Mechanisms of adhesion G protein–coupled receptor activation

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Adhesion G protein–coupled receptors (AGPCRs) are a thirty-three-member subfamily of Class B GPCRs that control a wide array of physiological processes and are implicated in disease. AGPCRs uniquely contain large, self-proteolyzing extracellular regions that range from hundreds to thousands of residues in length. AGPCR autoproteolysis occurs within the extracellular GPCR autoproteolysis-inducing (GAIN) domain that is proximal to the N terminus of the G protein–coupling seven-transmembrane–spanning bundle. GAIN domain–mediated self-cleavage is constitutive and produces two-fragment holoreceptors that remain bound at the cell surface. It has been of recent interest to understand how AGPCRs are activated in relation to their two-fragment topologies. Dissociation of the AGPCR fragments stimulates G protein signaling through the action of the tethered-peptide agonist stalk that is occluded within the GAIN domain in the holoreceptor form. AGPCRs can also signal independently of fragment dissociation, and a few receptors possess GAIN domains incapable of self-proteolysis. This has resulted in complex theories as to how these receptors are activated in vivo, complicating pharmacological advances. Currently, there is no existing structure of an activated AGPCR to support any of the theories. Further confounding AGPCR research is that many of the receptors remain orphans and lack identified activating ligands. In this review, we provide a detailed layout of the current theorized modes of AGPCR activation with discussion of potential parallels to mechanisms used by other GPCR classes. We provide a classification means for the ligands that have been identified and discuss how these ligands may activate AGPCRs in physiological contexts.

G protein–coupled receptors (GPCRs) are the largest class of membrane receptors, comprising over 800 members in humans. The GPCR seven-transmembrane helical bundle (7TM) allows for regulation of distinct G protein signaling cascades in response to diverse extracellular stimuli. Due to a broad influence on health and disease, GPCRs are heavily investigated for pharmacological intervention and are the targets of many approved drugs (1). Consequently, study of each individual GPCR subclass will provide unique angles that are beneficial to the development of therapeutics. Canonically, GPCR signaling is initiated by agonist binding to its orthosteric site, which results in rearrangements of the transmembrane helices of the 7TM bundle to allow efficient heterotrimeric G protein coupling and activation. G protein α subunits exchange GDP for GTP, allowing for functional dissociation of Gβγ and activation of downstream effectors. GPCRs are divided into six classes as follows with in-class examples: Class A (rhodopsin-like), Class B (secretin), Class C (metabotropic glutamate), Class D (pheromone), Class E (cAMP), and Class F (Frizzled) (2–4). Within this naming system, the adhesion GPCRs (AGPCRs) are family B members but have been more aptly termed subfamily B2, whereas the traditional Class B peptide hormone-binding GPCRs comprise subfamily B1.

AGPCRs are distinguished not only by their large extracellular regions (ECRs) that contain a wide variety of adhesive subdomains, but also by the highly conserved GPCR autoproteolysis–inducing (GAIN) domain that constitutively self-cleaves the receptors into two fragments (5). Whereas extensive work has been done to characterize AGPCRs, it is largely uncertain how they are activated in endogenous tissues. How protein ligand–ECR binding regulates the activation state of the 7TM bundle is arguably the most intensely studied problem in current AGPCR research. To date, mechanisms involving AGPCR fragment dissociation and modes of allosteric modulation in response to endogenous ligands have been proposed. Here, we sought to provide clarity to these activation mechanisms by detailing the structural topologies of AGPCRs, while examining the prospective actions of endogenous ligands. AGPCR activation models will be compared with established modes of activation of other GPCR classes. Select aspects of AGPCR physiological regulation will also be discussed as routes to receptor activation. The theories outlined in this review will provide a consistent framework for classification of endogenous AGPCR ligands as they are identified and develop thought of mechanistic considerations in advance of AGPCR structures that await solution. As knowledge of AGPCR function increases, the realization that this class of receptors are untapped therapeutic targets will increase, prompting efforts to target them.

The unique structural topology of adhesion GPCRs

The 33 human adhesion GPCRs (ADGRs) are divided among nine subfamilies, ADGRA–G, -L, and -V, based on sequence similarity (6, 7). AGPCRs possess 7TM domains that are known to signal through heterotrimeric G proteins in many cases. The N-terminal ECRs range from hundreds to thousands of residues and often share common characteristics among receptors in the same subfamily. The ECRs contain a variety of adhesion related subdomains that are often repeated (Fig. 1A, multicolored nodules). For example, group E AGPCRs, which include...
the ADGRE (EMR (EGF-like module–containing mucin-like hormone receptor)) receptors, contain epidermal growth factor–like repeats that are found in many types of proteins that mediate cell-adhesive interactions (8, 9). Twelve AGPCRs contain a ~70-residue hormone-binding (HormR) domain located N-terminally to their GAIN domain. There has yet to be a report of a hormone that binds to an AGPCR, leading many to believe that the HormR domain has additional functions beyond hormone binding (Fig. 1E) (10). Another interesting motif found in select adhesion GPCR ECRs is the sperm protein, enterokinase, and agrin (SEA) domain. This domain is found in ADGRF1 (GPR110), ADGRF5 (GPR116), and ADGRG6 (GPR126) (11–13). SEA domains mediate a second autoproteolytic cleavage event that is distinct from GAIN domain self-cleavage. Not much is known about the role of the SEA domain, but its function leaves open the possibility that these particular receptors have alternative modes of signaling regulation.

