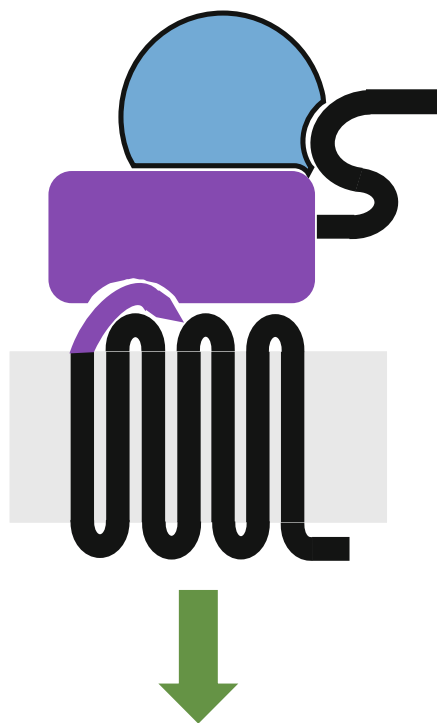# Versatile Signaling Activity of Adhesion GPCRs

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Graphical Abstract

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Contents

Abstract

The adhesion G protein-coupled receptors (aGPCRs) are a family of 33 receptors in humans that are widely expressed in various tissues and involved in many diverse biological processes. These receptors possess extremely large N-termini (NT) containing a variety of adhesion domains. A distinguishing feature of these receptors is the presence within the NT of a highly conserved *G*PCR *a*utoproteolysis-*in*ducing (GAIN) domain, which mediates autoproteolysis of the receptors into N-terminal and C-terminal fragments that stay non-covalently associated. The downstream signaling pathways and G protein-coupling preferences of many aGPCRs have recently been elucidated, and putative endogenous ligands for some aGPCRs have also been discovered and characterized in recent years. A pivotal observation for aGPCRs has been that deletion or removal of the NT up the point of GAIN cleavage results in constitutive receptor activation. For at least some aGPCRs, this activation is dependent on the unmasking of specific agonistic peptide sequences within the N-terminal stalk region (i.e., the region between the site of GAIN domain cleavage and the first transmembrane domain). However, the specific peptide sequences involved and the overall importance of the stalk region for activation can vary greatly from receptor to receptor. An emerging theme of work in this area is that aGPCRs are capable of versatile signaling activity that may be fine-tuned to suit the specific physiological roles played by the various members of this family.

Keywords

Adhesion • Receptor • GPCR • G protein • Signaling • Activity • Agonist • Ligand • Arrestin • Pathway

1 Adhesion G Protein-Coupled Receptors Are a Diverse Group of Self-Cleaving Cell Surface Receptors

G protein-coupled receptors (GPCRs) form the largest superfamily of cell surface signaling proteins in vertebrates [1]. Within this superfamily, the adhesion GPCRs (aGPCRs) represent the second largest family, encompassing 33 receptors in humans. These receptors are broadly expressed in different tissues and involved

in many diverse processes including neural development, immunity, myelination, and angiogenesis [2]. Via their large extracellular N-termini (NT), which range from 200 to 5600 amino acids in length and harbor a variety of adhesion domains, aGPCRs are thought to survey the surrounding cellular environment and transduce signals from the extracellular milieu into intracellular signaling [3]. The tremendous diversity in the NT regions of aGPCRs has led to the further categorization of the 33 aGPCRs into 9 distinct subfamilies [4] (see [5]). A new nomenclature for aGPCRs based on these subfamilies was recently approved by IUPHAR [4], and both the new names (all starting with "ADGR") and the traditional names for each receptor will be used in this review. The most commonly shared protein-protein interaction domains found in the N-termini of each of the nine aGPCR families are olfactomedin (OLF) and rhamnose-binding lectin-like (RBL) domains (subfamily I); epidermal growth factor (EGF)-like repeats (subfamily II); leucine-rich repeats (LRRs; subfamily III); cadherin repeats (subfamily IV); pentraxin domains (PTX; subfamily V); sea urchin sperm protein, enterokinase, and agrin (SEA) domains (subfamily VI); thrombospondin type 1 repeats (TSRs; subfamily VII); pentraxin domains (subfamily VIII); and calx-β repeats (subfamily IX) [4] (see [6]).

A unique feature of the aGPCRs is their autoproteolytic activity at a membrane-proximal motif of the NT called the GPS or GPCR proteolysis site motif [7, 8] (see also [6, 9]). This ~50-amino acid, cysteine- and tryptophan-rich motif is located within a much larger functional domain that is both necessary and sufficient for aGPCR self-cleavage called the GPCR autoproteolysis-inducing (GAIN) domain [10]. The GAIN domain is the only commonly shared domain in the NT of aGPCRs (with the exception of ADGRA1/GPR123) [11]. Moreover, the GAIN domain is also one of the most ancient domains found in aGPCRs, existing in the genomes of more primitive organisms such as *Dictyostelium discoideum* and *Tetrahymena thermophila* [10, 12]. Structural studies by Arac and colleagues showed that the GAIN domain stays intact following cleavage through an extensive network of hydrogen bonding and hydrophobic side-chain interactions [10]. These insights confirmed prior biochemical observations that autoproteolysis does not necessarily result in the dissociation of the N-terminal fragment (NTF) and C-terminal fragment (CTF) that result from GAIN domain cleavage of a given aGPCR.

2 Evidence for G Protein-Mediated Signaling by Adhesion GPCRs

Notwithstanding their N-terminal diversity, all members of the aGPCR family share a similar seven-transmembrane (7TM) domain architecture, which is the molecular signature of GPCRs. However, in the early years of aGPCR research, it was not known whether these proteins were bona fide GPCRs. In studies that were facilitated by the serendipitous discovery of a potent and high-affinity agonist, ADGRL1 (latrophilin-1) was one of the first aGPCRs characterized in terms of its signaling activity [13]. It was found that α-latrotoxin (α-LTX), a component of black widow spider venom, stimulated increases in intracellular cAMP and IP3 levels in ADGRL1-transfected COS7 cells in a receptor-dependent manner

[14]. However, in addition to binding to ADGRL1, α-LTX can also form calcium-permeable pores in the plasma membrane and trigger exocytosis [15]. Therefore, a mutant version of the toxin was generated, α-LTX^{N4C}, which does not cause exocytosis but still binds to and activates ADGRL1 [15]. Further studies showed that ADGRL1 could activate phospholipase C (PLC) and increase intracellular Ca $^{2+}$ within minutes of α-LTX^{N4C} treatment, suggesting coupling of the receptor to Gα$_q$ [16]. Moreover, ADGRL1 could be co-purified with Gα$_o$ [14, 17] and Gα$_{q/11}$ [17] using α-LTX affinity chromatography.

Unlike ADGRL1, the majority of aGPCRs do not have known ligands. Thus, a common method of discerning the signaling pathways downstream of aGPCRs has been to overexpress the receptors in heterologous systems and measure their constitutive activities in assays of specific G protein signaling. For example, overexpression of ADGRG1 (GPR56), a receptor that is critically involved in the development of the cerebral cortex [18, 19], was shown to robustly stimulate the activation of RhoA via coupling to the Gα$_{12/13}$ signaling pathway [20, 21]. Subsequent studies have demonstrated that ADGRG1 expression can upregulate the activity of a variety of downstream transcription factors, including NFκB [22], PAI-1 [22], TCF [22], SRE [20, 23–25], SRF [26], and NFAT [23, 26]. Other outputs influenced by ADGRG1 include PKCα [27], VEGF [25], and TGFα shedding [26]. In addition to these results, other lines of evidence supporting receptor G protein coupling have been provided by several groups. For example, it was demonstrated that Gα$_{q/11}$ could be co-immunoprecipitated with ADGRG1 in heterologous cells [28]. This interaction, however, depended on the presence of the tetraspanin CD81, which may act as a scaffold for the ADGRG1/Gα$_{q/11}$ signaling complex. In agreement with these data, stimulation of ADGRG1 in U87-MG cells was found to raise intracellular Ca^{2+} levels in a manner that was blocked by YM-245890, an inhibitor of Gα$_{q/11}$-mediated signaling [29]. Additionally, ADGRG1 has been shown to activate Gα$_{13}$ in a reconstituted GTPγS-binding assay [24], and an association between ADGRG1 and Gα$_{13}$ has also been shown via a co-immunoprecipitation approach [26].

