Activators of G-protein Signaling Exhibit Broad Functionality and Define a Distinct Core Signaling Triad

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ABSTRACT

Activators of G-protein signaling (AGS), initially discovered in the search for receptor-independent activators of G-protein signaling, define a broad panel of biological regulators that influence signal transfer from receptor to G-protein, guanine nucleotide binding and hydrolysis, G-protein subunit interactions and/or serve as alternative binding partners for G\(\alpha\) and G\(\beta\gamma\) independent of the classical heterotrimeric G\(\alpha\beta\gamma\). AGS proteins generally fall into three groups based upon their interaction with and regulation of G-protein subunits: Group I – guanine nucleotide exchange factors (GEF), Group II – guanine nucleotide dissociation inhibitors, Group III – bind to G\(\beta\gamma\). Group I AGS proteins may engage all subclasses of G-proteins, whereas Group II AGS proteins primarily engage the Gi/Go/transducin family of G-proteins. A fourth group of AGS proteins, with selectivity for G\(\alpha\)16 may be defined by the Mitf-Tfe family of transcription factors. Groups I-III may act in concert generating a core signaling triad analogous to the core triad for heterotrimeric G-proteins (GEF – G-proteins – Effector). These two core triads may function independently of each other or actually cross-integrate for additional signal processing. AGS proteins have broad functional roles and their discovery has advanced new concepts in signal processing, cell and tissue biology, receptor pharmacology and system adaptation providing unexpected platforms for therapeutic and diagnostic development.
Introduction

Since the initial discovery of the class of proteins defined as Activators of G-protein signaling in 1999, concepts defining the biology of G-proteins as signal transducers have evolved to embrace surprising mechanisms of regulation and diverse functional roles for the “G-switch.” AGS and related entities may have evolved to provide a mechanism for cells to adapt acutely and longer term to physiological and pathological challenges without altering the core components of a long-established signaling triad – receptor, G-protein, effector. During the course of evolving such adaptation or modulatory mechanisms, the component units of such (e.g. specific AGS proteins) may have been “hijacked” to also serve as core entities of signaling pathways that are not yet fully defined or may actually represent a window into a signaling system that is itself in the process of evolving. This mini-review touches upon the diverse functional roles of AGS proteins and expands recent concepts related to these proteins and signal processing.

Activators of G-protein Signaling: Mechanistic and Functional Diversity for the “G-Switch”

Activators of G-protein signaling refer to a class of proteins initially defined in a yeast-based functional screen for mammalian cDNAs that activated G-protein signaling in the absence of a G-protein coupled receptor (Cismowski et al., 1999; Takesono et al., 1999). AGS proteins fall into three groups based upon their engagement with different G-protein subunits and the biochemical consequences of this engagement with respect to Gα nucleotide exchange and/or subunit interactions. Serendipitously, these three groups were apparent with the discovery of the first three AGS proteins – AGS1, 2 and 3 – each of which exhibited different mechanisms of action. This grouping then also captures other proteins with similar actions or functional domains. Sato et al. recently defined a fourth group of AGS proteins (AGS11-13) that activate G-protein
signaling in the yeast-based functional assay with Gα16, but not with yeast strains expressing Gpa1, Gαi2 or Gα3 (Sato et al., 2011). Although not as robust as the activation signal involving Gα16, AGS11-13 also activated G-protein signaling in yeast strains expressing Gαs (Sato et al., 2011). AGS11-13 actually encode three members of the Mitf-Tfe family of basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factors: transcription factor E3, microphthalmia-associated transcription factor, and transcription factor EB (Sato et al., 2011).

The biochemical properties of the G-protein regulation by AGS11-13 have not yet been fully characterized; however, TFE3 and Gα16 are both upregulated in the hypertrophic mouse heart and TFE3 coexpression with Gα16 resulted in the nuclear localization of Gα16 and marked elevation of the mRNA encoding the tight junction protein claudin 14 (Sato et al., 2011).