At the C-terminal end of nearly every AGPCR ECR, ending 7–18 residues prior to the start of the first transmembrane span of the 7TM bundle, is the GAIN domain (5) (Fig. 1A). A seminal finding in the AGPCR field was the X-ray crystallographic solution of GAIN domain structures (5). GAIN domains are divided into two subdomains: an α-helix–rich GAINa and β-sandwich GAINb. Complete GAIN domains are ~320 amino acids with variability typically observed within the GAINa subdomain. As indicated by its name, the GAIN domain is a fully self-sufficient protease that catalyzes constitutive autoproteolysis that splits the receptors into two fragments. Autoproteolytic cleavage is
believed to be constitutive in most cases, but some studies have raised the possibility that cleavage might be regulated, although consideration of overexpression artifacts is also merited (5, 14, 15). The receptor fragments remaining after self-cleavage are the extracellular N-terminal fragment (NTF) or ECR and the membrane-intercalated C-terminal fragment (CTF), which is also referred to as the GPCR or 7TM domain. A dense network of hydrogen bonds within the GAIN domain allows the NTF (also referred to as the GPCR or 7TM domain. A dense network of hydrogen bonds within the GAIN domain allows the NTF and CTF of the receptor to become dissociated to liberate the agonist peptide from the interior core of the GAIN domain. Many within the field are currently deciphering exactly how AGPCR tethered agonism intersects with the varied AGPCR activation mechanisms, which are discussed below. Interestingly, not all AGPCRs undergo autoproteolysis; some receptors, including ADGRC1 (cadherin EGF LAG seven-pass G-type receptor 1 (CELSR1)), ADGRA2 (GPR124), ADGRF2 (GPR111), ADGRA3 (GPR125), and ADGRF4 (GPR115), may be activated by alternative modes that do not involve fragment dissociation and tethered-peptide agonism (23–25). Impaired self-cleavage is typically attributed to alterations of the GPS; receptors lacking a basic residue at the P2 position (e.g., ADGRF2 or ADGRF4) or a polar residue at the P1 position (e.g., ADGRC1) demonstrate minimal or no autoproteolysis (25). Impaired self-cleavage is typically attributed to alterations of the GPS; receptors lacking a basic residue at the P2 position (e.g., ADGRF2 or ADGRF4) or a polar residue at the P1 position (e.g., ADGRC1) demonstrate minimal or no autoproteolysis (25). Differences in post-translational modifications have also been proposed to regulate GAIN-mediated cleavage, such as N-linked glycosylation events within the GAIN domain (5, 14, 15). However, observations of inefficient cleavage in these instances may be manifestations of experimental receptor overexpression that impart improper receptor trafficking or processing. Noncleaved AGPCRs are still capable of signaling, leaving open the question of how these receptors become activated.

### The self-proteolytic reaction of adhesion GPCRs

The AGPCR self-proteolysis reaction requires proper folding of the GAIN domain and occurs within GAIN$_b$ when a conserved basic residue at the P2 position of the cleavage site abstracts a hydrogen from the side chain of the conserved, polar threonine (or serine) at the P1’ position (Fig. 1B) (17). AGPCR P2 site basic residues are most commonly histidines, such as His-836 in ADGRL1 (latrophilin (LPHN1)), but can be arginine, such as Arg-855 in ADGRB5 (BAI3) (5). The proton abstraction initiates a nucleophilic attack of the carbonyl group of the P1 residue, which is most commonly leucine. The resulting ester intermediate is resolved by a final nucleophilic attack of a water molecule. The consensus self-cleavage site within the GAIN domains of most AGPCRs is HL/T. Prior to solution of the GAIN domain structure, the HL/T site and surrounding sequence was termed the GPCR proteolysis site (GPS), reflecting the idea that the minimal sequence was sufficient for proteolysis rather than the larger structure of the GAIN domain (18–20). Given that this original definition has changed, GPS has now commonly come to mean the HL/T consensus site within the GAIN domain.

The GPS is located 14–25 residues N-terminal to the start of the first transmembrane span (TM1). The start of the CTF, the P1’ threonine, is also immediately N-terminal to the first residue of the final (13th) β-strand of the sandwich structure that comprises the GAIN$_b$ subdomain. Therefore, β-strand 13 is part of the CTF, but it is embedded within GAIN$_b$, and the GPS (i.e., HL/T) is essentially the loop that links β-strands 12 and 13 (Fig. 1C). β-Strand 13 sequences of adhesion GPCRs are highly conserved and very hydrophobic, which aligns with their location within the interior core of GAIN$_b$ subdomain (Fig. 1(C–E) and Table 1). β-Strand 13 is noncovalently bound by a dense network of ~20 hydrogen bonds that hold it firmly within the GAIN$_b$ subdomain (5).

Within recent years, the stalk that connects TM1 to the GAIN domain and includes β-strand 13 has been named the adhesion GPCR tethered-peptide agonist (also referred to as the tethered agonist). This conserved sequence within multiple AGPCRs was shown independently by two groups to play a pivotal role in mediating receptor activation (21, 22). One feature that seems to be an obvious requirement for tethered-peptide agonism is that the NTF and CTF of the receptor must become dissociated to liberate the agonist peptide from the interior core of the GAIN domain. Many within the field are currently deciphering exactly how AGPCR tethered agonism intersects with the varied AGPCR activation mechanisms, which are discussed below. Interestingly, not all AGPCRs undergo autoproteolysis; some receptors, including ADGRC1 (cadherin EGF LAG seven-pass G-type receptor 1 (CELSR1)), ADGRA2 (GPR124), ADGRF2 (GPR111), ADGRA3 (GPR125), and ADGRF4 (GPR115), may be activated by alternative modes that do not involve fragment dissociation and tethered-peptide agonism (23–25). Impaired self-cleavage is typically attributed to alterations of the GPS; receptors lacking a basic residue at the P2 position (e.g., ADGRF2 or ADGRF4) or a polar residue at the P1’ position. (e.g., ADGRC1) demonstrate minimal or no autoproteolysis (Table 1) (23–25). Differences in post-translational modifications have also been proposed to regulate GAIN-mediated cleavage, such as N-linked glycosylation events within the GAIN domain (5, 14, 15). However, observations of inefficient cleavage in these instances may be manifestations of experimental receptor overexpression that impart improper receptor trafficking or processing. Noncleaved AGPCRs are still capable of signaling, leaving open the question of how these receptors become activated.

