MINIREVIEW

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 biologic 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 $G\beta\gamma$ independently of the classic 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; and group III, entities that bind to $G\beta\gamma$. Group I AGS proteins can engage all subclasses of G proteins, whereas

group II AGS proteins primarily engage the G_i/G_o /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 (AGS) 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 in an acute and long-term manner to physiologic and pathologic challenges without altering the core components of a long-established signaling triad: receptor, G protein, and effector. During the course of evolving such adaptation or modulatory mechanisms, the component units (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 upon 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 (GPCR) (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 $G\alpha$ nucleotide exchange and/or subunit interactions. Serendipitously, these three groups were apparent with the discovery of the first three AGS proteins—AGS1, AGS2, and AGS3—each of which exhibited different mechanisms of action. This grouping then also captures other proteins with similar actions or functional domains. Sato et al. (2011) recently defined a fourth group of AGS proteins (AGS11–13) that activate G protein signaling in

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ABBREVIATIONS: AGS, activators of G protein signaling; AR, adrenergic receptor; GEF, guanine nucleotide exchange factor; GIV, $G\alpha$ -interacting, vesicle-associated protein; GPCR, G protein–coupled receptor; GPR, G protein regulatory; GPSM, G protein signaling modulator; PAR, partitioning defective; RGS, regulator of G protein signaling; Rhes, Ras homolog enriched in striatum; Ric, resistance to inhibitors of cholinesterase.

the yeast-based functional assay with $\text{G}\alpha 16$, but not with yeast strains expressing $\text{G}\alpha 1$, $\text{G}\alpha 2$, or $\text{G}\alpha 3$. Although not as robust as the activation signal involving $\text{G}\alpha 16$, AGS11–13 also activated G-protein signaling in yeast strains expressing $\text{G}\alpha s$ (Sato et al., 2011). The AGS11–13 proteins actually encode three members of the Mitf-Tfe family of basic helix-loop-helix-leucine zipper 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 $\text{G}\alpha 16$ are both upregulated in the hypertrophic mouse heart, and TFE3 coexpression with $\text{G}\alpha 16$ resulted in the nuclear localization of $\text{G}\alpha 16$ 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 include AGS1 (Dexras1, RASD1); resistance to inhibitors of cholinesterase (Ric)-8A and -8B; $\text{G}\alpha$ -interacting, vesicle-associated protein (GIV)/Girdin/Akt-phosphorylation enhancer, act as nonreceptor guanine nucleotide exchange factors (GEFs) for $\text{G}\alpha$ and/or $\text{G}\alpha\beta\gamma$ (Cismowski et al., 1999, 2000; Tall et al., 2003; Tall and Gilman, 2005; Garcia-Marcos et al., 2009; Chan et al., 2011a, 2013; Tall, 2013). Ras homolog enriched in striatum (Rhes) or RASD2, which exhibits 66% amino acid similarity to AGS1, interacts with $\text{G}\alpha i$, 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; Vargiu et al., 2004; Thapliyal et al., 2008; Harrison and He, 2011). 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 $\text{G}\alpha\text{GDP}$ 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 (Tall and Gilman, 2005; Thomas et al., 2008; Garcia-Marcos et al., 2011a).

The group I AGS proteins exhibit selectivity in their interaction with G proteins: AGS1 ($\text{G}\alpha i$, $\text{G}\alpha o$, $\text{G}\alpha i/o\beta\gamma$) (Cismowski et al., 1999, 2000); Ric-8A ($\text{G}\alpha i$, $\text{G}\alpha o$, $\text{G}\alpha q$, $\text{G}\alpha 13$) (Tall et al., 2003; Tall and Gilman, 2005; Thomas et al., 2008; Chan et al., 2011a, 2013; Gabay et al., 2011); Ric-8B ($\text{G}\alpha s$, $\text{G}\alpha olf$) (Chan et al., 2011a,b); GIV/Girdin ($\text{G}\alpha i$, $\text{G}\alpha s$) (Le-Niculescu et al., 2005; Garcia-Marcos et al., 2009, 2010, 2011a; Ghosh et al., 2010; Beas et al., 2012); and Rhes ($\text{G}\alpha i$) (Harrison and He, 2011). The group I proteins have broad functional impact influencing tissue development, cell growth, and/or neuronal signaling (Fig. 1). Disruption of the Ric-8A gene, but not that of AGS1, Rhes, or GIV, is embryonic lethal (Cheng et al., 2004; Spano et al., 2004; Tonissoo et al., 2006; Kitamura et al., 2008; Enomoto et al., 2009; Tonissoo et al., 2010; Gabay et al., 2011; Wang et al., 2011; Chen et al., 2013).