In addition to ADGRG1, evidence for G protein coupling has also been provided for several other members of aGPCR subfamily VIII. For example, ADGRG2 (GPR64) expression in transfected cells has been demonstrated to stimulate the SRE and NFκB pathways [30], raise intracellular cAMP, and elevate IP3 levels in the presence of the chimeric G protein Gα$_{qi4}$, suggesting promiscuous coupling to both G$_{αs}$ and G$_{αi}$ [31]. Similarly, it was shown that overexpression of ADGRG3 (GPR97) in HEK293 cells stimulated IP3 accumulation only in the presence of chimeric G protein Gα$_{qo3}$, which converts Gα$_o$ signaling into Gα$_q$ activity, suggesting natural coupling of the receptor to Gα$_o$ [32]. ADGRG5 (GPR114) overexpression was shown to potentiate cAMP levels, an effect that could be blocked via knockdown of endogenous Gα$_s$ or overexpression of the chimeric G protein Gα$_{qs4}$, which converts Gα$_s$ signaling into Gα$_q$-mediated activity [33]. Another member of the subfamily, ADGRG6 (GPR126), which plays an important role in regulating peripheral nerve myelination [34], was also found to raise intracellular cAMP [35–37] as well as stimulate IP3 accumulation in the

presence of chimeric G proteins to redirect either $G_{\alpha s}$ or $G_{\alpha i}$ activity toward $G_{\alpha q}$ pathways [36]. Thus, both ADGRG2 and ADGRG6 may couple to $G\alpha_s$ to raise cAMP levels while also exhibiting coupling to other G proteins to mediate pleiotropic effects on cellular physiology.

ADGRB1 (BAI1), a receptor that regulates phagocytosis [38–41], myogenesis [42], and synaptic plasticity [43, 44], has been shown to constitutively activate RhoA [45], Rac1 [41], ERK [45], SRF [26], NFAT [26], and TGFα shedding [26] when overexpressed in heterologous cells. ADGRB1 signaling to most of these downstream readouts can be greatly attenuated by co-expression of the RGS domain of p115-RhoGEF, suggesting a predominant coupling of the receptor to $G\alpha_{12/13}$. These functional data are consistent with co-immunoprecipitation data revealing the existence of cellular complexes between ADGRB1 and $G\alpha_{12/13}$ [26]. Expression of ADGRB2 (BAI2), a close relative of ADGRB1, was found to also stimulate the NFAT pathway and additionally induce IP3 accumulation in HEK293 cells, indicating a likely coupling to $G\alpha_{q/11}$ [46].

ADGRE2 (EMR2), a receptor highly enriched in immune cells, was demonstrated to stimulate IP3 accumulation in transiently transfected HEK293 cells, indicative of $G_{\alpha q}$ coupling [32]. Expression of another receptor from the same subfamily, ADGRE5 (CD97), was found to activate the SRE pathway in transfected COS7 cells in a manner that was sensitive to the presence of RGS-p115-RhoGEF, suggesting receptor coupling to $G\alpha_{12/13}$ [47]. Receptors ADGRF1 (GPR110) and ADGRF4 (GPR115) were both shown to stimulate IP3 accumulation in transiently transfected HEK293 cells [32]. In separate studies that confirmed some of these findings, ADGRF1 was shown to activate $G\alpha_q$ in a GTPγS assay [24].

ADGRV1 (VLGR1), a receptor that has a crucial role in hearing and vision and whose dysfunction is associated with the human disease known as Usher syndrome, was shown to inhibit isoproterenol-induced cAMP levels in HEK293 cells, indicative of $G\alpha_i$ coupling [48]. Moreover, co-expression of the chimeric G protein $G\alpha_{qi5}$ was able to reroute receptor activity toward a $G\alpha_{q/11}$ readout (NFAT activation), thereby providing further evidence for $G\alpha_i$ coupling. In contrast, expression of ADGRD1 (GPR133) has been shown to raise cAMP levels in multiple studies [32, 37, 49]. Moreover, it was demonstrated that ADGRD1-mediated cAMP elevation could be blocked by knocking down $G\alpha_s$ [32].

3 Ligands for Adhesion GPCRs

Potential ligands have been identified for a number of members of the aGPCR family (Table 1). As mentioned previously, α-LTX is a high-affinity agonist of ADGRL1 that has been shown to stimulate several readouts of receptor activity. Another reported ligand for ADGRL1 is teneurin-2, a large (~2800 residue) glycoprotein with a single transmembrane region that is found predominantly in the brain [50]. Teneurin-2 was first identified as a binding partner of ADGRL1 through pull-down studies in which rat brain lysates were subjected to α-LTX affinity chromatography [50]. Treatment of cultured neurons expressing ADGRL1 with a soluble,

Table 1 Adhesion GPCR ligands and/or agonists

Receptor	Ligand	Binding region	Downstream activity
Family I			
ADGRL1	α-Latrotoxin	NT (GAIN domain)	Increased cAMP [14], IP3 [14], Ca^{2+} [16], and PLC activation [16]
ADGRL1	Teneurin-2	NT	Increased Ca^{2+} in cultured hippocampal neurons [50]
ADGRL1	Neurexin1α	NT	Regulation of α-latrotoxin-mediated glutamate release [51]
ADGRL3	FLRT3	NT	Regulation of synaptic density [52]
ADGRL3	FLRT2	NT (OLF domain)	Regulation of cell adhesion/repulsion [53]
Family II			
ADGRE2	NT antibody (2A1)	NT	Increased production of inflammatory cytokines [54]
ADGRE2/ ADGRE5	Chondroitin sulfate	? (likely NT region)	Mediates cell adhesion [55]
ADGRE5	CD55	NT (EGF domains)	Alteration in ADGRE5 NT-CTF interaction [56]
ADGRE5	α5β1/αvβ3	NT	Mediates endothelial cell migration [57]
ADGRE5	CD90	NT	Mediates cell adhesion [58]
Family V			
ADGRD1	Stalk peptide(s)	? (likely 7TM region)	Increased cAMP levels [37]
Family VI			
ADGRF1	Stalk peptide(s)	? (likely 7TM region)	Increased GTPγS binding [24]
Family VII			
ADGRB1	Phosphatidylserine	NT (TSR domains)	Enhanced Rac1-dependent uptake of apoptotic cells [39]
ADGRB3	C1ql1	NT (CUB domain)	Regulation of dendritic spine density [59]
ADGRB3	C1ql3	NT (TSR domains)	Regulation of synaptic density [60]
Family VIII			
ADGRG1	Tissue transglutaminase 2	NT (STP region)	Regulation of VEGF secretion [27]
ADGRG1	Collagen III	NT (aa 27–160)	Stimulation of RhoA activation [61]
ADGRG1	NT antibody	NT	Stimulation of SRE and RhoA activity [20]
ADGRG1	Stalk peptide(s)	? (likely 7TM region)	Stimulation of SRE luciferase [24]
ADGRG2	Stalk peptide(s)	? (likely 7TM region)	Increased cAMP and IP3 accumulation [31]
ADGRG3	Beclomethasone dipropionate	?	Increased GTPγS binding [32]

Table 1 (continued)

Receptor	Ligand	Binding region	Downstream activity
ADGRG5	Stalk peptide(s)	? (likely 7TM region)	Increased cAMP levels [33]
ADGRG6	Collagen IV	NT (CUB and PTX domains)	Increased cAMP levels [35]
ADGRG6	Laminin-211	NT (aa 446–807)	Increased cAMP levels upon mechanical shaking [62]
ADGRG6	Stalk peptide(s)	? (likely 7TM region)	Increased cAMP levels [37] and IP3 accumulation when co-expressed with chimeric Gqi [36]

? unavailable

C-terminal fragment of teneurin-2 was found to trigger the release of intracellular Ca^{2+}, possibly through a G protein-dependent mechanism [50]. In another study, coculturing cells expressing either ADGRL1 or teneurin-2 resulted in the formation of large cell aggregates, indicating that the specific interaction between the two proteins may mediate cell adhesion [64]. In the brain, ADGRL1 and teneurin-2 are enriched in the presynaptic and postsynaptic membranes, respectively. The extracellular NT of ADGRL1, however, may be large enough to span the synaptic cleft to mediate interneuronal contact through its high-affinity interaction with teneurin-2.