### Table 1

| Receptor | HLT Cleavage Site and CTF Stalk |
|----------|---------------------------------|
| **ADGR1L (LPHN1)** | **CXC** |
| **ADGR2L (LPHN2)** | **CXXX** |
| **ADGR3L (LPHN3)** | **CXXX** |
| **ADGR4L (ELD1)** | **CXXX** |
| **ADGRE1 (EMR1)** | **CXXX** |
| **ADGRE2 (EMR2)** | **CXXX** |
| **ADGRE3 (EMR3)** | **CXXX** |
| **ADGRE4 (EMR4)** | **CXXX** |
| **ADGRE5 (CGD7)** | **CXXX** |

* ADGRA1 (GPR123) does not have a GAIN domain and thus does not have a GPS.
Adhesion GPCR activation mechanisms

GPCRs exhibit different basal activity levels that depend on the individual characteristics of each receptor. Basal activity is one state that GPCRs occupy within a dynamic energy landscape of active and inactive conformations (26–28). Fig. 2 depicts four proposed activity states of adhesion GPCRs with cartoon diagrams that the field has used with representation of G protein–binding site dynamism as a function of receptor activation. Accompanying the diagrams are activity profiles of relative signaling strength. An understanding of the ways that AGPCRs become activated is emerging. There has been a broad and imaginative variety of proposed AGPCR activation schemes (10, 29–34). The current evidence supports two fundamental modes of AGPCR modulation: orthosteric agonism (i.e. tethered-peptide agonism), in which NTF/CTF dissociation is required, and allosteric regulation, which has also been termed the tunable model and does not require receptor subunit dissociation (21, 30, 31, 33, 35). Both fundamental activation modes are supported through several lines of evidence, and it is likely that individual AGPCRs can be activated in both manners.

Orthosteric agonism (tethered-peptide agonism)

Orthosteric agonism is a receptor activation model that depends on the action of the AGPCR tethered agonist. The most important residues, or the core of the tethered agonist, are the seven residues located immediately C-terminal to the GPS. These residues mostly overlap with β-strand 13, the conformation the sequence adopts in the holoreceptor form. The core residues share the consensus sequence TXXFAVLM, with the T, F, and M residues showing the highest conversation across all AGPCRs (Table 1). Prior to understanding that this sequence was a tethered-peptide agonist, a study by Hall and colleagues (36) provided evidence that an isolated CTF was more active than its cognate holoreceptor. The signaling strengths of ADGRG1 (GPR56) and an engineered ADGRG1 construct in which the entirety of the NTF was deleted (ΔNTF, or CTF) were compared. The ΔNTF receptor exhibited substantially higher G protein–dependent signaling than the full-length receptor (36). Since then, ΔNTF variants of several AGPCRs were found to have increased signaling capacity (21, 36–39). A subsequent study used urea to operationally dissociate the two

Figure 2. Models of adhesion GPCR activation. A, adhesion GPCRs, like other GPCRs, occupy a range of activated and inhibited states. Consequently, AGPCRs possess varying levels of basal G protein signaling. The adhesion GPCR N-terminal subdomains (dark green, yellow, and brown modules) are portrayed to reflect the potential variety within adhesion GPCR ECRs. B, in the orthosteric agonism model of activation, NTF/CTF dissociation via an anchored ligand (depicted by the green star) results in exposure of the tethered-peptide agonist (orange), allowing it to bind to an orthosteric site that is predicted to lie within the 7TM helical bundle. Orthosteric agonism is proposed to be a threshold response (all or none) due to forced NTF dissociation, which results in stabilization of highly active states of the receptors and maximal signaling. C and D, in allosteric modes of AGPCR regulation, ligands (indicated with a blue or red star) can interact with various AGPCR N-terminal adhesive motifs to stabilize active (activation, C) or inactive states (inhibition, D), respectively. Allosteric activation and inhibition mechanisms are unknown but may be mediated by GAIN-7TM interactions that favor stabilization of specific receptor conformations. E, relative signaling strength outputs in response to stimulus for each of the receptor modulation modes.
fragments of the AGDRG1 in membrane homogenates. The isolated CTF was markedly more efficacious at activating G proteins than the holoreceptor (21). At this point, the aspect that rendered AGPCR CTFs to be activated was not clear. It was found that the TM1 N-terminal stalk sequence consisting of ~20 residues behaved as a tethered-peptide agonist and dramatically activated AGPCRs. Deletion of tethered agonist residues dramatically lowered receptor activities in vitro, as shown for ADGRG1, ADGRG2 (GPR64), ADGRG6, ADGRD1 (GPR133), and ADGFR1 (21, 22, 40, 41). Partial deletion of the zebrafish ADGRG6 tethered agonist resulted in a puffy ear phenotype and impairment of nerve cell myelination, essentially phenocopying the fish model deletion of the ADGRG6 CTF (22, 42). Sequential N-terminal truncations to the first residues of ΔNTF ADGDRG1 and ADFGR1 receptors resulted in incremental loss of G protein signaling activity in vitro (21). AGPCRs with substitution mutations to critical residues within the tethered agonist also had reduced activities (21, 22, 43, 44). In contrast, deletion of the entire stalk of a ΔNTF version of ADGRB1 surprisingly had little impact on signaling compared with a version with an intact stalk (40). This led to the proposal of stalk-independent signaling, which may align with the concept that GPCRs are diverse and have a broad range of basal signaling capacities and that AGPCRs can be activated by means that do not always rely on the tethered agonist (40, 45). Full-length, WT ADGRB1 did respond in vivo to synthetic peptide agonists, indicating the probability of bimodal ADGRB1 activation (46).