AGS1 (RASD1, DexRas1) and Rhes (RASD2) are two related members of the Ras family of small G proteins (Kempainen and Behrend, 1998; Falk et al., 1999). Both AGS1 and Rhes have an extended carboxyl terminus as compared with Ras family proteins, and both proteins interact with G_i/G_o and regulate G protein signaling (Cismowski et al., 1999, 2000; Graham et al., 2002, 2004; Takesono et al., 2002; Vargiu et al., 2004; Nguyen and Watts, 2005; Harrison and He, 2011). 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 (Fang et al., 2000; Graham et al., 2001; Jaffrey et al., 2002; Takahashi et al., 2003; Cheng et al., 2004; Vaidyanathan et al., 2004; Lellis-Santos et al., 2012; McGrath et al., 2012; Chen et al., 2013; Harrison et al., 2013). Rhes

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

interacts with mutant huntingtin, influencing its cytotoxicity and the associated neurodegeneration in Huntington disease (Okamoto et al., 2009; Subramaniam et al., 2009; Baiaumont et al., 2013; Lu and Palacino, 2013; Mealer et al., 2013). Both AGS1 and Rhes increased the basal activity of N-type calcium channels via apparent activation of $G\alpha\beta\gamma$, and both proteins antagonized the channel activation elicited by activation of a GPCR 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 M_2 -muscarinic receptors in *Xenopus* oocytes (Takesono et al., 2002). AGS1 and/or Rhes also influence the regulation of extracellular signal-regulated kinase 1/2 and adenylyl cyclase by heterotrimeric G proteins (Cismowski et al., 2000; Graham et al., 2001, 2002, 2004; Nguyen and Watts, 2005; Harrison and He, 2011). Both proteins bind to $G\alpha$ (Cismowski et al., 1999, 2000; Harrison and He, 2011) and perhaps $G\beta\gamma$ (Hiskens et al., 2005; Hill et al., 2009). 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 $G\alpha_i$, regulating $G\alpha$ folding and processing during its biosynthesis; this regulatory action requires its GEF activity (Gabay et al., 2011; Chan et al., 2013). The interaction of Ric-8A with $G\alpha$ stabilizes the G protein subunit, and in its absence $G\alpha$ levels are markedly reduced. Ric-8A may also play a role in signal processing independent of its chaperone function. The Ric-8A- $G\alpha$ GPR pathway regulates asymmetrical 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., 2005; Woodard et al., 2010). As is also the case for GPCR coupling to $G\alpha\beta\gamma$, the action of Ric-8A on the $G\alpha$ GPR signaling module is blocked by cell treatment with pertussis toxin (Woodard et al., 2010; Oner et al., 2013a). It is not clear what lies downstream of the Ric-8A- $G\alpha$ GPR module, and it is not clear what provides signal input to the module.

GIV/Girdin/Akt-phosphorylation enhancer was first identified as an Akt substrate and a $G\alpha$ -interacting protein by yeast two hybrid screens (Anai et al., 2005; Enomoto et al., 2005; Le-Niculescu et al., 2005). GIV is involved in signals from the epidermal growth factor receptor, the insulin receptor, and GPCRs that regulate cell migration, proliferation, and autophagy (Anai et al., 2005; Enomoto et al., 2005; Garcia-Marcos et al., 2009, 2010, 2011a; Ghosh et al., 2010). Elevated levels of GIV are associated with increased incidence of colon and breast cancer metastasis (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 $G\alpha$ GPR 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 partitioning defective (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 (Lechler and Fuchs, 2005; Zigman et al., 2005; Izaki et al., 2006; Yuzawa et al., 2011; Kamakura et al., 2013). As $G\alpha_i$ -AGS3 is a substrate for the GEF activity of GIV (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 $G\alpha\beta\gamma$ and/or $G\alpha$ GPR.