**Group I AGS Proteins**

Group I AGS proteins, which includes AGS1 (Dexras1, RASD1), Ric-8A and Ric-8B (resistance to inhibitors of cholinesterase) and GIV (Gα Interacting, Vesicle-associated protein, Girdin, APE - Akt-phosphorylation enhancer), act as non-receptor guanine nucleotide exchange factors (GEF) for Gα and/or Gαβγ (Chan et al., 2011a; Chan et al., 2013; Cismowski et al., 2000; Cismowski et al., 1999; Garcia-Marcos et al., 2009; Tall, 2013; Tall and Gilman, 2005; Tall et al., 2003). Rhes (Ras homologue enriched in striatum, RASD2) which exhibits 66% amino acid similarity to AGS1 and interacts with G0i and regulates G-protein signaling, may be included in this Group of AGS proteins, although it has not yet been shown to exhibit GEF activity in experiments with purified proteins (Falk et al., 1999; Harrison and He, 2011; Thapliyal et al., 2008; Vargiu et al., 2004). The Saccharomyces cerevisiae protein Arr4 also acts as a GEF for the yeast G-protein GPA1 (Lee and Dohlman, 2008). Of particular interest, both
GIV and Ric-8A act as GEFs for G_αGDP when it is bound to Group II AGS proteins (see below) containing G-protein Regulatory (GPR) motifs providing connectivity between Group I and Group II AGS proteins (Garcia-Marcos et al., 2011a; Tall and Gilman, 2005; Thomas et al., 2008).

The Group I proteins exhibit selectivity in their interaction with G-proteins: AGS1 (G_αi, G_αo, G_αi/o_βγ) (Cismowski et al., 2000; Cismowski et al., 1999); Ric-8A (G_αi, G_αo, Gq, G_α13) (Chan et al., 2011a; Chan et al., 2013; Gabay et al., 2011; Tall and Gilman, 2005; Tall et al., 2003; Thomas et al., 2008), Ric-8B (G_αs, G_αolf) (Chan et al., 2011a; Chan et al., 2011b); GIV/Girdin (G_αi, G_αs) (Beas et al., 2012; Garcia-Marcos et al., 2011a; Garcia-Marcos et al., 2010; Garcia-Marcos et al., 2009; Ghosh et al., 2010; Le-Niculescu et al., 2005); Rhes (G_αi) (Harrison and He, 2011). The Group I proteins have broad functional impact influencing tissue development, cell growth and/or neuronal signaling (Figure 1). Disruption of the Ric-8A gene, but not that of AGS1, Rhes or GIV, is embryonic lethal (Chen et al., 2013; Cheng et al., 2004; Enomoto et al., 2009; Gabay et al., 2011; Kitamura et al., 2008; Spano et al., 2004; Tonissoo et al., 2006; Tonissoo et al., 2010; Wang et al., 2011).

AGS1 (RASD1, DexRas1) and Rhes (RASD2) are two related members of the Ras family of small G-proteins (Kemppainen and Behrend, 1998) (Falk et al., 1999). Both AGS1 and Rhes have an extended carboxyl terminus as compared to Ras family proteins and both proteins interact with Gi/Go and regulate G-protein signaling (Cismowski et al., 2000; Cismowski et al., 1999; Graham et al., 2002; Graham et al., 2004; Harrison and He, 2011; Nguyen and Watts, 2005; Takesono et al., 2002; Vargiu et al., 2004). AGS1 has a range of functional roles including inhibition of cell growth, N-methyl-D-aspartate receptor signaling, regulation of the circadian rhythm and regulation of hormone secretion (Chen et al., 2013; Cheng...
et al., 2004; Fang et al., 2000; Graham et al., 2001; Harrison et al., 2013; Jaffrey et al., 2002; Lellis-Santos et al., 2012; McGrath et al., 2012; Takahashi et al., 2003; Vaidyanathan et al., 2004). Rhes interacts with mutant huntingtin influencing its cytotoxicity and the associated neurodegeneration in Huntington’s disease (Baiamonte et al., 2013; Lu and Palacino, 2013; Mealer et al., 2013; Okamoto et al., 2009; Subramaniam et al., 2009). Both AGS1 and Rhes increased the basal activity of N-type calcium channels via apparent activation of Gαiβγ and both proteins antagonized the channel activation elicited by activation of a G-protein coupled receptor coupled to pertussis toxin sensitive G-proteins (Thapliyal et al., 2008). The inhibition of receptor-mediated events by AGS1 is similar to that observed for AGS1 in the regulation of potassium channels by M2-muscarinic receptors in Xenopus oocytes (Takesono et al., 2002). AGS1 and/or Rhes also influence the regulation of ERK1/2 and adenylyl cyclase by heterotrimeric G-proteins (Cismowski et al., 2000; Graham et al., 2001; Graham et al., 2002; Graham et al., 2004; Harrison and He, 2011; Nguyen and Watts, 2005). Both proteins bind to Gα (Cismowski et al., 2000; Cismowski et al., 1999; Harrison and He, 2011) and perhaps Gβγ (Hill et al., 2009; Hiskens et al., 2005). The mechanistic aspects of AGS1- and Rhes-mediated effects on heterotrimeric G-protein signaling systems are not fully understood. It is likely that there are actions of AGS1 and Rhes that are G-protein independent.

