happen,

though, as full length RGS14 actually displays a better GAP effect on GPCR-activated G proteins than the isolated RGS domain.[157] The GDI function of the RGS14 GPSM domain, in contrast, appears to predominate under other conditions.[156] Whether RGS12 functions in the same way, and how the switch between RGS14 G protein-regulatory effects is accomplished, remains to be explained.

7. CONCLUSIONS

The studies summarized herein provide compelling evidence that GPCR signaling is influenced by proteins that modify G protein-activation states, and to a large extent the observed changes in signaling are consistent with the known biochemical effects of these proteins on isolated Gα. GPCR signaling is limited by RGS proteins that promote G protein deactivation, and by GPSM proteins that impede activation; nonreceptor GEFs activate Gα and in some cases augment GPCR signaling. Still, it is seldom clear to what degree changes in the activation state of Gα may underlie the alterations in receptor responses caused by RGS proteins, GPSM proteins, and nonreceptor GEFs, as the corresponding nucleotide-binding state and hydrolytic activity of the G protein are generally not monitored. With all three types of Gα modulators, it seems clear that their ability to physically disrupt interactions between a target Gα protein and its affiliated GPCR, Gβγ, or effector protein can also strongly influence receptor signals. These tendencies are illustrated in Fig. 10.2.

Steric or competitive effects on signaling are most widely recognized with GPSM proteins, which were identified as Group II AGS proteins based on their ability to promote Gβγ signaling and in spite of their propensity to impede Gα activation.[10] Combined, these effects tend to promote Gβγ-dependent signaling under basal conditions but interfere with activation by GPCRs due to heterotrimer disruption and/or negative effects on nucleotide exchange. Nonreceptor GEFs may produce similar stimulatory and inhibitory effects but for partly different reasons, and as noted the effects on heterotrimer stability and binding to targets other than Gα can vary considerably from one nonreceptor GEF to the next. Apart from their incompletely understood effects on Gαs-mediated signaling, RGS proteins are generally presumed to inhibit GPCR signals by virtue of their GAP effects; however, a direct demonstration of this in any system is still lacking and

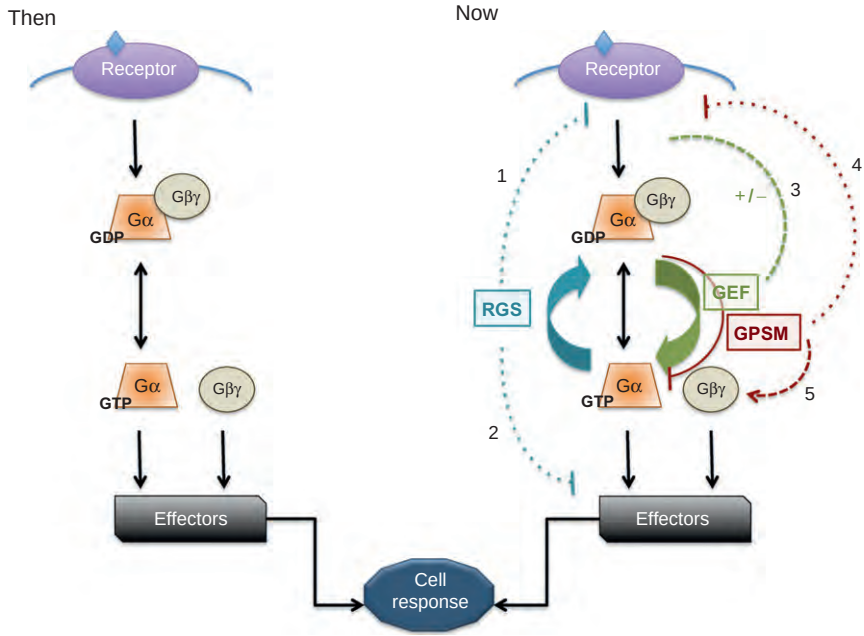

Figure 10.2 Model illustrating the effects of RGS, GPSM and nonreceptor GEF proteins on GPCR signaling. Solid lines indicate effects on G protein activity and dashed lines show potential additional effects on receptor signaling as follows: 1, Direct/indirect interactions between RGS proteins and the receptor may inhibit signaling. 2, RGS proteins can inhibit effector regulation by blocking G protein–effector interactions. 3, Nonreceptor GEFs may either potentiate or decrease receptor signaling respectively by maintaining GPCR-activated Gα in the activated state or by competing with the GPCR for Gα. 4, GPSM proteins may inhibit receptor signaling by interfering with receptor–G protein coupling. 5, GPSM proteins may activate Gβγ signaling.

some findings suggest that non-GAP effects can also play a substantial role. In time hopefully such ambiguities will be resolved, and the impact of these proteins on GPCR signaling will be better understood.

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