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), regulator of G protein signaling (RGS)12 (AGS6), Rap1Gap (Transcript Variant 1), RGS14, and PCP2/L7 (GPSM4), each of which contains one to four GPR motifs for docking of $G\alpha$, serving as alternative binding partners for specific subtypes of $G\alpha$. Group II AGS proteins engage the Gi/Go/transducin family of G proteins. The three types of mammalian group II AGS proteins are distinguished by the number of GPR motifs and/or the presence of defined regulatory protein domains (Sato et al., 2006a; Blumer et al., 2007, 2012). 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 $G\alpha$ -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 $G\alpha$ subunit in its GDP-bound conformation and inhibits GTP γ S binding (see references in Willard et al., 2004; Sato et al., 2006a), and thus group II proteins behave as guanine nucleotide dissociation inhibitors in a manner analogous to $G\beta\gamma$. Group II AGS proteins with multiple GPR motifs can bind a corresponding number of $G\alpha$ proteins. Thus, for AGS3 and AGS5, up to four $G\alpha$ proteins 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 (Zhao et al., 2010; Giguère et al., 2012). Selected GPR motifs in *D. melanogaster* and *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, mInsc, and Frmpd1 were recently determined, as were the complexes of AGS5/LGN and RGS14 GPR domains with $G\alpha_{iGDP}$ (Kimple et al., 2002; Culurgioni et al., 2011; Yuzawa et al., 2011; Zhu et al., 2011; Jia et al., 2012; Pan et al., 2013).

The $G\alpha$ GPR complex participates in an increasingly fascinating set of regulatory functions that we are just beginning to

understand (Fig. 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, insulin-like growth factor-1 mediated ciliary resorption, cardiovascular regulation, and metabolism (Blumer et al., 2002, 2006, 2008; Blumer and Lanier, 2003; Ghosh et al., 2003; Gotta et al., 2003; Pattingre et al., 2003; Bowers et al., 2004, 2008; Sato et al., 2004; Yao et al., 2005, 2006; Groves et al., 2007; Willard et al., 2008; Groves et al., 2010; Nadella et al., 2010; Vural et al., 2010; Hofler and Koelle, 2011; Regner et al., 2011; Chauhan et al., 2012; Kwon et al., 2012; Conley and Watts, 2013; Kamakura et al., 2013; Yeh et al., 2013). AGS3 single nucleotide polymorphisms are associated with diabetes and glucose handling (Scott et al., 2012; Hara et al., 2014; Huyghe et al., 2013). AGS4 (GPM3), 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 (Du et al., 2001; Du and Macara, 2004; Lechler and Fuchs, 2005; Fukukawa et al., 2010; Walsh et al., 2010; Zheng et al., 2010, 2013; Williams et al., 2011; Yariz et al., 2012; Diaz-Horta et al., 2012; Doherty et al., 2012; Almomani et al., 2013; Ezan et al., 2013). 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 (Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012). 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 $G\beta\gamma$, 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 $G\beta\gamma$ -mediated signaling (Takesono et al., 1999; Sato et al., 2004; Sanada and Tsai, 2005; Nadella et al., 2010; Regner et al., 2011; Kwon et al., 2012).

The number of GPR proteins has expanded with evolution. *D. melanogaster* has one GPR protein (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 (Parmentier et al., 2000; Schaefer et al., 2000, 2001; Yu et al., 2000; Nipper et al., 2007; Cabernard et al., 2010; Yoshiura et al., 2012; Bergstralh et al., 2013). *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 $G\alpha$ signaling.

The expansion of the GPR family with evolution reflects important roles in complex biologic 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 (Fig. 2A), function as part of a distinct signaling pathway (e.g., $G\alpha$ GPR signaling module) (Fig. 2B), serve as a chaperone for $G\alpha$, and/or impact basic cellular events such as autophagy (Pattingre et al., 2003; Groves et al., 2010; Vural et al., 2010; Garcia-Marcos et al., 2011a) and secretory pathway dynamics (Groves et al., 2007; Oner et al., 2013b).