Further evidence supporting AGPCR tethered agonism was demonstrated through the use of synthetic tethered agonist-mimetic peptides that activated their corresponding receptors in vitro and, in some cases, in vivo. ADGRG1, ADGRG2, ADGRG5 (GPR114), ADGRG6, ADGRD1, ADGFR1, ADGFR4, and ADGFR5 all demonstrated increases in signaling when exposed to these synthetic activating peptides (21, 22, 43, 44, 47, 48). As with the tethered agonist stalks from which they are derived, synthetic peptide agonists have a critical dependence on their N-terminal residues. Differences as small as single-residue substitutions or deletions at the N terminus can severely abrogate the agonistic properties of the peptides (21, 22). Receptor specificity of synthetic peptide agonists also depends on the sequence similarities shared among the tethered agonists. For example, synthetic peptide agonist cross-reactivity was exhibited among members of the Group VI (Group F) AGPCR subfamily that share a highly conserved tethered-peptide agonist and include ADGFR1, ADGFR4, and ADGFR5 (Table 1) (47).

The conformation that tethered-peptide agonists adopt once released from the GAIN domain core is not known, but it is interesting that many seven-residue tethered-peptide agonist sequences are followed C-terminally by predicted β-turn elements within the middle of the stalk regions (21). Performing turn element predictions on all tethered agonist–containing AGPCRs via the Chou and Fasman Secondary Structure Prediction (CFSSP) server revealed that turn elements are common and often conserved among receptors within the same subfamily (Table 1, red) (49). The presence of the mid-stalk turns suggests that when the tethered agonist is exposed by NTF/CTF dissociation, it may serve as a flexible point to allow the tethered agonist to bind intramolecularly back toward its proposed orthosteric site within the CTF. Point mutations of synthetic peptide agonists near the prospective turn points alter their capacity to stimulate signaling. For example, a 13-residue synthetic peptide that was derived from the stalk sequence of ADGRF4, a noncleaved receptor with an unusually small CTF stalk (Table 1), was incapable of promoting signaling (47). However, mutating 2–3 residues around the predicted turn element of the peptide, so that it now matched the ADGRF5 sequence, restored its ability to activate ADGRF4. This suggests that the turn regions of AGPCR tethered-peptide agonists may be critical for receptor activation.

The presence of the turn elements may also help to account for the sprawling evidence that synthetic peptides modeled after AGPCR tethered agonists critically depend upon length. Most studies found that longer peptides with lengths of 12–20 residues, which in most cases include the predicted turn motifs, exhibit the highest efficacies (21, 22, 47, 48). The lone reported exception to this is the 7-mer peptide derived from the ADGRG1 tethered agonist, which is the only ADGRG1-modeled peptide capable of activating the receptor in vitro (21). Intriguingly, the ADGRG1 7-mer peptide does not activate ADGRG5, even though ADGRG5 has this identical sequence (Table 1). However, longer ADGRG5-mimetic peptides (18–20 residues, comprising the complete stalk) will activate both ADGRG1 and -G5 in vitro and in cells, even though, beyond the first seven residues, the sequences have no conservation to the ADGRG1 stalk (22, 50). The C-terminal ends of longer peptides may be necessary for proper folding or bending about the predicted turn element to accommodate binding to the AGPCR orthosteric site, whereas the 7-mer ADGRG1 peptide may be a rare perfect fit that requires only the core tethered agonist sequence. It is also plausible that the C-terminal ends of activating peptides help stabilize them in solution, as the C termini are far more hydrophilic than the hydrophobic 7-mer N termini.

The current leading model of adhesion GPCR activation is that the tethered-peptide agonist binds intramolecularily to its orthosteric site following receptor NTF/CTF dissociation. Upon NTF dissociation, the hydrophobic agonist residues are exposed to the aqueous extracellular environment, resulting in a thermodynamically unfavorable condition. Hydrophobic effects may be a driving force for the tethered agonist to rapidly bind to its orthosteric binding pocket within the 7TM bundle (Fig. 2B). Given that the core seven residues comprising the tethered agonist are completely embedded within the GAIN domain in the holo-receptor form, it is likely that orthosteric agonism is an abrupt, threshold-like response. In other words, receptor fragment dissociation is a binary off/on switch and results in full agonist-driven signaling once the NTF is removed (Fig. 2F). The rapid onset of signaling is predicted because tethered agonist binding to its orthosteric site is a first-order event; the agonist and receptor are tethered together in extreme proximity, and binding of the tethered agonist within the 7TM may be driven to overcome the disfavored hydrophilic environment. This mechanism has some parallels with and distinctions from protease-activated receptor (PAR) activation. PARs are Class A GPCRs and are activated when exogenous proteases (e.g. thrombin or trypsin) cleave their N-terminal stalk leader sequences.
PARs are distinguished from AGPCRs in that they are proteolyzed in trans, rather than autoproteolytically. Following proteolysis, the new N terminus of the TM1 stalk is proposed to bind to an orthosteric site that includes extracellular loop 2 (51–53). A fundamental difference between PARs and AGPCRs is that PAR stalk sequences are typically longer, and the tethered agonists are less hydrophobic than those of AGPCRs. Consequently, whereas PAR tethered agonists are proposed to bind to PAR 7TM extracellular loops, it would make sense that AGPCR tethered agonists might instead bind deeper within the hydrophobic core of the 7TM bundle. However, it is currently unsolved precisely where the tethered agonists of both receptor classes bind to their respective 7TMs.

Given the strong noncovalent interactions that hold the tethered-peptide agonist (as β-strand 13) within the GAIN domain, it is expected that a substantial force would be needed to break them to liberate the agonist. However, the means by which force-mediated AGPCR fragment dissociation occurs are largely unknown and likely to vary. Many adhesion GPCRs have been observed to undergo fragment dissociation, which has been referred to as NTF shedding. Freed NTFs in various tissues or cell culture models were observed for ADGRG1, ADGRA2, ADGRB1 (BA11), and ADGRE5 (CD97) (24, 54–57). Whereas shedding may imply spontaneous dissociation of the NTFs, these observations could also be remnants of ligand-induced NTF dissociation events (24, 58). AGPCR fragment dissociation could be achieved by NTF binding to its ligands that are anchored to the extracellular matrix (ECM) or adjacent cells. Cell movement in relation to the anchored ligands would generate a sufficient shear force to dissociate the NTF from the CTF to initiate G protein signaling. In line with this, adhesion GPCRs were recently considered to be a group of metabotropic mechanosensors (30, 59–61). Ligand-mediated shear force dissociation of the NTF is discussed further under “How do AGPCR ligands modulate receptor activity?”

