Versatile Signaling Activity of Adhesion GPCRs

Ayush Kishore and Randy A. Hall

Graphical Abstract

A. Kishore • R.A. Hall (✉)
Department of Pharmacology, Emory University School of Medicine, 1510 Clifton Road NE,
Atlanta, GA 30322, USA
e-mail: rhall3@emory.edu

© Springer International Publishing AG 2016
T. Langenhan, T. Schöneberg (eds.), Adhesion G Protein-coupled Receptors,
Handbook of Experimental Pharmacology 234, DOI 10.1007/978-3-319-41523-9_7
Contents

1 Adhesion G Protein-Coupled Receptors Are a Diverse Group of Self-Cleaving Cell Surface Receptors ................................................................. 128
2 Evidence for G Protein-Mediated Signaling by Adhesion GPCRs .................................................. 129
3 Ligands for Adhesion GPCRs ...................................................................................... 131
4 Adhesion GPCR Models of Activation .............................................................................. 136
5 Adhesion GPCR N-Termini as Sensors of Mechanical Force .............................................. 139
6 Associations of aGPCRs with Signaling Proteins Other Than G Proteins .............................. 139
7 Concluding Remarks ................................................................................................. 140
References .................................................................................................................... 141

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 GPCR autoproteolysis-inducing (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 to 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.
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-LTXN4C, 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-LTXN4C treatment, suggesting coupling of the receptor to Goq [16]. Moreover, ADGRL1 could be co-purified with Go6 [14, 17] and Goq/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 Go12/13 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 Goq/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/Goq/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 Goq/11-mediated signaling [29]. Additionally, ADGRG1 has been shown to activate Go13 in a reconstituted GTPγS-binding assay [24], and an association between ADGRG1 and Go13 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 Goq14, suggesting promiscuous coupling to both Gs and Gq [31]. Similarly, it was shown that overexpression of ADGRG3 (GPR97) in HEK293 cells stimulated IP3 accumulation only in the presence of chimeric G protein Goq3, which converts Go signaling into Gq activity, suggesting natural coupling of the receptor to Go6 [32]. ADGRG5 (GPR114) overexpression was shown to potentiate cAMP levels, an effect that could be blocked via knockdown of endogenous Go or overexpression of the chimeric G protein Goq4, which converts Go signaling into Gq-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\(\alpha_q\) 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 TGF\(\alpha\) 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 GTP\(\gamma\)S 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\(\alpha_{qS}\) 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, \(\alpha\)-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 \(\alpha\)-LTX affinity chromatography [50]. Treatment of cultured neurons expressing ADGRL1 with a soluble,
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Binding region</th>
<th>Downstream activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADGRL1</td>
<td>α-Latrotoxin</td>
<td>NT (GAIN domain)</td>
<td>Increased cAMP [14], IP3 [14], Ca²⁺ [16], and PLC activation [16]</td>
</tr>
<tr>
<td>ADGRL1</td>
<td>Teneurin-2</td>
<td>NT</td>
<td>Increased Ca²⁺ in cultured hippocampal neurons [50]</td>
</tr>
<tr>
<td>ADGRL1</td>
<td>Neurexin1α</td>
<td>NT</td>
<td>Regulation of α-latrotoxin-mediated glutamate release [51]</td>
</tr>
<tr>
<td>ADGRL3</td>
<td>FLRT3</td>
<td>NT</td>
<td>Regulation of synaptic density [52]</td>
</tr>
<tr>
<td>ADGRL3</td>
<td>FLRT2</td>
<td>NT (OLF domain)</td>
<td>Regulation of cell adhesion/repulsion [53]</td>
</tr>
<tr>
<td><strong>Family II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADGRE2</td>
<td>NT antibody (2A1)</td>
<td>NT</td>
<td>Increased production of inflammatory cytokines [54]</td>
</tr>
<tr>
<td>ADGRE2/ADGRE5</td>
<td>Chondroitin sulfate</td>
<td>(likely NT region)</td>
<td>Mediates cell adhesion [55]</td>
</tr>
<tr>
<td>ADGRE5</td>
<td>CD55</td>
<td>NT (EGF domains)</td>
<td>Alteration in ADGRE5 NT-CTF interaction [56]</td>
</tr>
<tr>
<td>ADGRE5</td>
<td>α5β1/αvβ3</td>
<td>NT</td>
<td>Mediates endothelial cell migration [57]</td>
</tr>
<tr>
<td>ADGRE5</td>
<td>CD90</td>
<td>NT</td>
<td>Mediates cell adhesion [58]</td>
</tr>
<tr>
<td><strong>Family V</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADGRD1</td>
<td>Stalk peptide(s)</td>
<td>(likely 7TM region)</td>
<td>Increased cAMP levels [37]</td>
</tr>
<tr>
<td><strong>Family VI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADGRF1</td>
<td>Stalk peptide(s)</td>
<td>(likely 7TM region)</td>
<td>Increased GTPγS binding [24]</td>
</tr>
<tr>
<td><strong>Family VII</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADGRB1</td>
<td>Phosphatidylserine</td>
<td>NT (TSR domains)</td>
<td>Enhanced Rac1-dependent uptake of apoptotic cells [39]</td>
</tr>
<tr>
<td>ADGRB3</td>
<td>C1q11</td>
<td>NT (CUB domain)</td>
<td>Regulation of dendritic spine density [59]</td>
</tr>
<tr>
<td>ADGRB3</td>
<td>C1q13</td>
<td>NT (TSR domains)</td>
<td>Regulation of synaptic density [60]</td>
</tr>
<tr>
<td><strong>Family VIII</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADGRG1</td>
<td>Tissue transglutaminase 2</td>
<td>NT (STP region)</td>
<td>Regulation of VEGF secretion [27]</td>
</tr>
<tr>
<td>ADGRG1</td>
<td>Collagen III</td>
<td>NT (aa 27–160)</td>
<td>Stimulation of RhoA activation [61]</td>
</tr>
<tr>
<td>ADGRG1</td>
<td>NT antibody</td>
<td>NT</td>
<td>Stimulation of SRE and RhoA activity [20]</td>
</tr>
<tr>
<td>ADGRG1</td>
<td>Stalk peptide(s)</td>
<td>(likely 7TM region)</td>
<td>Stimulation of SRE luciferase [24]</td>
</tr>
<tr>
<td>ADGRG2</td>
<td>Stalk peptide(s)</td>
<td>(likely 7TM region)</td>
<td>Increased cAMP and IP3 accumulation [31]</td>
</tr>
<tr>
<td>ADGRG3</td>
<td>Beclomethasone dipropionate</td>
<td></td>
<td>Increased GTPγS binding [32]</td>
</tr>
</tbody>
</table>