Group III AGS Proteins. Group III AGS proteins interact with $G\beta\gamma$ or perhaps $G\alpha\beta\gamma$ (Cismowski et al., 1999; Sato et al., 2006b, 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 $G\beta\gamma$ and exhibit nonselectivity for $G\alpha$ subunits in the yeast-based functional screen. It was hypothesized that group III AGS proteins influence subunit ($G\alpha$ and $G\beta\gamma$) interactions independent of nucleotide exchange via an interaction with $G\alpha\beta\gamma$ or $G\beta\gamma$ leading to dissociation of $G\alpha\beta\gamma$, resulting in increased “free” $G\beta\gamma$ for effector engagement in the yeast-based functional screen. Many of the group III AGS proteins play important functional roles in different systems (Fig. 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 $G\beta\gamma$ effector regulating neurite outgrowth and neurogenesis (Sachdev et al., 2007; Gauthier-Fisher et al., 2009; 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), $G\alpha$ GPR (GPR as a guanine nucleotide dissociation inhibitor, group II), and proteins interacting with $G\beta\gamma$ (group III), which is analogous to the core signaling triad for heterotrimeric G proteins: GEFs (receptor), $G\alpha\beta\gamma$ ($G\beta\gamma$ as a guanine nucleotide dissociation inhibitor) and effector (e.g., $G\beta\gamma$ binding proteins) (Figs. 2 and 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 biologic events. Extending this thought, one may also imagine communication between the two core signaling triads. Such cross-talk may be sequential or coordinated as a cycle and may

occur at different points across the two triads (Figs. 2, A and B, and 3).

Certainly the regulation of the $G\alpha$ GPR complex by Ric-8A and GIV is an example of cross-talk among groups I and II AGS proteins. Cross-talk may also occur across the two core signaling triads. Group I AGS proteins may regulate $G\alpha$ and/or $G\alpha\beta\gamma$ (Fig. 3). Another example of cross-talk between the two core signaling triads is illustrated in Fig. 2A. The $G\alpha$ GPR complex generated subsequent to receptor activation of $G\alpha\beta\gamma$ may impede the reassociation of $G\alpha$ and $G\beta\gamma$ augmenting $G\beta\gamma$ -mediated signaling events (Fig. 2A). In addition, the newly generated $G\alpha$ -GPR may initiate the formation of a signaling complex that acts independently of $G\beta\gamma$ -mediated signaling. For example, during neutrophil chemotaxis the $G\alpha$ AGS3 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 $G\alpha$ GPR 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 $G\alpha$ and $G\beta\gamma$ by subunit exchange (Fig. 3).

Group II and III AGS proteins may also work together, providing another platform for system cross-talk. In neuronal stem cells, the insulin-like growth factor-1 receptor couples to heterotrimeric G proteins. Subsequent to dissociation of $G\alpha$ and $G\beta\gamma$, the group II protein AGS3 interacts with $G\alpha$ -GDP, allowing $G\beta\gamma$ 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,

Fig. 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 $G\alpha_i/\beta\gamma$ and releases $G\beta\gamma$ for effector engagement. Upon GTP hydrolysis (either by intrinsic GTPase activity or accelerated by regulators of G protein signaling), the $G\alpha_{iGDP}$ may reassociate with $G\beta\gamma$. As GPR motifs compete with $G\beta\gamma$ for $G\alpha_{iGDP}$ binding (Bernard et al., 2001; Ghosh et al., 2003; Oner et al., 2010a,b), GPR proteins may actually bind $G\alpha_{iGDP}$ prior to its reassociation with $G\beta\gamma$ during receptor-mediated G protein cycling. $G\alpha_{iGDP}$ GPR complexes formed in this context may: 1) enhance or prolong $G\beta\gamma$ -mediated effector activation, 2) serve as targets for nonreceptor GEFs (group I AGS proteins), and/or 3) initiate the formation of a larger signaling complex whose action is distinct from $G\beta\gamma$ -mediated signaling. (B) Working hypotheses for $G\alpha$ GPR complexes as direct targets for receptor (1) or nonreceptor (2) GEFs. In this scenario, the GPR protein serves a role analogous to that of $G\beta\gamma$ in the heterotrimer $G\alpha\beta\gamma$. GEF action on this $G\alpha$ GPR complex is depicted at the plasma membrane, but could also ostensibly occur at other subcellular compartments. As in A, the $G\alpha$ GPR complex can initiate the formation of noncanonical 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 $G\alpha$ GPR is presented as a hypothesis to be tested. The hypothesis is based upon the following: 1) the ability of both the GPR motif and $G\beta\gamma$ to stabilize $G\alpha$ in the GDP-bound conformation, 2) the regulation of AGS3-Rluc- $G\alpha$ -YFP and AGS4-Rluc- $G\alpha$ -YFP by a cell-surface G protein-coupled receptor as determined by bioluminescence resonance energy transfer (BRET) (Oner et al., 2010a,b), and 3) the $G\alpha$ -dependent BRET between a cell-surface G protein-coupled receptor tagged with Venus and AGS3-, AGS4-, and RGS14-Rluc (Oner et al., 2010a,b; Vellano et al., 2011, 2013). (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 $G\alpha$ GPR docking sites for a GPCR.