Tethered agonist activation of AGPCRs does not account for all means of AGPCR activation, with the most obvious examples being the noncleaved receptors that are incapable of undergoing NTF/CTF dissociation. Noncleaved receptors and many engineered uncleavable mutants of cleaved receptors are still capable of signaling through G proteins (40, 44, 62, 63). In these instances, it is unreasonable to predict that the tethered agonist directly regulates signaling as it is covalently bound to the NTF within the interior of the GAIN domain. Consequently, an alternative model has been proposed whereby AGPCR CTFs may be activated allosterically via ligand-induced structural changes of the NTFs.

**Allosteric activation and inhibition**

In allosteric models of adhesion GPCR activation, the NTF and CTF remain bound, and changes in signaling may be induced by receptor conformational changes upon agonist binding to the NTF, rather than orthosteric engagement by the tethered-peptide agonist. The exact mechanisms of allosteric activation or inhibition are not clear, as the N-terminal ligand-binding domains and the G protein-coupling 7TM domain are distal, separated by hundreds or even thousands of residues. Binding of an allosteric ligand must somehow transmit an activation signal over a large structural space. The GAIN domain, located immediately N-terminal to the 7TM domain, is the best candidate to transmit the allosteric signals. Following binding of an allosteric ligand or antagonist, the GAIN domain could help to stabilize active- or restricted-conformation states of the 7TM domain to promote or inhibit signaling, respectively (Fig. 2, C and D).

Beyond the β-strand 13/tethered agonist linkage, interactions between the GAIN or other ECR elements and 7TM domains are not well-characterized and have only been observed indirectly in a few isolated studies. In HEK293T cells co-expressing Myc-tagged ADGRB1 NTF and a CTF ADGRB1 variant (ΔNTF), anti-Myc was used to co-immunoprecipitate the ADGRB1 CTF (64). ADGRG1 harbors two disease-causing mutations, R565W and L640R, in extracellular loops 2 and 3 (ECL2 and -3) of the 7TM, respectively (65). Relative cell surface abundance of the mutant receptors was impaired, but it was not when the mutants were expressed as CTF-only variants (66). The differential effect of the NTF on WT and mutant receptor trafficking suggests that the mutant residues may alter critical interactions between the ECLs and the NTF that are important for trafficking and possibly receptor activity. Additional evidence comes from a study that reported that NTFs and CTFs of chimeric AGPCRs had the ability to exchange or to swap via a “split personality” model (67). Given the intricacies of how the tethered agonist is embedded within the GAIN domain, it is unlikely that AGPCRs are capable of NTF exchange that includes re-embedding of the tethered agonist, especially considering that GAIN domains are destabilized following NTF/CTF dissociation or when constructed recombinantly to lack β-strand 13. These observations of NTF swapping may, however, be evidence of additional, conserved GAIN-CTF contact points that are distinct from β-strand 13.

Whereas additional GAIN or NTF direct interactions with the CTF remain undefined, evidence for them would help explain how AGPCRs are allosterically activated by ligands to produce more modest signaling strength outputs than orthosteric agonism (Fig. 2E). Several endogenous and engineered soluble or diffusible binding partners of AGPCRs have been identified that bind to AGPCR NTFs and induce signaling changes. One example is the docosahexaenoic acid metabolite synaptamide, which was identified as an ADGRF1 ligand and proposed to activate cAMP signaling to promote synaptogenesis and anti-neuroinflammatory responses (68–70). Synaptamide binds directly to the GAIN domain and results in a modest increase in cAMP levels, as shown by gene reporter assays (69). Functional studies of ADGRG1 in neural progenitor cells demonstrated that ADGRG1 NTF-targeting antibodies could stimulate G12/13 signaling (71). Recent structural studies of the ADGRG1 NTF corroborated this, showing that NTF-targeted antibodies could induce small, antibody-specific decreases or increases in G protein signaling (45, 72). The changes in signaling were modest and within 0.5-fold of basal levels as opposed to tethered agonist– or synthetic peptide agonist–activated receptors that exhibit manyfold increases in signaling over holoreceptors (21, 22, 36, 40, 44, 47). It is difficult to envision how soluble antibody ligands alone could support the anchoring and
force requirements that are proposed to be needed for NTF dissociation, and there was no observation of NTF dissociation following the ADGRG1 antibody treatments. Therefore, AGPCR NTF-directed antibodies may be useful probes for understanding allosteric regulation of AGPCR activation that is independent of receptor fragment dissociation.

Allosteric modulation of AGPCRs also occurs through NTF interactions with anchored protein ligands in cis (on the same cell) or in trans (from an adjacent cell). Distinct from those mentioned above, these ligands are anchored binding partners, often receptors themselves, and may convey conformational change to the 7TM via the NTF/GAIN. ADGRA2 interacts in cis with the protein RECK to regulate Wnt7/Frizzled receptor activation for modulation of angiogenesis in the central nervous system (73, 74). ADGRA2 is predicted to be noncleaved due to its atypical GPS (Table 1), so it makes sense for the receptor to mediate signaling allosterically in a large “signalosome” complex rather than through tethered agonism. Additional in cis interactions with AGPCRs have been observed, directly or indirectly, for ADGRB3 and stabilin-2, ADGRC1 and Vangl-2 or Frizzled-6, and ADGR1L and contactin-6 (75–77). Most ADGR (latrophilin) receptors interact in trans with teneurin and fibronectin leucine-rich repeat transmembrane protein (FLRT) ligands to form trans-synaptic signaling complexes that in some contexts are implied to be stable due to their role in maintaining the architecture of the synapse (59–61, 78–82). The stabilities of these cis or trans ligand/NTF complexes provide additional evidence for the idea that AGPCRs function in adhesion capacities and in doing so may allosterically regulate signaling in the absence of fragment dissociation.