Ric-8A acts as a chaperone for Gαi regulating Gα folding and processing during its biosynthesis and this regulatory action requires its GEF activity (Chan et al., 2013; Gabay et al., 2011). The interaction of Ric-8A with Gα stabilizes the G-protein subunit and in its absence Gα levels are markedly reduced. Ric-8A may also play a role in signal processing independent of its chaperone function. The Ric-8A – GαGPR pathway regulates asymmetric cell division and mitotic spindle orientation in model organisms and mammalian cells (Afshar et al., 2004;
Couwenbergs et al., 2004; David et al., 2005; Hampoelz et al., 2005; Wang et al., 2005b; Woodard et al., 2010). As is also the case for GPCR coupling to Goβγ, the action of Ric-8A on the GoGPR signaling module is blocked by cell treatment with pertussis toxin (Oner et al., 2013a; Woodard et al., 2010). It is not clear what lies downstream of the Ric-8A – GoGPR module and it is not clear what provides signal input to the module.

GIV/Girdin/APE was first identified as an Akt substrate and a Goα interacting protein by yeast two hybrid screens (Anai et al., 2005; Enomoto et al., 2005; Le-Niculescu et al., 2005) and it mediates signals from the epidermal growth factor receptor, the insulin receptor and G-protein coupled receptors that regulate cell migration, proliferation and autophagy (Anai et al., 2005; Enomoto et al., 2005; Garcia-Marcos et al., 2011a; Garcia-Marcos et al., 2010; Garcia-Marcos et al., 2009; Ghosh et al., 2010). GIV is associated with increased incidence of colon and breast cancer (Garcia-Marcos et al., 2011b; Liu et al., 2012). The regulation of autophagy by several cell-surface receptors apparently requires the GEF activity of GIV acting upon a GoGPR complex at autophagic vesicles (Garcia-Marcos et al., 2011a). GIV also integrates signals processed through heterotrimeric G-proteins subsequent to GPCR activation by acting as a putative rheostat to provide graded signal integration across different pathways (Ghosh et al., 2011). GIV/Girdin knockout mice exhibit defects in angiogenesis, neurogenesis and cell motility (Enomoto et al., 2005; Kitamura et al., 2008; Wang et al., 2011). The action of GIV on cell motility may relate in part to its ability to interact with the PAR protein complex (Ohara et al., 2012). GIV is also localized to the centrosome and the midbody as observed for AGS5 (Blumer et al., 2006; Mao et al., 2012). Interestingly, Group II AGS proteins AGS5/LGN and AGS3 are also able to interact with and influence the subcellular distribution of the PAR complex to regulate cell polarity and in some cases orient the mitotic spindle (Izaki et al., 2006; Kamakura et
al., 2013; Lechler and Fuchs, 2005; Yuzawa et al., 2011; Zigman et al., 2005). As Gαi-AGS3 is a substrate for GIV-induced GEF activity (Garcia-Marcos et al., 2011a), this may illustrate yet another area of possible connectivity between Group I and Group II AGS proteins. Finally, as with AGS1 and Rhes, it is not clear if all of the actions of GIV/Girdin involve the regulation of Gαβγ and/or GαGPR.

**Group II AGS Proteins**

Mammalian Group II AGS proteins consist of seven proteins [AGS3 (G-protein signaling modulator (GPSM) 1), LGN (GPSM2, AGS5), AGS4 (GPSM3), RGS12 (AGS6), Rap1Gap (Transcript Variant 1), RGS14, PCP2/L7 (GPSM4)] each of which contain 1-4 GPR motifs for docking of Gα serving as alternative binding partners for specific subtypes of Gα. Group II AGS proteins engage the Gi/Go/transducin family of G-proteins. There are three types of mammalian Group II AGS proteins distinguished by the number of GPR motifs and/or the presence of defined regulatory protein domains (Blumer et al., 2012; Blumer et al., 2007; Sato et al., 2006a). AGS3 and LGN (AGS5) have four GPR motifs downstream of a tetratricopeptide repeat domain, whereas RGS12 (AGS6), RGS14 and Rap1GAP have one GPR motif plus other defined domains that act to accelerate Gα−GTP hydrolysis. AGS3-SHORT, AGS4 (GPSM3) and PCP2/L7 (GPSM4) have multiple GPR motifs without any other clearly defined regulatory protein domains.