ADGRL1 has also been shown to interact with neurexin, a presynaptic protein implicated in synaptogenesis and function [65]. Neurexin is a binding partner of α-LTX, as is ADGRL1 [66]. A particular neurexin isoform (1α) binds α-LTX in a Ca^{2+}-dependent fashion, while the α-LTX-ADGRL1 interaction is Ca^{2+} independent [66]. Interestingly, in the absence of Ca^{2+}, knockdown of neurexin in cultured hippocampal neurons significantly diminished the α-LTX response compared to wild-type neurons, suggesting that while ADGRL1 and neurexin can independently associate with α-LTX, their interaction may synergistically enhance α-LTX-induced signaling by ADGRL1 [51]. Moreover, coculture of cells expressing either ADGRL1 or neurexin resulted in numerous cell aggregates, providing evidence that the interaction promotes adhesion complexes [67]. More work must be done, however, to demonstrate whether neurexins directly stimulate receptor signaling activity.

The fibronectin leucine-rich repeat transmembrane (FLRT) proteins are an additional class of ligands for ADGRL1 and the related receptor ADGRL3 (latrophilin-3) [52]. Direct interactions between the NT of ADGRL3 and FLRT3 were demonstrated in a non-cell-based assay [52]. In vivo, both proteins are enriched in cell-to-cell junctions and regulate synaptic density [52]. In another study, a high-affinity interaction was demonstrated for ADGRL3 and FLRT2 [53]. This interaction was found to be mediated by the OLF domain on the ADGRL3 NT and, intriguingly, promoted either adhesion of FLRT2-expressing HeLa cells or repulsion of FLRT2-expressing cultured cortical neurons. These

results potentially highlight the influence that cellular environment may have on the relationship between receptor and ligand. At present, however, there is no evidence that FLRT proteins can directly instigate signaling by the latrophilin receptors.

The association between ADGRE5 and CD55 was one of the first confirmed protein-protein interactions involving an aGPCR [68]. This interaction was found to be mediated by the EGF domains on the receptor's NT [69]. Recently, it was shown that CD55 does not modulate ADGRE5-mediated signaling to ERK or Akt [56]. It remains to be determined whether CD55 can modulate other receptor-controlled pathways, such as perhaps the RhoA signaling pathway. ADGRE2 is a close relative of ADGRE5 with highly homologous EGF domains, but nonetheless ADGRE2 has been found to have a much lower binding affinity for CD55 than ADGRE5 [70]. Both ADGRE5 and ADGRE2 have also been shown to bind to extracellular matrix (ECM) components known as chondroitin sulfates [55]. These interactions are generally low affinity and Ca^{2+} dependent and have not yet been demonstrated to instigate G protein-mediated signaling for either receptor.

A number of ligands have been identified for subfamily VII aGPCRs. ADGRB1 was found to bind externalized phosphatidylserine on apoptotic cells through the thrombospondin type 1 repeat domains on its NT [38]. This interaction promoted the engulfment of the apoptotic cells in a mechanism reliant on the adaptor protein ELMO1 and signaling by the small GTPase Rac1 [38]. Another receptor from this subfamily, ADGRB3 (BAI3), was shown to bind to C1q-like (C1ql) proteins [60, 71]. Similar to the interaction of ADGRB1 and phosphatidylserine, the interaction between ADGRB3 and C1ql3 was found to be mediated by thrombospondin repeats on the receptor's NT [60]. In cultured neurons, submicromolar C1ql3 treatment significantly reduced synaptic density, an effect readily blocked by exogenous addition of purified ADGRB3 NT [60]. In a similar study, it was shown that ADGRB3 binds C1ql1 via its N-terminal CUB domain and that both proteins were necessary for normal spine density of cerebellar neurons [59]. Furthermore, the interaction between C1ql1 and ADGRB3 was demonstrated to regulate pruning in mouse cerebellum, with knockout of either protein resulting in severe motor learning deficits [72]. Future studies in this area will likely examine whether C1ql proteins have similar binding affinities for other members of subfamily VII and whether those interactions can stimulate receptor-mediated activity.

Several ligands have been identified for ADGRG1, including tissue transglutaminase 2 (TG2), a major cross-linking enzyme of the extracellular matrix implicated in cancer progression [63, 73]. TG2 binds a ~70-residue region on the NT of ADGRG1; deletion of this TG2-binding region was found to enhance receptor-mediated VEGF production in vitro and significantly increase tumor growth and angiogenesis in vivo, whereas expression of the wild-type receptor reduced both measures [27]. In a more recent study, it was demonstrated that the antagonistic relationship between ADGRG1 and TG2 may be attributed to internalization and lysosomal degradation of extracellular TG2 in a receptor-dependent mechanism [74]. It is unclear at present whether interaction with TG2 stimulates G protein-mediated signaling by ADGRG1.

Collagen III is another ligand for ADGRG1 [61]. ADGRG1 loss-of-function mutations cause the human disease bilateral frontoparietal polymicrogyria (BFPP). Patients with BFPP have a cortical malformation due to aberrant neural stem cell migration [75]. Remarkably, knockout of collagen III in mice results in a cortical phenotype similar to that observed in mice lacking ADGRG1 as well as human BFPP patients [75]. Collagen III binds a ~130-residue region in the distal half of the receptor's NT [76]. Moreover, nanomolar concentrations of collagen III have been shown to significantly reduce migration of mouse neurospheres (masses of cells containing neural stem cells) in a receptor-dependent fashion [61]. Biochemical studies revealed that collagen III could stimulate RhoA signaling in a mechanism dependent on receptor expression and likely mediated by $G\alpha_{12/13}$ [61].

Another subfamily VIII receptor, ADGRG6, has also been shown to be stimulated by collagen interactions, albeit with a distinct type of collagen. The association between ADGRG6 and collagen IV was found to be mediated by a region of the ADGRG6 NT containing the CUB and PTX domains [35]. Furthermore, the association was shown to be specific, as other types of collagen, including collagen III, did not bind the receptor. In heterologous cells, collagen IV stimulated receptor-dependent cAMP elevation. The half-maximal effective concentration for this response was 0.7 nM, indicating that collagen IV is a potent agonist for ADGRG6.

An additional ligand for ADGRG6 is laminin-211, an extracellular matrix protein that is involved in Schwann cell development and peripheral nervous system myelination [62]. Interestingly, laminin-211 was found to antagonize receptor-mediated cAMP elevation in a dose-dependent fashion in heterologous cells. Furthermore, cAMP inhibition was due to antagonism of receptor-mediated $G\alpha_s$ activity rather than through differential activation of $G\alpha_i$. Remarkably, laminin-211 treatment under the condition of mechanical shaking had the opposite effect of boosting receptor-mediated cAMP levels. Thus, laminin-211 may serve as a unique ligand that can differentially modulate receptor activity depending upon other physical cues and mechanical forces in the extracellular environment.