NTF allosteric regulation of the 7TM is also supported by evidence that the GAIN domain may act as an autoinhibitor of the 7TM and that this inhibition can be relieved or enhanced by ligand binding to NTF sites (Fig. 2D) (31, 32). Autoinhibition experiments can be difficult to interpret while excluding instances of NTF shedding and resultant tethered agonism. NTF autoinhibition was inferred from observed increases in signaling following NTF deletion for multiple receptors (21, 31, 32, 36–39). As aforementioned, some antibodies directed toward the ADGRG1 NTF imparted subtle signaling decreases (45). Although not direct evidence, the inhibitory effects of these antibodies supports the idea that in specific contexts, the NTF may repress activity of the CTF (red star in Fig. 2D). An ADGR1G1 construct with an intact GAIN domain but deleted N-terminal pentraxin/jaminin/neurexin/sx hormone–binding globulin-like (PLL) adhesive domain had enhanced signaling over full-length ADGRG1 (45, 72). This implies that the PLL may enforce GAIN autoinhibition of the CTF or inhibit the CTF directly. However, exposure of recombinant ADGRG1 NTF to a ΔNTF ADGRG1 receptor resulted in no change in activated receptor signaling, indicating that the NTF may not provide inhibition unless it is noncovalently bound through the TM1 stalk (21).

Whereas many details remain unknown for understanding orthosteric agonism and allosteric modulation, it is clear that the AGPCR NTFs are critical for regulating 7TM signaling. The extracellular domains (ECDs) of Class B1 and Class C GPCRs are also critical regulators of 7TM activation. Consequently, drawing parallels to these receptors may aid in understanding of how adhesion GPCRs are capable of transducing distal extracellular signals across the receptor to impart G protein activation.

**Class B1 GPCR-activating transitions and peptide agonism: Parallels for AGPCR activation?**

The Class B1 or secretin-like GPCR family is most closely related to adhesion GPCRs. There have been substantial structural determination efforts and insight into the transitions that occur between the inactive and active states of family B1 members in recent years (Fig. 3A) (83–89). As opposed to tethered-peptide agonists, family B1 members are activated by soluble peptide hormone agonists. Prominent examples include secretin, parathyroid hormone, glucagon, and glucagon-like peptides, among others (90–92). Family B1 members have ~120–160 amino acid N-terminal ECDs that extend from stalks emanating from TM1, akin, although not homologous, to the GAIN domains of AGPCRs. Family B1 ECDs do not undergo autoproteolysis. In the unliganded state, family B1 ECDs were proposed to contact the three extracellular loops and are thought to constrain movements of the 7TM helical bundle, thereby stabilizing receptor low-activity states (Fig. 3A, left) (93, 94). Family B1 ECDs serve as the initial docking site for the C termini of the peptide hormone agonists (92, 95). Upon peptide binding, the orientation of the ECD is thought to change such that it is released from its strong contacts with the ECLs (Fig. 3A) (93). This opens the extracellular face of the 7TM bundle to allow entry of the N terminus of the agonist peptide hormone and its binding to the interior core. Thus, the orthosteric site of family B1 peptide agonists consist of two components, the ECD/C-terminal binding site and the 7TM bundle/N-terminal binding site.

Current structural information of adhesion GPCRs is limited to views of extracellular portions of the receptor, including GAIN domains, the GAIN plus adjacent NTF subdomains, or, for ADGRG1, the entire NTF (5, 13, 72, 96). If the ECRs or NTFs of adhesion GPCRs parallel the function of family B1 ECDs, then they may bind the ECLs and act to constrain the 7TM bundle in a low-activity state (Fig. 3C). Multiple lines of evidence were discussed above demonstrating that distal ligand binding events can allosterically induce receptor activation in the absence of AGPCR NTF/CTF dissociation (44, 59–61, 69, 72, 97). The combined actions of ligand binding and force may alter the conformation of the CTF toward higher-activity states. The only known point of contact between the NTF and CTF is the stalk region that is contiguous with TM1. Conformational changes to TM1 that are induced allosterically through the stalk may account for instances of 7TM helical bundle activation, but the evidence for interactions between the NTF or GAIN domain and the AGPCR ECLs must also be considered (Fig. 3C). Ligand engagement events could alter these putative interactions and release constraints on receptor activation, paralleling the first step of family B1 receptor activation (derepression; Fig. 3A).

AGPCR orthosteric agonism may also share parallels to family B1 soluble peptide hormone agonism. If AGPCR NTFs/
GAINs serve to constrain the 7TM bundle, then upon force-mediated dissociation of the NTF from the CTF, this inhibition is relieved while tethered-peptide agonists are released from the interior core of the GAIN domain. The partially activated state of the receptor (after NTF disinhibition) would favor the fully active form and drive first-order binding of the tethered-peptide agonist to its orthosteric site. Akin to family B1 receptors, the N termini of AGPCR tethered-peptide agonists may be expected to bind within the interior of the 7TM bundle, and the C-terminal elements of the tethered-peptide agonist/stalk region might bind to the ECLs. The N-terminal ~7 amino acids of adhesion GPCR tethered-peptide agonists are much more hydrophobic than family B1 peptide agonist N termini, suggesting an orthosteric site position that lies deeper within the interior of the 7TM bundle. Family B1 peptide agonists are highly conserved at their N termini, with receptor-specific variations occurring at the C termini (99). This feature is shared with adhesion GPCR tethered-peptide agonists that also have highly conserved N termini and divergent C termini.

Class C GPCRs as a parallel for transmission of distal activation signals?