The GPR motif stabilizes the Gα subunit in its GDP bound conformation and inhibits GTPγS binding [See references in (Sato et al., 2006a; Willard et al., 2004)] and thus Group II proteins behave as guanine nucleotide dissociation inhibitors in a manner analogous to Gβγ. Group II AGS proteins with multiple GPR motifs can bind a corresponding number of Gα.
Thus, for AGS3 and AGS5 up to four G\(\alpha\) may be docked to the GPR protein at any given moment. The structural organization and intramolecular dynamics of such a complex is of interest. Different members of the G\(\alpha\)i/o family may be docked to the protein and there may be cooperativity among the multiple GPR motifs with respect to their interaction with G\(\alpha\). Different GPR motifs may exhibit G\(\alpha\) selectivity within the family of pertussis toxin sensitive G-proteins (Mittal and Linder, 2004). AGS4, which has three GPR motifs, is also reported to interact with G\(\beta\)\(\gamma\) and its amino terminal region exhibits apparent GEF activity (Giguere et al., 2011; Zhao et al., 2010). Selected GPR motifs in \textit{D. melanogaster} and \textit{C. elegans} AGS3 orthologs are reported to also engage G\(\alpha\)GTP (Kopein and Katanaev, 2009; Yoshiura et al., 2012), although it is not clear how these data are reconciled with the large majority of information indicating that GPR motifs stabilize the GDP-bound conformation of G\(\alpha\). The x-ray crystal structures of AGS5/LGN in complex with binding partners NuMA, mNsc and Frmpd1 were recently determined as were the complexes of AGS5/LGN and RGS14 GPR domains with G\(\alpha\)iGDP (Culurgioni et al., 2011; Jia et al., 2012; Kimple et al., 2002; Pan et al., 2013; Yuzawa et al., 2011; Zhu et al., 2011).

The G\(\alpha\)GPR complex participates in an increasingly fascinating set of regulatory functions that we are just beginning to understand (Figure 1). In mammalian systems, AGS3 is implicated in asymmetric cell division, neuronal plasticity and addiction, autophagy, membrane protein trafficking, polycystic kidney disease, renal response to ischemia, immune cell chemotaxis, IGF-1 mediated ciliary resorption, cardiovascular regulation and metabolism (Blumer et al., 2002; Blumer et al., 2006; Blumer and Lanier, 2003; Blumer et al., 2008; Bowers et al., 2008; Bowers et al., 2004; Chauhan et al., 2012; Conley and Watts, 2013; Ghosh et al., 2003; Gotta et al., 2003; Groves et al., 2010; Groves et al., 2007; Hofler and Koelle, 2011; Kamakura et al., 2013; Kwon et al., 2012; Nadella et al., 2010; Pattingre et al., 2003; Regner et
al., 2011; Sato et al., 2004; Vural et al., 2010; Willard et al., 2008; Yao et al., 2005; Yao et al., 2006; Yeh et al., 2013). AGS3 SNPs are associated with diabetes and glucose handling (Hara et al., 2013; Huyghe et al., 2013; Scott et al., 2012). AGS4 (GPSM3), which is enriched in immune cells and regulates immune cell chemotaxis is associated with autoimmune diseases (Cao et al., 2004; Giguere et al., 2013). AGS5, which exhibits a similar domain structure as AGS3, also plays important functional roles in asymmetric cell division and morphogenesis and was recently identified as a responsible gene for certain types of nonsyndromic hearing loss, planar cell polarity in cochlear hair cells and the brain malformations and hearing loss observed as part of the Chudley-McCullough syndrome (Almomani et al., 2013; Diaz-Horta et al., 2012; Doherty et al., 2012; Du and Macara, 2004; Du et al., 2001; Ezan et al., 2013; Fukukawa et al., 2010; Lechler and Fuchs, 2005; Walsh et al., 2010; Williams et al., 2011; Yariz et al., 2011; Zheng et al., 2013; Zheng et al., 2010). The functional role of AGS5 and G-proteins in coordinating planar cell polarity of the cochlear hair cell may involve both the PAR polarity protein complex and positioning of the kinocilium (Ezan et al., 2013). Both AGS5 and AGS3 also interact with guanylate cyclase and AGS5 appears to regulate guanylate cyclase activity (Chauhan et al., 2012).