Most of the putative aGPCR endogenous ligands described thus far are large, ECM-derived molecules. Nonetheless, it has been shown that small molecules can be developed as aGPCR ligands. For example, screening studies revealed beclomethasone dipropionate as a ligand for ADGRG3 [32]. Beclomethasone dipropionate is a glucocorticoid steroid that can stimulate ADGRG3 with nanomolar potency. The region of the receptor that interacts with beclomethasone is unknown, but considering the molecule's hydrophobicity, it would not be surprising if it were found in future studies to directly interact with the receptor's 7TM region to modulate receptor activity.

An intriguing observation made for several aGPCRs has been that these receptors may be activated by antibodies directed against their NT regions. Antibodies may be able to mimic the binding of endogenous ligands to aGPCRs and thus may represent powerful research tools for studying aGPCR signaling, especially for those receptors with no identified ligands. An N-terminal activating antibody of ADGRG1 was first described in 2008 by Itoh and colleagues. Studies in

heterologous cells revealed that antibody treatment could dose-dependently stimulate receptor signaling in the SRE luciferase assay (a commonly used assay for $G\alpha_{12/13}$ activity) [20]. Moreover, stimulation was readily blocked by exogenous addition of the receptor's NT, which presumably competed for antibody binding. Moreover, in a later study it was shown that other newly generated N-terminal antibodies for ADGRG1 could inhibit cell migration in a manner that was sensitive to inhibition of either $G\alpha_q$ or $G\alpha_{12/13}$ signaling [29]. In another example, an antibody directed against the N-terminal region of ADGRE2 was shown to dose-dependently increase inflammatory cytokine production in receptor-mediated neutrophil activation [54].

Given the importance of aGPCR N-termini in mediating binding to extracellular ligands, it is perhaps not surprising that mutations to the aGPCR N-termini can oftentimes lead to loss of receptor function and human disease. For example, there are several reported N-terminal disease-causing mutations to ADGRG1 that result in reduced plasma membrane expression of the receptor [77, 78] and/or disruption of the receptor's ability to bind collagen III [76]. Another prominent example is of ADGRV1, where several NT mutations cause cochlear and retinal defects in humans [79]. Moreover, missense NT mutations to ADGRC1 (CELSR1) impair surface trafficking of the protein and are implicated in a severe neural tube defect in humans known as craniorachischisis [80].

4   Adhesion GPCR Models of Activation

With the idea that aGPCR ligands mainly bind to the large extracellular NT regions and that the NT regions are cleaved in the GAIN domain and may be removed at some point following ligand binding, a number of groups have generated truncated versions of aGPCRs lacking most of their NT regions up to the sites of predicted GAIN cleavage. The first studies of this type were performed independently for a trio of receptors—ADGRB2 [46], ADGRG1 [21], and ADGRE5 [47]—and in each case the truncation was found to result in a substantial increase in the receptors' constitutive signaling activity. Subsequently, this phenomenon has been reported for a number of other aGPCRs, including ADGRB1 [45], ADGRG6 [35], ADGRG2 [30, 31], ADGRD1 [37], ADGRF1 [24], and ADGRV1 [48]. In light of these findings, a general model of aGPCR activation was proposed wherein the tethered NTF behaves as an antagonist of CTF-mediated signaling, with N-terminal deletion mimicking ligand-mediated removal of the NTF to result in receptor activation [81]. This model of activation, termed the *disinhibition model*, was a general model that left open the mechanistic question of precisely how removal of aGPCR NT regions might activate receptor signaling.

Subsequently, a more mechanistically specific model of aGPCR activation, termed the *tethered agonist model*, was proposed (Fig. 1; see also [82]). In this model, GAIN domain autoproteolysis (and/or conformational change) reveals a tethered cryptic agonist sequence contained within the NT region between the site of cleavage and the first transmembrane domain (i.e., the stachel or stalk region).
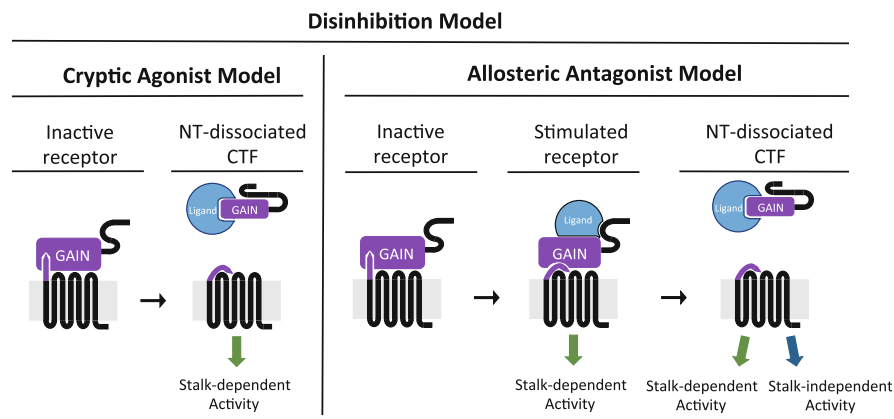
Fig. 1 Models of adhesion GPCR activation. Cryptic agonist model—*Inactive receptor*: The GAIN domain antagonizes receptor activity by concealing a cryptic agonist found in the N-terminal stalk region between the site of autoproteolysis and the first transmembrane domain. *NTF-dissociated CTF*: Following ligation of the N-terminal fragment (NTF) with an extracellular ligand and subsequent removal from the plasma membrane, the cryptic agonist sequence (the stachel) is unveiled and stimulates activity through interactions with the remaining C-terminal fragment (CTF). Allosteric antagonist model—*Inactive receptor*: In the absence of ligand engagement, the GAIN domain can inhibit receptor activity in two distinct ways: by concealing a cryptic agonist on the N-terminal stalk and also by dampening the inherent constitutive activity of the CTF. *Stimulated receptor*: Ligation of the NTF with an extracellular ligand induces a conformational change to allow for stimulation by the cryptic agonist within the stalk, even though the NTF may stay associated with the CTF for some time. *NTF-dissociated CTF*: If and when ligand binding induces NTF dissociation from the CTF, another wave of receptor activity may be unleashed, with the inherent, stalk-independent activity of the CTF being stimulated. In this stage, the receptor may achieve its maximal activity due to the summation of signals from both stalk-dependent and stalk-independent mechanisms

This mechanism of activation is conceptually similar to that of the protease-activated receptors, for which proteolysis of the N-terminal domain by an extracellular protease unveils an agonist in the remaining NT [83]. Evidence in favor of the cryptic agonist model was provided by two independent groups: Liebscher et al. and Stoveken et al. First, Liebscher et al. showed that deletion of the remaining NT (i.e., the stachel or stalk region) from constitutively active NTF-lacking versions of ADGRG6 and ADGRD1 ablated activity of both receptors in cAMP accumulation assays [37]. Moreover, synthetic peptides corresponding to the stalk regions of each receptor were able to restore activity of the stalkless mutants with varying degrees of efficacy. The most potent peptides displayed half-maximal effective concentrations in the high micromolar range. Further studies from Liebscher et al. along similar lines provided evidence for tethered agonist-mediated activation of ADGRG2 [31] and ADGRG5 [33]. Additionally, Stoveken et al. showed that stalkless versions of ADGRG1 and ADGRF1 lacked activity in reconstitution assays examining GTP binding to purified $G\alpha_{13}$ and $G\alpha_q$,

respectively [24]. Synthetic peptides fashioned after the stalk of each receptor were shown to resuscitate their cognate stalkless receptors in a dose-dependent manner, with the most potent peptides displaying submicromolar half-maximal effective concentrations. Moreover, the most potent stalk peptide of ADGRG1 was shown to stimulate receptor-mediated activity in cellular SRE luciferase assays in addition to the $G\alpha_{13}$ reconstitution studies.