Crosstalk between two core signaling triads

Fig. 3. Cross-talk between two core signaling triads. This schematic presents a working model for AGS proteins as a core signaling triad and for potential cross-talk between the core signaling triads involving G proteins. The direct coupling of a G protein-coupled receptor to $G\alpha$ GPR is presented as a hypothesis to be tested. The schematic representation of a GPR protein interacting with an effector and the presentation of $G\alpha$ exchange (red lines) between $G\alpha\beta\gamma$ and $G\alpha$ GPR subsequent to GEF activation are speculative.

together with $G\alpha$, 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; Hess et al., 2004; Hampoelz et al., 2005; Wang et al., 2005).

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

Both the GPR motif and $G\beta\gamma$ stabilize $G\alpha$ in its GDP-bound conformation, which is the conformation of $G\alpha$ when initially engaged by a GPCR. Thus, the hypothesis that a GPCR couples directly to the $G\alpha$ GPR complex presents another path for cross-talk between the two core signaling triads (Figs. 2B and 3). Although it is generally accepted that $G\beta\gamma$ plays a role in receptor recognition of $G\alpha\beta\gamma$, the X-ray crystal structure of the β_2 -adrenergic receptor (AR)- $G\alpha\beta\gamma$ complex indicates no direct contacts of the receptor with $G\beta\gamma$ (Rasmussen et al., 2011). These data do not rule out an interaction of β_2 -AR with the $G\beta\gamma$ subunits as the β_2 -AR- $G\alpha\beta\gamma$ complex is “stabilized,” but they also do not rule out a primary interaction of the receptor with $G\alpha$ —an interaction that might be mimicked in the context of a $G\alpha$ GPR complex (see Discussion in Oner et al., 2010a).

A series of studies involving bioluminescence resonance energy transfer in transfected cell models indicates that $G\alpha$ GPR complexes ($G\alpha$ AGS3, $G\alpha$ AGS4, $G\alpha$ RGS14) at the plasma membrane are indeed regulated by a subgroup of G protein-coupled receptors, presenting an unexpected mechanism for signal input to $G\alpha$ GPR complex (Oner et al., 2010a,b, 2013a; Vellano et al., 2011, 2013) (Fig. 2B). Activation of the

α_2 -AR leads to dissociation of the $G\alpha$ AGS3 and the $G\alpha$ AGS4 complex and release of the GPR protein from the inner face of the plasma membrane (Oner et al., 2010a,b, 2013b). Both transfected and endogenous AGS3 translocate to the Golgi apparatus subsequent to dissociation from $G\alpha$ (Oner et al., 2013b).

It is not known if the regulation of the $G\alpha$ GPR complex by a G protein-coupled receptor reflects direct coupling of the receptor to the $G\alpha$ GPR complex or involves cycling of G protein subunits subsequent to GPCR activation of $G\alpha\beta\gamma$ within a larger signaling complex. An extension of this hypothesis regarding direct coupling of a GPCR to $G\alpha$ GPR is that a GPCR may couple to $G\alpha$ GPR in a ligand-dependent, conformationally-selective manner analogous to ligand-bias as described for GPCR coupling to $G\alpha\beta\gamma$ and β -arrestin, offering substantial flexibility for systems to adapt and respond to extracellular stimuli (Fig. 2C) (Brink et al., 2000; Gesty-Palmer and Luttrell, 2011; Blattermann et al., 2012; Kenakin, 2012; Pradhan et al., 2012; Reiter et al., 2012; Rivero et al., 2012; Ahn et al., 2013; DeWire et al., 2013; Katritch et al., 2013; Kelly, 2013; Kenakin and Christopoulos, 2013; Wehbi et al., 2013; Wootten et al., 2013). 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 $G\alpha$ units within a larger scaffold with receptor (Fig. 2D), providing additional interesting mechanisms for sensing receptor activation and influencing 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 has 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 $G\alpha$ GPR complex?
- Is the core signaling triad defined by AGS proteins a therapeutic target?

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