The functional role of AGS3 in the kidney is of particular interest. AGS3 is expressed at low or undetectable levels in the normal kidney, but when the organ is challenged by ischemia or polycystic kidney disease, AGS3 expression is markedly upregulated (Kwon et al., 2012; Nadella et al., 2010; Regner et al., 2011). The upregulation of AGS3 may serve as a “protective adaptation” by facilitating epithelial cell repair in both types of organ stress. The loss of AGS3 delays the recovery from the ischemic challenge and results in a dramatic increase in cyst formation in animal models of polycystic kidney disease. Mechanistically, this action of AGS3
may be mediated through Gβγ, which may regulate the polycystins PC1/PC2 (Kwon et al., 2012). These data provide another example of an action of AGS3 on G-protein subunit interactions that augments Gβγ-mediated signaling (Kwon et al., 2012; Nadella et al., 2010; Regner et al., 2011; Sanada and Tsai, 2005; Sato et al., 2004; Takesono et al., 1999).

The number of GPR proteins has expanded with evolution. D. melanogaster has one GPR protein (Pins – Partner of Inscuteable) that has a domain structure similar to mammalian AGS3 and AGS5 and plays an important functional role in asymmetric cell division and cell polarity (Bergstralh et al., 2013; Cabernard et al., 2010; Nipper et al., 2007; Parmentier et al., 2000; Schaefer et al., 2001; Schaefer et al., 2000; Yoshiura et al., 2012; Yu et al., 2000). C. elegans has two GPR proteins, one of which is similar to AGS3 and AGS5 and may integrate neural signals required for system adaptation (Hofler and Koelle, 2011). A second C. elegans GPR protein, GPR1/2, is required for asymmetric cell division during early development (Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003). In both model organisms, the functional roles of the GPR proteins involve the regulation of Gα signaling.

The expansion of the GPR family with evolution reflects important roles in complex biological events. The mechanism by which the GPR protein AGS3 influences a range of cell and tissue behaviors is not clear. The protein may influence specific signaling events through heterotrimeric G-proteins (Figure 2A), function as part of a distinct signaling pathway (e.g. GαGPR signaling module) (Figure 2B), serve as a chaperone for Gα and/or impact basic cellular events such as autophagy (Garcia-Marcos et al., 2011a; Groves et al., 2010; Pattingre et al., 2003; Vural et al., 2010) and secretory pathway dynamics (Groves et al., 2007; Oner et al., 2013b).
Group III AGS Proteins

Group III AGS proteins interact with Gβγ or perhaps Goβγ (Cismowski et al., 1999; Sato et al., 2006b; Sato et al., 2009; Yuan et al., 2007). The Group III AGS proteins are a diverse group and their roles in G-protein signaling systems are not well-defined. In general, the Group III proteins interact with Gβγ and exhibit nonselectivity for Gα subunits in the yeast-based functional screen. It was hypothesized that Group III AGS proteins influence subunit (Gα and Gβγ) interactions independent of nucleotide exchange via an interaction with Goβγ or Gβγ leading to dissociation of Goβγ resulting in increased “free” Gβγ for effector engagement in the yeast-based functional screen. Many of the Group III AGS proteins play important functional roles in different systems (Figure 1). AGS8 was actually identified as part of a strategy to identify disease- or challenge-specific AGS proteins in different model systems (Sato et al., 2006b). AGS8, which is upregulated in a rat model of cardiac hypertrophy, is required for hypoxia-induced apoptosis of cardiomyocytes although the mechanism involved is not fully defined (Sato et al., 2009). AGS2/Tctex1/DYNLT1 is a component of the cytoplasmic motor protein dynein and actually functions in one capacity as a Gβγ effector regulating neurite outgrowth and neurogenesis (Gauthier-Fisher et al., 2009; Sachdev et al., 2007; Yeh et al., 2013).

Two Core Signaling Triads

The observation that AGS proteins fell into three distinct functional groups when initially discovered may have greater significance than first appreciated. The three defined groups revealed unexpected regulatory mechanisms for G-proteins actually suggesting a previously undefined path for signal processing. The three functional groups of AGS proteins are GEFs (nonreceptor, Group I), GoαGPR (GPR as a GDI, Group II) and proteins interacting with Gβγ.
(Group III), which is analogous to the core signaling triad for heterotrimeric G-proteins - GEFs (Receptor), Gαβγ (Gβγ as a GDI) and effector (e.g. Gβγ binding proteins) (Figures 2,3). Thus, one might imagine that there may be opportunities for the three groups of AGS proteins to align their different functional mechanisms and act in concert to regulate biological events. Extending this thought, one may also imagine communication between the two core signaling triads. Such crosstalk may be sequential or coordinated as a cycle and may occur at different points across the two triads (Figure 2A,B; Figure 3).