The finding from Stoveken et al. that stalk-deficient ADGRG1 is unable to activate SRE luciferase was confirmed in recent studies using a similar readout, SRF luciferase [26]. However, the stalkless ADGRG1 was found in these studies to be functional in other readouts of receptor signaling activity including TGFα shedding, NFAT luciferase, beta-arrestin recruitment, and receptor ubiquitination [26]. In parallel, a stalkless truncated version of ADGRB1 was examined in the same battery of assays and found to have nearly identical activity to the constitutively active truncated version of ADGRB1 that retained the stalk. A conclusion from this work was that aGPCRs are capable of both stalk-dependent and stalk-independent signaling, with the relative contribution of the stalk varying between different receptors and even between different readouts for the same receptor. These findings led to the proposal of the *allosteric antagonist model* of aGPCR activation (Fig. 1), in which aGPCR NT regions can dampen receptor activity in at least two distinct ways: (1) by masking the stalk region to prevent stalk-dependent signaling and (2) by allosterically antagonizing the inherent, stalk-independent activity of the 7TM region.

Further evidence that a proteolytically liberated agonist in the stalk region may not be required for all aspects of aGPCR signaling comes from studies on non-cleavable aGPCR mutants. The GAIN domain crystal structures from Arac et al. revealed how mutation of a key catalytic threonine in the GPS motif could block GAIN domain cleavage but allow for normal GAIN domain folding [10]. Such non-cleaving mutants of ADGRD1 [49], ADGRG1 [26], and ADGRG2 [30] have been studied and found to be capable of robust constitutive signaling, although in the case of ADGRG2 the non-cleavable mutant receptor exhibited signaling comparable to the wild-type receptor in one pathway but reduced signaling when a distinct pathway was measured. There is also evidence that certain aGPCRs may not undergo GAIN cleavage at all [84]. ADGRG5 and ADGRB1 are examples of aGPCRs that are naturally cleavage deficient (at least in some cellular contexts) and yet retain signaling ability [33, 45]. Moreover, in vivo studies on lat-1, the *C. elegans* ortholog of ADGRL1, revealed that wild-type and mutant non-cleavable versions of the receptor performed just as well in the transgenic rescue of deficits resulting from receptor knockout [85]. The requirement of the stalk region for aGPCR signaling is also uncertain due to observations that individual aGPCRs, such as ADGRL1, undergo additional proteolytic processing wherein GAIN autoproteolysis is followed by one or more additional cleavage events that remove the stalk region [86] (see also [9] for an in-depth discussion on the relationship between proteolytic processing and aGPCR activity). These findings taken together suggest that neither GAIN domain autoproteolysis nor the

presence of the stalk region are absolutely required for aGPCR signaling activity but rather may be important for some receptors and certain downstream pathways.

## 5 Adhesion GPCR N-Termini as Sensors of Mechanical Force

There is emerging evidence that aGPCRs may be involved in sensing mechanical forces. For example, it was shown that the ADGRE5 NTF is released from the CTF after engagement with the ligand CD55, but only under mechanical shaking conditions that are meant to recapitulate the shear stress associated with circulating blood [56]. In a similar vein, laminin-211, a ligand of ADGRG6 as mentioned above, was found to only stimulate the receptor under shaking conditions and actually antagonized receptor activity under static conditions [62]. In these studies, the mechanical forces may have helped laminin-211 to disengage the NTF from its CTF, whereas without shaking, the ligand binding may have actually stabilized the inhibitory NTF-CTF interaction. These examples support the idea that, for at least some ligand-receptor pairs, mechanical force may be a key determinant of the signaling output that results from the interaction. In a key in vivo study on aGPCR-mediated mechanosensation, Scholz et al. recently demonstrated that *Drosophila* larvae lacking the ADGRL1 ortholog CIRL exhibited diminished sensitivity to mechanical stimuli [87]. The role of aGPCRs in sensing mechanical force is likely to be an active area of research in the coming years and discussed in detail in [88].

6 Associations of aGPCRs with Signaling Proteins Other Than G Proteins

In addition to the aforementioned examples of aGPCR coupling to G proteins, there have also been a number of cytoplasmic proteins other than G proteins that have been found to interact with aGPCRs (see [89] for more on this topic). In some cases, these interactions appear to modulate G protein-mediated signaling, while in other cases these associations appear to mediate G protein-independent signaling (Fig. 2). One example of the regulation of G protein signaling comes from work on ADGRV1, which was found to interact with the PDZ domain-containing protein PDZD7, a key scaffold protein in the USH2 protein complex that is known to be pivotal for stereocilial development and function [48]. Association with PDZD7 was found to antagonize ADGRV1 activity, likely by competitively disrupting receptor association with $G\alpha_i$ [48, 90]. ADGRB1 is another aGPCR that has been found to associate with PDZ scaffold proteins. One such PDZ protein, MAGI-3, was found to potentiate receptor-mediated ERK signaling, possibly by recruiting positive regulators of the pathway [45].

In terms of G protein-independent signaling by aGPCRs, ADGRB1 and ADGRB3 have both been shown to bind to the intracellular adaptor protein ELMO1 [38, 91]. For ADGRB1, this interaction has been demonstrated to result in the formation of a complex at the plasma membrane capable of activating the
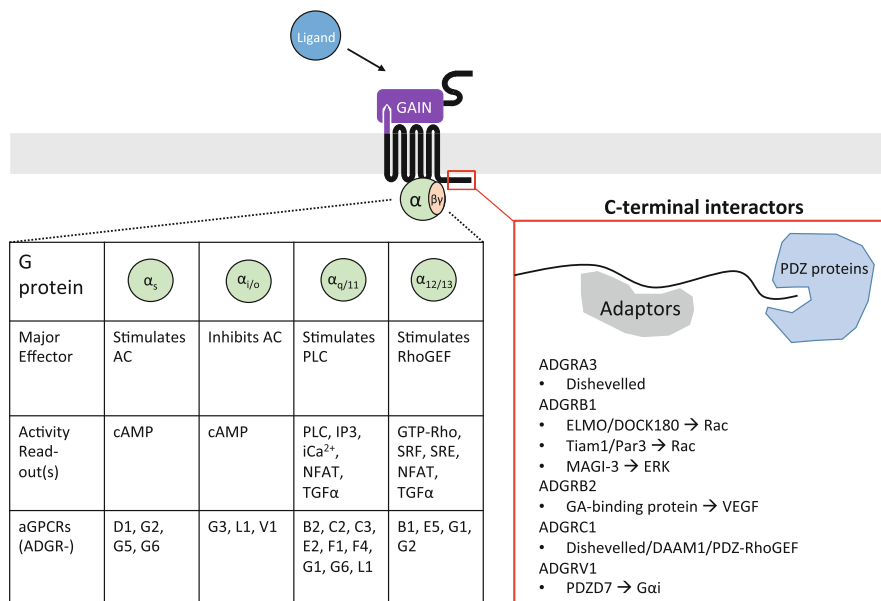
Fig. 2 G protein-dependent and G protein-independent signaling by adhesion GPCRs. The *left panel* shows the various G protein-dependent pathways that can be activated by aGPCRs. Also shown are the probable G protein-coupling preferences for selected members of the aGPCR family. The *right panel* displays various aGPCR C-terminal binding partners and briefly describes their influence on aGPCR signaling pathways (both G protein dependent and G protein independent)

small GTPase Rac1 in a G protein-independent manner [38]. ADGRB1-mediated activation of Rac1 has been implicated in phagocytosis and myoblast fusion [38, 42]. Intriguingly, ADGRB1 can also activate Rac in a distinct G protein-independent manner through association with the RacGEF Tiam1 [43]. Other examples of G protein-independent signaling by aGPCRs include ADGRB2 interaction with GA-binding protein (GABP) gamma to regulate VEGF expression [92]; ADGRC1 association with dishevelled, DAAM1, and PDZ-RhoGEF to regulate neural tube closure [93]; and ADGRA3 (GPR125) interaction with dishevelled to mediate the recruitment of planar cell polarity components [94].