(continued)
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 \(\alpha\)-LTX, as is ADGRL1 [66]. A particular neurexin isoform (1\(\alpha\)) binds \(\alpha\)-LTX in a Ca\(^{2+}\)-dependent fashion, while the \(\alpha\)-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 \(\alpha\)-LTX response compared to wild-type neurons, suggesting that while ADGRL1 and neurexin can independently associate with \(\alpha\)-LTX, their interaction may synergistically enhance \(\alpha\)-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

### Table 1 (continued)

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

? unavailable

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 Go12/13 [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 Go activity rather than through differential activation of Goi. 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 stimu-
late receptor signaling in the SRE luciferase assay (a commonly used assay for 
G\textsubscript{α12/13} 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\textsubscript{αq} or G\textsubscript{α12/13} 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 neu-
trophil 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], ADGFR1 [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).
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 found in the N-terminal stalk region between the site of autoproteolysis and the first transmembrane domain. 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. 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 and ADGRG5. Additionally, Stoveken et al. showed that stalkless versions of ADGRG1 and ADGRF1 lacked activity in reconstitution assays examining GTP binding to purified Gα₁₃ and Gα₆₅.

**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.
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 Go13 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 Goi [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
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.

Acknowledgments  The authors’ research is supported by the National Institutes of Health.

References