Certainly the regulation of the GαGPR complex by Ric-8A and GIV is an example of crosstalk among Groups I and II AGS proteins. Crosstalk may also occur across the two core signaling triads. Group I AGS proteins may regulate Gα and/or Gαβγ (Figure 3). Another example of crosstalk between the two core signaling triads is illustrated in Figure 2A. The GαGPR complex generated subsequent to receptor activation of Gαβγ may impede the reassociation of Gα and Gβγ augmenting Gβγ-mediated signaling events (Figure 2A). In addition, the newly generated Gα-GPR may initiate the formation of a signaling complex that acts independent of Gβγ-mediated signaling. For example, during neutrophil chemotaxis the GαiAGS3 complex acts as a docking site for polarized formation of a mInsc – Par3 – Par6 – aPKC complex, which is important for efficient chemokine-directed migration (Kamakura et al., 2013). Such a GαGPR complex could also serve as a target for Group I AGS proteins as suggested for signaling events associated with feeding behavior in C. elegans (Hofler and Koelle, 2011). Such signaling events may be cyclic and could result in the generation of different combinations of Gα and Gβγ by subunit exchange (Figure 3).

Groups II and III AGS proteins may also work together providing another platform for system crosstalk. In neuronal stem cells, activation of the IGF-1 receptor couples to
heterotrimeric G-proteins and subsequent to dissociation of $\text{G}_\alpha$ and $\text{G}_\beta\gamma$, the Group II protein AGS3 interacts with $\text{G}_\alpha$-GDP allowing $\text{G}_\beta\gamma$ to engage the dynein light chain AGS2 (Group III AGS protein) as an effector to regulate cilia resorption and cell differentiation (Yeh et al., 2013). There may be additional points of “cross talk” involving AGS2. Dynein also regulates the movement of proteins through the aggresome pathway and the regulation of spindle pulling forces during cell division. The GPR protein AGS3 actually traffics into the aggresome pathway and the GPR proteins AGS3 and AGS5, together with $\text{G}_\alpha$, also regulate spindle orientation and spindle dynamics during asymmetric cell division. The Group I AGS protein Ric-8A also regulates asymmetric cell division (Afshar et al., 2004; Hampoelz et al., 2005; Hess et al., 2004; Wang et al., 2005a).

Although the majority of $\text{G}_\alpha$ within the cell likely exists as part of the $\text{G}_\alpha\beta\gamma$ heterotrimer at the plasma membrane, a subpopulation of $\text{G}_\alpha$ is apparently complexed with GPR proteins (Figure 2B) (Afshar et al., 2004; Bernard et al., 2001; Blumer et al., 2003; Garcia-Marcos et al., 2011a; Nair et al., 2005; Schaefer et al., 2000; Wiser et al., 2006). The $\text{G}_\alpha$GPR complex may be generated by direct action of a GPR protein on $\text{G}_\alpha\beta\gamma$ independent of receptor activation or subsequent to dissociation of $\text{G}_\alpha\beta\gamma$ in response to receptor activation as noted above. Alternatively, $\text{G}_\alpha$ and GPR may associate with each other during their biosynthesis or via colocalization in microdomains of the cell independent of any engagement with $\text{G}_\alpha\beta\gamma$.

Both the GPR motif and $\text{G}_\beta\gamma$ stabilize $\text{G}_\alpha$ in its GDP-bound conformation, which is the conformation of $\text{G}_\alpha$ when initially engaged by a GPCR. Thus, the hypothesis that a GPCR couples directly to the $\text{G}_\alpha$GPR complex presents another path for crosstalk between the two core signaling triads (Figure 2B, Figure 3). Although it is generally accepted that $\text{G}_\beta\gamma$ plays a role in receptor recognition of $\text{G}_\alpha\beta\gamma$, the x-ray crystal structure of the $\beta_2$-AR - Gs$\alpha$ complex indicates
no direct contacts of the receptor with Gβγ (Rasmussen et al., 2011). These data do not rule out an interaction of β2-AR with the Gβγ subunits as the β2-AR-Gαβγ complex is “stabilized,” but also do not rule out a primary interaction of the receptor with Gα – an interaction that might be mimicked in the context of a GαGPR complex (See discussion in (Oner et al., 2010a).