7 Concluding Remarks

The versatility of aGPCR signaling described here highlights the need to comprehensively study the members of this family on a receptor-by-receptor basis in order to delineate the diversity of metabotropic pathways they serve. Further insights gained into the mechanisms of aGPCR activation will have important implications for drug development efforts aimed at these receptors. Given the number of human diseases linked to aGPCR mutations and the intriguing phenotypes observed upon

genetic deletion of aGPCRs [4], there are compelling reasons to believe that elucidation of the activation mechanisms and downstream pathways of aGPCRs will allow for an enhanced understanding of human disease and promote the development of novel classes of therapeutics.

References

1. Pierce KL, Premont RT, Lefkowitz RJ (2002) Seven-transmembrane receptors. Nat Rev Mol Cell Biol 3(9):639–650
2. Fredriksson R, Gloriam DE, Hoglund PJ, Lagerstrom MC, Schioth HB (2003) There exist at least 30 human G-protein-coupled receptors with long Ser/Thr-rich N-termini. Biochem Biophys Res Commun 301(3):725–734
3. Langenhan T, Aust G, Hamann J (2013) Sticky signaling–adhesion class G protein-coupled receptors take the stage. Sci Signal 6(276):re3
4. Hamann J, Aust G, Arac D, Engel FB, Formstone C, Fredriksson R et al (2015) International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. Pharmacol Rev 67(2):338–367
5. Krishnan A, Nijmeijer S, de Graaf C, Schiöth HB (2016) Classification, nomenclature and structural aspects of adhesion GPCRs. In: Langenhan T, Schöneberg T (eds) Adhesion G protein-coupled receptors: molecular, physiological and pharmacological principles in health and disease. Springer, Heidelberg
6. Araç D, Sträter N, Seiradake E (2016) Understanding the structural basis of adhesion GPCR functions. In: Langenhan T, Schöneberg T (eds) Adhesion G protein-coupled receptors: molecular, physiological and pharmacological principles in health and disease. Springer, Heidelberg
7. Krasnoperov VG, Bittner MA, Beavis R, Kuang Y, Salnikow KV, Chepurny OG et al (1997) alpha-Latrotoxin stimulates exocytosis by the interaction with a neuronal G-protein-coupled receptor. Neuron 18(6):925–937
8. Lin HH, Chang GW, Davies JQ, Stacey M, Harris J, Gordon S (2004) Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. J Biol Chem 279(30):31823–31832
9. Nieberler M, Kittel RJ, Petrenko AG, Lin H-H, Langenhan T (2016) Control of adhesion GPCR function through proteolytic processing. In: Langenhan T, Schöneberg T (eds) Adhesion G protein-coupled receptors: molecular, physiological and pharmacological principles in health and disease. Springer, Heidelberg
10. Arac D, Boucard AA, Bolliger MF, Nguyen J, Soltis SM, Sudhof TC et al (2012) A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. EMBO J 31(6):1364–1378
11. Liebscher I, Ackley B, Arac D, Ariestanti DM, Aust G, Bae BI et al (2014) New functions and signaling mechanisms for the class of adhesion G protein-coupled receptors. Ann N Y Acad Sci 1333:43–64
12. Promel S, Langenhan T, Arac D (2013) Matching structure with function: the GAIN domain of adhesion-GPCR and PKD1-like proteins. Trends Pharmacol Sci 34(8):470–478
13. Davletov BA, Shamotienko OG, Lelianova VG, Grishin EV, Ushkaryov YA (1996) Isolation and biochemical characterization of a Ca2+-independent alpha-latrotoxin-binding protein. J Biol Chem 271(38):23239–23245
14. Lelianova VG, Davletov BA, Sterling A, Rahman MA, Grishin EV, Totty NF et al (1997) Alpha-latrotoxin receptor, latrophilin, is a novel member of the secretin family of G protein-coupled receptors. J Biol Chem 272(34):21504–21508

15. Ichtchenko K, Khvotchev M, Kiyatkin N, Simpson L, Sugita S, Sudhof TC (1998) alpha-Latrotoxin action probed with recombinant toxin: receptors recruit alpha-latrotoxin but do not transduce an exocytotic signal. EMBO J 17(21):6188–6199

16. Volynski KE, Silva JP, Lelianova VG, Atiqur Rahman M, Hopkins C, Ushkaryov YA (2004) Latrophilin fragments behave as independent proteins that associate and signal on binding of LTX(N4C). EMBO J 23(22):4423–4433

17. Rahman MA, Ashton AC, Meunier FA, Davletov BA, Dolly JO, Ushkaryov YA (1999) Norepinephrine exocytosis stimulated by alpha-latrotoxin requires both external and stored Ca2+ and is mediated by latrophilin, G proteins and phospholipase C. Philos Trans R Soc Lond B Biol Sci 354(1381):379–386

18. Piao X, Hill RS, Bodell A, Chang BS, Basel-Vanagaite L, Straussberg R et al (2004) G protein-coupled receptor-dependent development of human frontal cortex. Science 303 (5666):2033–2036

19. Bae BI, Tietjen I, Atabay KD, Evrony GD, Johnson MB, Asare E et al (2014) Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. Science 343(6172):764–768

20. Iguchi T, Sakata K, Yoshizaki K, Tago K, Mizuno N, Itoh H (2008) Orphan G protein-coupled receptor GPR56 regulates neural progenitor cell migration via a G alpha 12/13 and Rho pathway. J Biol Chem 283(21):14469–14478

21. Paavola KJ, Stephenson JR, Ritter SL, Alter SP, Hall RA (2011) The N terminus of the adhesion G protein-coupled receptor GPR56 controls receptor signaling activity. J Biol Chem 286(33):28914–28921

22. Shashidhar S, Lorente G, Nagavarapu U, Nelson A, Kuo J, Cummins J et al (2005) GPR56 is a GPCR that is overexpressed in gliomas and functions in tumor cell adhesion. Oncogene 24 (10):1673–1682

23. Wu MP, Doyle JR, Barry B, Beauvais A, Rozkalne A, Piao X et al (2013) G-protein coupled receptor 56 promotes myoblast fusion through serum response factor- and nuclear factor of activated T-cell-mediated signalling but is not essential for muscle development in vivo. FEBS J 280(23):6097–6113

24. Stoveken HM, Hajduczok AG, Xu L, Tall GG (2015) Adhesion G protein-coupled receptors are activated by exposure of a cryptic tethered agonist. Proc Natl Acad Sci U S A 112 (19):6194–6199

25. Kim JE, Han JM, Park CR, Shin KJ, Ahn C, Seong JY et al (2010) Splicing variants of the orphan G-protein-coupled receptor GPR56 regulate the activity of transcription factors associated with tumorigenesis. J Cancer Res Clin Oncol 136(1):47–53

26. Kishore A, Purcell RH, Nassiri-Toosi Z, Hall RA (2016) Stalk-dependent and stalk-independent signaling by the adhesion G protein-coupled receptors GPR56 (ADGRG1) and BAI1 (ADGRB1). J Biol Chem 291(7):3385–3394

27. Yang L, Chen G, Mohanty S, Scott G, Fazal F, Rahman A et al (2011) GPR56 regulates VEGF production and angiogenesis during melanoma progression. Cancer Res 71(16):5558–5568

28. Little KD, Hemler ME, Stipp CS (2004) Dynamic regulation of a GPCR-tetraspanin-G protein complex on intact cells: central role of CD81 in facilitating GPR56-Galpha q/11 association. Mol Biol Cell 15(5):2375–2387