Class C GPCRs are distantly related to AGPCRs and possess completely distinct ECR architectures. Class C receptors may nonetheless provide clues for understanding how AGPCRs allosterically regulate signaling without NTF/CTF dissociation (Fig. 3B). Class C receptors are dimers that possess large ECRs comprising a Venus flytrap (VFT) domain and an intervening rigid structure, such as a cysteine-rich domain (CRD), that are linked N-terminally to the 7TM bundle (Fig. 3B) (100, 101). Class C GPCR ligand binding occurs at the distal VFT domains (102). In the ligand-free state, a prototypical Class C receptor dimer possesses two VFT domains in an open conformation, and ligand binding causes domain closure. This precedes 7TM bundle structural shifts that facilitate G protein activation (103). The exact mechanisms of how the activation signal is transduced from the ligand-bound VFT to the 7TM bundle are emerging, and it is thought that ligand binding induces inter- and intraprotomer conformational changes that result in 7TM activation (Fig. 3B, right) (104, 105). Ligand binding draws the two VFT domains in close proximity, which alters the conformation of the intervening rigid body to impose rotational shifts of the 7TM bundles that stabilize an active state (106–108). Recent cryo-EM structural work with mGlu5 has helped to form this hypothesis while also revealing a critical interaction occurring at the CRD and ECL2, where the CRD-ECL2 interaction is proposed to serve as a rigid turning point to convey structural shifts between the CRD and 7TM domain (109). The inactive-state conformation. This is aided, in part, by a critical interaction with ECL2 of the 7TM domain that acts as a rigid linker for a subtle rotational shift in the 7TMs. C, it is not certain how AGPCR ligands allosterically modulate adhesion GPCR signaling in the absence of NTF/CTF dissociation. In the absence of ligand, the GAIN domain may interact with the ECLs of the 7TM bundle to repress active conformations (left), similarly to the ECDs of family B1 receptors. In the presence of an allosteric ligand, the activation signal may be transmitted through the GAIN domain to the 7TM (right). This could activate the receptor by relieving repression conferred by the NTF or by utilizing the CTF stalk as a fulcrum in a manner akin to class C receptors that use a rigid body to impart activating conformational changes to the 7TM.

Figure 3. Parallels of adhesion GPCR allosteric activation to Class B1 and Class C GPCRs. A, family B1 GPCRs are receptors for soluble peptide agonists and are closely related to adhesion GPCRs. B1 receptors possess ECDs that inhibit signaling of the 7TM domain in the absence of a ligand via interactions with ECLs. In the active state of the receptor, the N terminus of the peptide agonist (orange) binds within the 7TM bundle, whereas the C terminus binds to the ECD. Shown is the structure of active glucagon-like peptide-1 receptor (GLP1R) (gray and blue, ribbon) bound to glucagon-like peptide-1 (GLP1) peptide (orange, space-filled, PDB entry 5VAI). B, Class C GPCRs are distantly related to adhesion GPCRs but also possess large ECDs. Class C receptors are obligate dimers that possess an extracellular VFT domain (Fig. 3C) and intraprotomer conformational changes that result in 7TM bundle structural shifts that facilitate G protein activation (103). The exact mechanisms of how the activation signal is transduced from the ligand-bound VFT to the 7TM bundle are emerging, and it is thought that ligand binding induces inter- and intraprotomer conformational changes that result in 7TM activation (Fig. 3B, right) (104, 105). Ligand binding draws the two VFT domains in close proximity, which alters the conformation of the intervening rigid body to impose rotational shifts of the 7TM bundles that stabilize an active state (106–108). Recent cryo-EM structural work with mGlu5 has helped to form this hypothesis while also revealing a critical interaction occurring at the CRD and ECL2, where the CRD-ECL2 interaction is proposed to serve as a rigid turning point to convey structural shifts between the CRD and 7TM domain (109). The active-state conformation. This is aided, in part, by a critical interaction with ECL2 of the 7TM domain that acts as a rigid linker for a subtle rotational shift in the 7TMs. C, it is not certain how AGPCR ligands allosterically modulate adhesion GPCR signaling in the absence of NTF/CTF dissociation. In the absence of ligand, the GAIN domain may interact with the ECLs of the 7TM bundle to repress active conformations (left), similarly to the ECDs of family B1 receptors. In the presence of an allosteric ligand, the activation signal may be transmitted through the GAIN domain to the 7TM (right). This could activate the receptor by relieving repression conferred by the NTF or by utilizing the CTF stalk as a fulcrum in a manner akin to class C receptors that use a rigid body to impart activating conformational changes to the 7TM.
portance of the rigid linker-ECL2 interaction was affirmed in a recent structural determination of the heterodimeric GABA\textsubscript{B}\textsubscript{2} receptor that lacks typical CRDs (110). An ECL2 truncation of the ligand-bound protomer, GABA\textsubscript{B1}, destabilized this interaction and resulted in increased basal signaling, whereas ECL truncation of the other protomer, GABA\textsubscript{B2}, only increased GABA\textsubscript{B} efficacy, indicating that the rigid linker-ECL2 interaction mediates both inter- and intraprotomer interactions.

In instances where AGPCR ligands bind to the N-terminal adhesive domains and allosterically promote signaling without NTF/CTF dissociation, the information would also need to be transmitted distally across the receptor to the 7TM bundle. The most obvious domain to act as the transmitter of these intramolecular signals is the GAIN domain, which might function analogously to the Class C rigid body. The Class C rigid link between the VFT and 7TM generally possesses a cysteine-rich \(\beta\)-sheet architecture that confers ligand-mediated contortions to the 7TM, which may parallel the dense \(\beta\)-sheet architecture of the GAIN\textsubscript{B} domain of AGPCRs. In line with this, the ADGRG1 GAIN domain forms a critical disulfide link to the PLL adhesive domain (Fig. 1D) (72). Disruption of this disulfide bond via C-to-S mutations surprisingly increased basal signaling ~2-fold in a gene reporter readout (72). Additionally, an ADGRG6 splice variant that adopted more diverse ECR conformations as judged by negative-stain EM images also had greater basal signaling (13). These studies indicate that disruption of AGPCR NTF rigidity may result in a more dynamic GAIN domain that influences 7TM activation. Future studies may explore this putative function of the GAIN domain as conferring rigidity and stabilization of inactive states. Binding of allosteric ligands may impart flexibility, allowing for activating structural changes to be conferred to the 7TM bundle (Fig. 3C).