A series of studies involving bioluminescence resonance energy transfer in transfected cell models indicate that GαGPR complexes (Gai-AGS3, Gai-AGS4, Gai-RGS14) at the plasma membrane are indeed regulated by a subgroup of G-protein coupled receptors presenting an unexpected mechanism for signal input to GαGPR complex (Oner et al., 2010a; Oner et al., 2010b; Oner et al., 2013a; Vellano et al., 2013; Vellano et al., 2011) (Figure 2B). Activation of the α2-adrenergic receptor leads to dissociation of the GαAGS3 and the GαAGS4 complex and release of the GPR protein from the inner face of the plasma membrane (Oner et al., 2010a; Oner et al., 2010b; Oner et al., 2013b). Both transfected and endogenous AGS3 translocate to the Golgi apparatus subsequent to dissociation from Gα (Oner et al., 2013b).

It is not known if the regulation of the GαGPR complex by a G-protein coupled receptor reflects direct coupling of the receptor to the GαGPR complex or involves cycling of G-protein subunits subsequent to GPCR activation of Gαβγ within a larger signaling complex. An extension of this hypothesis regarding direct coupling of a GPCR to GαGPR is that a GPCR may couple to GαGPR in a ligand-dependent, conformationally-selective manner analogous to ligand-bias as described for GPCR coupling to Gαβγ and β-arrestin offering substantial flexibility for systems to adapt and respond to extracellular stimuli (Figure 2C) (Ahn et al., 2013; Blattermann et al., 2012; Brink et al., 2000; DeWire et al., 2013; Gesty-Palmer and Luttrell, 2011; Katritch et al., 2013; Kelly, 2013; Kenakin, 2012; Kenakin and Christopoulos, 2013; Pradhan et al., 2012; Reiter et al., 2012; Rivero et al., 2012; Wehbi et al., 2013; Wootten et al.,
One might imagine that receptor coupling to these three distinct transducer elements would regulate different signaling pathways and/or the strength and duration of generated signals and offer additional opportunities to expand upon the concept of pathway targeted drugs. Proteins with multiple GPR motifs may assemble Gai units within a larger scaffold with receptor (Figure 2D) providing additional interesting mechanisms for sensing receptor activation and influence signaling kinetics and specificity in ways not yet fully appreciated.

**Perspective**

The discovery of the family of AGS proteins and related accessory proteins revealed totally unexpected mechanisms for regulation of the G-protein activation cycle and have opened up new areas of research related to the cellular role of G-proteins as signal transducers with broad functional impact. Key questions going forward include the following.

- How is the biochemistry of AGS proteins translated into function?
- What regulates the interaction of AGS proteins with G proteins?
- Do cell surface receptors couple directly to a GoαGPR complex?
- Is the core signaling triad defined by AGS proteins a therapeutic target?
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AUTHOR CONTRIBUTIONS

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Diversity of functional roles for AGS and related proteins.