29. Ohta S, Sakaguchi S, Kobayashi Y, Mizuno N, Tago K, Itoh H (2015) Agonistic antibodies reveal the function of GPR56 in human glioma U87-MG cells. Biol Pharm Bull 38(4):594–600

30. Peeters MC, Fokkelman M, Boogaard B, Egerod KL, van de Water B, IJzerman AP et al (2015) The adhesion G protein-coupled receptor G2 (ADGRG2/GPR64) constitutively activates SRE and NFkB and is involved in cell adhesion and migration. Cell Signal 27 (12):2579–2588

31. Demberg LM, Rothemund S, Schoneberg T, Liebscher I (2015) Identification of the tethered peptide agonist of the adhesion G protein-coupled receptor GPR64/ADGRG2. Biochem Biophys Res Commun 464(3):743–747

32. Gupte J, Swaminath G, Danao J, Tian H, Li Y, Wu X (2012) Signaling property study of adhesion G-protein-coupled receptors. FEBS Lett 586(8):1214–1219

33. Wilde C, Fischer L, Lede V, Kirchberger J, Rothemund S, Schoneberg T et al (2016) The constitutive activity of the adhesion GPCR GPR114/ADGRG5 is mediated by its tethered agonist. FASEB J 30(2):666–673

34. Monk KR, Naylor SG, Glenn TD, Mercurio S, Perlin JR, Dominguez C et al (2009) A G protein-coupled receptor is essential for Schwann cells to initiate myelination. Science 325 (5946):1402–1405

35. Paavola KJ, Sidik H, Zuchero JB, Eckart M, Talbot WS (2014) Type IV collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. Sci Signal 7(338):ra76

36. Mogha A, Benesh AE, Patra C, Engel FB, Schoneberg T, Liebscher I et al (2013) Gpr126 functions in Schwann cells to control differentiation and myelination via G-protein activation. J Neurosci 33(46):17976–17985

37. Liebscher I, Schon J, Petersen SC, Fischer L, Auerbach N, Demberg LM et al (2014) A tethered agonist within the ectodomain activates the adhesion G protein-coupled receptors GPR126 and GPR133. Cell Rep 9(6):2018–2026

38. Park D, Tosello-Trampont AC, Elliott MR, Lu M, Haney LB, Ma Z et al (2007) BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. Nature 450(7168):430–434

39. Das S, Sarkar A, Ryan KA, Fox S, Berger AH, Juncadella IJ et al (2014) Brain angiogenesis inhibitor 1 is expressed by gastric phagocytes during infection with Helicobacter pylori and mediates the recognition and engulfment of human apoptotic gastric epithelial cells. FASEB J 28(5):2214–2224

40. Mazaheri F, Breus O, Durdu S, Haas P, Wittbrodt J, Gilmour D et al (2014) Distinct roles for BAI1 and TIM-4 in the engulfment of dying neurons by microglia. Nat Commun 5:4046

41. Das S, Owen KA, Ly KT, Park D, Black SG, Wilson JM et al (2011) Brain angiogenesis inhibitor 1 (BAI1) is a pattern recognition receptor that mediates macrophage binding and engulfment of Gram-negative bacteria. Proc Natl Acad Sci U S A 108(5):2136–2141

42. Hochreiter-Hufford AE, Lee CS, Kinchen JM, Sokolowski JD, Arandjelovic S, Call JA et al (2013) Phosphatidylserine receptor BAI1 and apoptotic cells as new promoters of myoblast fusion. Nature 497(7448):263–267

43. Duman JG, Tzeng CP, Tu YK, Munjal T, Schwechter B, Ho TS et al (2013) The adhesion-GPCR BAI1 regulates synaptogenesis by controlling the recruitment of the Par3/Tiam1 polarity complex to synaptic sites. J Neurosci 33(16):6964–6978

44. Zhu D, Li C, Swanson AM, Villalba RM, Guo J, Zhang Z et al (2015) BAI1 regulates spatial learning and synaptic plasticity in the hippocampus. J Clin Invest 125(4):1497–1508

45. Stephenson JR, Paavola KJ, Schaefer SA, Kaur B, Van Meir EG, Hall RA (2013) Brain-specific angiogenesis inhibitor-1 signaling, regulation, and enrichment in the postsynaptic density. J Biol Chem 288(31):22248–22256

46. Okajima D, Kudo G, Yokota H (2010) Brain-specific angiogenesis inhibitor 2 (BAI2) may be activated by proteolytic processing. J Recept Signal Transduct Res 30(3):143–153

47. Ward Y, Lake R, Yin JJ, Heger CD, Raffeld M, Goldsmith PK et al (2011) LPA receptor heterodimerizes with CD97 to amplify LPA-initiated RHO-dependent signaling and invasion in prostate cancer cells. Cancer Res 71(23):7301–7311

48. Hu QX, Dong JH, Du HB, Zhang DL, Ren HZ, Ma ML et al (2014) Constitutive Galphai coupling activity of very large G protein-coupled receptor 1 (VLGR1) and its regulation by PDZD7 protein. J Biol Chem 289(35):24215–24225

49. Bohnekamp J, Schoneberg T (2011) Cell adhesion receptor GPR133 couples to Gs protein. J Biol Chem 286(49):41912–41916

50. Silva JP, Lelianova VG, Ermolyuk YS, Vysokov N, Hitchen PG, Berninghausen O et al (2011) Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities. Proc Natl Acad Sci U S A 108(29):12113–12118

51. Tobaben S, Sudhof TC, Stahl B (2002) Genetic analysis of alpha-latrotoxin receptors reveals functional interdependence of CIRL/latrophilin 1 and neurexin 1 alpha. J Biol Chem 277 (8):6359–6365

52. O'Sullivan ML, de Wit J, Savas JN, Comoletti D, Otto-Hitt S, Yates JR III et al (2012) FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development. Neuron 73(5):903–910

53. Jackson VA, del Toro D, Carrasquero M, Roversi P, Harlos K, Klein R et al (2015) Structural basis of latrophilin-FLRT interaction. Structure 23(4):774–781

54. Huang YS, Chiang NY, Hu CH, Hsiao CC, Cheng KF, Tsai WP et al (2012) Activation of myeloid cell-specific adhesion class G protein-coupled receptor EMR2 via ligation-induced translocation and interaction of receptor subunits in lipid raft microdomains. Mol Cell Biol 32 (8):1408–1420

55. Stacey M, Chang GW, Davies JQ, Kwakkenbos MJ, Sanderson RD, Hamann J et al (2003) The epidermal growth factor-like domains of the human EMR2 receptor mediate cell attachment through chondroitin sulfate glycosaminoglycans. Blood 102(8):2916–2924

56. Karpus ON, Veninga H, Hoek RM, Flierman D, van Buul JD, Vandenakker CC et al (2013) Shear stress-dependent downregulation of the adhesion-G protein-coupled receptor CD97 on circulating leukocytes upon contact with its ligand CD55. J Immunol 190(7):3740–3748

57. Wang T, Ward Y, Tian L, Lake R, Guedez L, Stetler-Stevenson WG et al (2005) CD97, an adhesion receptor on inflammatory cells, stimulates angiogenesis through binding integrin counterreceptors on endothelial cells. Blood 105(7):2836–2844

58. Wandel E, Saalbach A, Sittig D, Gebhardt C, Aust G (2012) Thy-1 (CD90) is an interacting partner for CD97 on activated endothelial cells. J Immunol 188(3):1442–1450

59. Sigoillot SM, Iyer K, Binda F, Gonzalez-Calvo I, Talleur M, Vodjdani G et al (2015) The secreted protein C1QL1 and its receptor BAI3 control the synaptic connectivity of excitatory inputs converging on cerebellar Purkinje cells. Cell Rep 10(5):820–832

60. Bolliger MF, Martinelli DC, Sudhof TC (2011) The cell-adhesion G protein-coupled receptor BAI3 is a high-affinity receptor for C1q-like proteins. Proc Natl Acad Sci U S A 108 (6):2534–2539

61. Luo R, Jeong SJ, Jin Z, Strokes N, Li S, Piao X (2011) G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. Proc Natl Acad Sci U S A 108(31):12925–12930

62. Petersen SC, Luo R, Liebscher I, Giera S, Jeong SJ, Mogha A et al (2015) The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211. Neuron 85(4):755–769

63. Xu L, Hynes RO (2007) GPR56 and TG2: possible roles in suppression of tumor growth by the microenvironment. Cell Cycle 6(2):160–165

64. Boucard AA, Maxeiner S, Sudhof TC (2014) Latrophilins function as heterophilic cell-adhesion molecules by binding to teneurins: regulation by alternative splicing. J Biol Chem 289(1):387–402

65. Bang ML, Owczarek S (2013) A matter of balance: role of neurexin and neuroligin at the synapse. Neurochem Res 38(6):1174–1189

66. Geppert M, Khvotchev M, Krasnoperov V, Goda Y, Missler M, Hammer RE et al (1998) Neurexin I alpha is a major alpha-latrotoxin receptor that cooperates in alpha-latrotoxin action. J Biol Chem 273(3):1705–1710

67. Boucard AA, Ko J, Sudhof TC (2012) High affinity neurexin binding to cell adhesion G-protein-coupled receptor CIRL1/latrophilin-1 produces an intercellular adhesion complex. J Biol Chem 287(12):9399–9413

68. Hamann J, Vogel B, van Schijndel GM, van Lier RA (1996) The seven-span transmembrane receptor CD97 has a cellular ligand (CD55, DAF). J Exp Med 184(3):1185–1189

69. Hamann J, Stortelers C, Kiss-Toth E, Vogel B, Eichler W, van Lier RA (1998) Characterization of the CD55 (DAF)-binding site on the seven-span transmembrane receptor CD97. Eur J Immunol 28(5):1701–1707

70. Lin HH, Stacey M, Saxby C, Knott V, Chaudhry Y, Evans D et al (2001) Molecular analysis of the epidermal growth factor-like short consensus repeat domain-mediated protein-protein interactions: dissection of the CD97-CD55 complex. J Biol Chem 276(26):24160–24169

71. Iijima T, Miura E, Watanabe M, Yuzaki M (2010) Distinct expression of C1q-like family mRNAs in mouse brain and biochemical characterization of their encoded proteins. Eur J Neurosci 31(9):1606–1615

72. Kakegawa W, Mitakidis N, Miura E, Abe M, Matsuda K, Takeo YH et al (2015) Anterograde C1ql1 signaling is required in order to determine and maintain a single-winner climbing fiber in the mouse cerebellum. Neuron 85(2):316–329

73. Xu L, Begum S, Hearn JD, Hynes RO (2006) GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2, and inhibits melanoma tumor growth and metastasis. Proc Natl Acad Sci U S A 103(24):9023–9028

74. Yang L, Friedland S, Corson N, Xu L (2014) GPR56 inhibits melanoma growth by internalizing and degrading its ligand TG2. Cancer Res 74(4):1022–1031

75. Singer K, Luo R, Jeong SJ, Piao X (2013) GPR56 and the developing cerebral cortex: cells, matrix, and neuronal migration. Mol Neurobiol 47(1):186–196

76. Luo R, Jin Z, Deng Y, Strokes N, Piao X (2012) Disease-associated mutations prevent GPR56-collagen III interaction. PLoS One 7(1), e29818

77. Chiang NY, Hsiao CC, Huang YS, Chen HY, Hsieh IJ, Chang GW et al (2011) Disease-associated GPR56 mutations cause bilateral frontoparietal polymicrogyria via multiple mechanisms. J Biol Chem 286(16):14215–14225

78. Jin Z, Tietjen I, Bu L, Liu-Yesucevitz L, Gaur SK, Walsh CA et al (2007) Disease-associated mutations affect GPR56 protein trafficking and cell surface expression. Hum Mol Genet 16 (16):1972–1985

79. Weston MD, Luijendijk MW, Humphrey KD, Moller C, Kimberling WJ (2004) Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. Am J Hum Genet 74(2):357–366

80. Robinson A, Escuin S, Doudney K, Vekemans M, Stevenson RE, Greene ND et al (2012) Mutations in the planar cell polarity genes CELSR1 and SCRIB are associated with the severe neural tube defect craniorachischisis. Hum Mutat 33(2):440–447

81. Paavola KJ, Hall RA (2012) Adhesion G protein-coupled receptors: signaling, pharmacology, and mechanisms of activation. Mol Pharmacol 82(5):777–783

82. Liebscher I, Schöneberg T (2016) Tethered agonism: a common activation mechanism of adhesion GPCRs. In: Langenhan T, Schöneberg T (eds) Adhesion G protein-coupled receptors: molecular, physiological and pharmacological principles in health and disease. Springer, Heidelberg

83. Coughlin SR (2000) Thrombin signalling and protease-activated receptors. Nature 407 (6801):258–264

84. Promel S, Waller-Evans H, Dixon J, Zahn D, Colledge WH, Doran J et al (2012) Characterization and functional study of a cluster of four highly conserved orphan adhesion-GPCR in mouse. Dev Dyn 241(10):1591–1602

85. Promel S, Frickenhaus M, Hughes S, Mestek L, Staunton D, Woollard A et al (2012) The GPS motif is a molecular switch for bimodal activities of adhesion class G protein-coupled receptors. Cell Rep 2(2):321–331

86. Krasnoperov V, Deyev IE, Serova OV, Xu C, Lu Y, Buryanovsky L et al (2009) Dissociation of the subunits of the calcium-independent receptor of alpha-latrotoxin as a result of two-step proteolysis. Biochemistry 48(14):3230–3238

87. Scholz N, Gehring J, Guan C, Ljaschenko D, Fischer R, Lakshmanan V et al (2015) The adhesion GPCR latrophilin/CIRL shapes mechanosensation. Cell Rep 11(6):866–874

88. Scholz N, Monk KR, Kittel RJ, Langenhan T (2016) Adhesion GPCRs as a putative class of metabotropic mechanosensors. In: Langenhan T, Schöneberg T (eds) Adhesion G protein-coupled receptors: molecular, physiological and pharmacological principles in health and disease. Springer, Heidelberg

89. Knapp B, Wolfrum U (2016) Adhesion G protein-coupled receptor-related protein networks. In: Langenhan T, Schöneberg T (eds) Adhesion G protein-coupled receptors: molecular, physiological and pharmacological principles in health and disease. Springer, Heidelberg

90. Zou J, Zheng T, Ren C, Askew C, Liu XP, Pan B et al (2014) Deletion of PDZD7 disrupts the Usher syndrome type 2 protein complex in cochlear hair cells and causes hearing loss in mice. Hum Mol Genet 23(9):2374–2390

91. Lanoue V, Usardi A, Sigoillot SM, Talleur M, Iyer K, Mariani J et al (2013) The adhesion-GPCR BAI3, a gene linked to psychiatric disorders, regulates dendrite morphogenesis in neurons. Mol Psychiatry 18(8):943–950

92. Jeong BC, Kim MY, Lee JH, Kee HJ, Kho DH, Han KE et al (2006) Brain-specific angiogenesis inhibitor 2 regulates VEGF through GABP that acts as a transcriptional repressor. FEBS Lett 580(2):669–676

93. Nishimura T, Honda H, Takeichi M (2012) Planar cell polarity links axes of spatial dynamics in neural-tube closure. Cell 149(5):1084–1097

94. Li X, Roszko I, Sepich DS, Ni M, Hamm HE, Marlow FL et al (2013) Gpr125 modulates Dishevelled distribution and planar cell polarity signaling. Development 140(14):3028–3039