Figure 2. Schematic illustrations of the influence of Group I and II AGS proteins on G-protein signal processing and signal integration. (A) Influence of GPR proteins (Group II AGS proteins) on subunit interactions subsequent to receptor activation. In this scenario, agonist-bound receptor catalyzes nucleotide exchange on Gαi/oβγ and releases Gβγ for effector engagement. Upon GTP hydrolysis (either by intrinsic GTPase activity or accelerated by regulators of G-protein signaling), the GαiGDP may re-associate with Gβγ. As GPR motifs compete with Gβγ for GαiGDP binding (Bernard et al., 2001; Ghosh et al., 2003; Oner et al., 2010a; Oner et al., 2010b), GPR proteins may actually bind GαiGDP prior to its re-association with Gβγ during receptor-mediated G-protein cycling. GαGDP-GPR complexes formed in this context may: (1) enhance or prolong Gβγ-mediated effector activation; (2) serve as targets for non-receptor GEFs (Group I AGS proteins); and/or (3) initiate the formation of a larger signaling complex that acts distinct from Gβγ-mediated signaling. (B) Working hypotheses for GαGPR complexes as direct targets for receptor (1) or non-receptor (2) GEFs. In this scenario, the GPR protein serves a role analogous to that of Gβγ in the heterotrimer Gαβγ. GEF action on this GαGPR complex is depicted at the plasma membrane, but could also ostensibly occur at other subcellular compartments. As in (A), the GαGPR complex can initiate the formation of non-canonical signaling complexes (e.g. those involved in the regulation of mitotic spindle dynamics and cell polarity). (C) Conformation-selective receptor coupling to different candidate signal transducers. Receptor coupling to these three distinct transducer elements is hypothesized to regulate different signaling pathways (depicted as “A-F”) and/or differentially regulate the
strength and duration of generated signals. The direct coupling of a G-protein coupled receptor to GαGPR is presented as a hypothesis to be tested. The hypothesis is based upon the following: a) the ability of both the GPR motif and Gβγ to stabilize Gα in the GDP-bound conformation; b) the regulation of AGS3-Rluc–Gαi-YFP and AGS4-Rluc-Gαi-YFP by a cell surface G-protein coupled receptor as determined by bioluminescence resonance energy transfer (BRET) (Oner et al., 2010a; Oner et al., 2010b); and c) the Gαi-dependent BRET between a cell surface G-protein coupled receptor tagged with Venus and AGS3-, AGS4- and RGS14-Rluc (Oner et al., 2010a; Oner et al., 2010b; Vellano et al., 2013; Vellano et al., 2011). (D) Hypothetical assembly of higher-order signaling complexes by proteins with multiple GPR motifs. AGS3, which contains four GPR motifs, is used as an example of a multi-GPR motif protein that may provide multiple GαGPR docking sites for a GPCR.

**Figure 3. Crosstalk between two core signaling triads.** This schematic presents a working model for AGS proteins as a core signaling triad and for potential crosstalk between the core signaling triads involving G-proteins. The direct coupling of a G-protein coupled receptor to GαGPR is presented as a hypothesis to be tested. The schematic representation of a GPR protein interacting with an effector and the presentation of Gα exchange between Gαβγ and GαGPR subsequent to GEF activation are speculative.
Figure 1

Cell growth inhibition
Breast Cancer
Circadian rhythm
NMDA signaling
Hormone secretion
Glutamate neurotoxicity
Cell division, Gα chaperone

<table>
<thead>
<tr>
<th>Group I (GEF)</th>
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<tbody>
<tr>
<td>AGS1 (RASD1)</td>
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<td>Ric-8</td>
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<td>GIV/Girdin</td>
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Autophagy, Neurogenesis
Angiogenesis
Colon and breast cancer
Cell migration and proliferation

Craving behavior
Autophagy
Polycystic kidney disease
Renal injury
Golgi dynamics
Chemotaxis

Group II (GDI)

AGS3 (GPSM1)
AGS4 (GPSM3/G18.1)
AGS5 (LGN/GPSM2)
AGS6 (RGS12)
PCP2/L7 (GPSM4)
Rap1-Gap1 TV1
RGS14

Neuronal differentiation
Asymmetric cell division
Mitotic spindle dynamics
“Stemness”
Epithelial morphogenesis
Cell division, Hearing
Brain development

Learning and memory

Group III (Gβγ interaction)

Aggresomal protein transport
Neurite outgrowth
Cilia resorption
Neurogenesis

AGS2 (DYNLT1/Tctex1)
AGS7 (Trip13)
AGS8 (FNDC1)
AGS9 (Rpn10)

Cardiac myocyte apoptosis
Proteasome component

Group IV (Gα16 AGS)

AGS11 (TFE3)
AGS12 (TFEB)
AGS13 (MITF)

(HLH transcription factors)
Figure 2

A. Influence of GPR proteins on subunit interactions subsequent to receptor activation

B. Direct coupling of receptor or non-receptor GEFs to a GPR-Gαi complex

C. Conformation-selective receptor coupling

D. Assembly of higher order signaling complexes by proteins with multiple GPR motifs
Figure 3

*Figure 3*

*Crosstalk between two core signaling triads*

GEF (Receptor) → $G_\alpha_{GDP} \beta\gamma$

GEF (Group I AGS) → $G_\alpha_{GDP} GPR$ [Group II AGS]

Effectors $\leftarrow G_\alpha_{GTP}$

Effectors (e.g. Group III AGS proteins)

$G_\beta\gamma$ → $G_\alpha_{GDP} \beta\gamma$

$G_\alpha_{GDP} GPR$ → Effectors?

$G_\alpha_{GDP} GPR$ → $G_\alpha_{GDP} \beta\gamma$