Given the lack of hard evidence for NTF/ECL interactions, it is plausible that the tethered agonist, which links the two domains together in the holoreceptor conformation, may also be key in regulating allosteric modulation via 7TM conformational shifts. The tethered agonist stalk (beyond the core seven residues embedded within the GAIN) may serve as a pivot point to convey 7TM contortions via TM1. This would allow for receptor activation if NTF/ECL interactions do not exist, but it is also possible that both types of interactions are relevant and may work together (Fig. 3C). Supporting this role for the stalk, reduced basal signaling of an ADGRG5 SNP was attributed to its missing glutamine residue within the middle of the stalk apart from the core tethered agonist. A synthetic peptide agonist derived from this stalk enhanced signaling independent of the glutamine (44). This implies that the stalk of the ADGRG5 SNP that contains the glutamine positively impacts holoreceptor basal activity independent of the tethered agonist, possibly by conveying structural information to the 7TM. In sum, there is much to learn about the nature of AGPCR ECR interactions with the 7TM domain, and the potential parallels to Class B1 and Class C mechanisms may help guide research design.

**How do AGPCR ligands modulate receptor activity?**

Insights into AGPCR activation mechanisms may be gleaned from the endogenous ligands that target them, some of which were introduced previously. Wide-ranging deorphanization studies have uncovered several endogenous ligands that bind to individual AGPCRs (reviewed in Refs. 29–31, 33, 35, 111, and 112). The identities of the ligands provide useful clues for understanding receptor activation mechanisms in physiological contexts. About half of all AGPCRs remain orphans, and of those with identified ligands, it is unanimously unclear how the ligands impart receptor activation. Table 2 and Fig. S1 detail the currently known endogenous AGPCR ligands and proposes a means to classify adhesion GPCRs based on the type(s) of ligand that each receptor binds. Some AGPCRs have multiple ligands and span multiple categories of the classification system: 1) trans-cell–presented proteins, 2) extracellular matrix components, and 3) soluble proteins, peptides, lipids, and small molecules. 

**Trans-cell–presented proteins**

Trans-signaling complexes are formed through interactions of the extracellular extensions of protein ligands presented by neighboring cells and the N-terminal adhesive modules of AGPCRs to provide modes of cell-to-cell adhesion and communication. As discussed previously, stable trans-cell AGPCR and protein ligand complexes may signal through allosteric activation modes and/or tethered agonism. Tethered agonism imparted by trans-cell–adhesive ligands is thought to occur by ligand binding to its AGPCR binding site(s) in a manner that is tighter than the strength of the noncovalent contacts that embed \(\beta\)-strand 13 within the GAIN domain. Consequently, shear force created by the two cells moving in relation to each other serves to dissociate the ligand-anchored NTF from the CTF to promote signaling. Oftentimes multiple trans-cell ligands bind simultaneously to AGPCR N-terminal adhesive modules, which is thought to provide strong multivalent binding that is sufficient to anchor the NTF. Prominent examples of AGPCRs that utilize trans-cell ligands are those present at synaptic junctions that bind to ligands spanning the synapse. ADGRL (latrophilin) and ADGRB (BAI) receptors are both enriched in synaptic junctions and serve as models of trans-cell synaptic AGPCR signaling.

Most ADGRL receptors (ADGRL1–4), excluding ADGRL4, are localized on axons, axonal growth cones, and nerve terminals (78, 112–114). They are structurally distinguished from other AGPCRs by the presence of an N-terminal olfactomedin (Olf) domain and a lectin-like (Lec) domain. ADGRL receptors regulate interneuron adhesion and the migration of growth cones (actin-rich neuronal extensions) while promoting synapse formation and remodeling through control of cytoskeletal rearrangements (78, 79, 81, 82, 113). Defects or variants in ADGRL genes are associated with neuronal disorders including attention deficit and hyperactivity disorder, autism spectrum disorder, schizophrenia, rhombencephalosynapsis, and microcephaly (115–120). ADGRL receptors bind to three classes of single-membrane pass, trans-presented protein ligands: ten-eurins, neurexins, and FLRTs. Teneurins bind to the ADGRL Lec domain, whereas neurexins and FLRTs interact with the ADGRL Olf domain (82, 96, 114, 121). These proteins comprise...
trans-synaptic signaling complexes and provide models for understanding how AGPCRs can be activated by trans-presented ligands. For instance, ADGRL1 interacts with both teneurin-2 (also known as Lasso) and FLRT simultaneously to regulate dendritic arborization, axonal extension, and synaptogenesis (82, 96, 121). These neuronal reshaping processes are thought to result from Rho/Rac-mediated actin cytoskeletal changes downstream of $\text{G}_{12/13}$ signaling, through which ADGRL3 was recently shown to signal (122–128). ADGRLs also couple to $\text{G}_{i/o}$ and $\text{G}_{q}$, which may also influence neuronal migration and synaptogenesis, as the asymmetric production of cAMP or $\text{Ca}^{2+}$ within the cell influences growth cone guidance (112, 129–132).

As with many AGPCRs, ADGRLs are self-cleaved receptors (Table 1) and will activate signaling following NTF/CTF dissociation via tethered-peptide agonism (5, 128, 133). The receptors may utilize allosteric and/or tethered agonism modes of activation. For allosteric activation, teneurins and FLRTs may convey conformational modulation through the ADGRL NTF to elicit signaling (Fig. 4, left). Studies in Drosophila neurons showed that mechanically induced ionotropic channel currents were dependent on the Drosophila homolog of ADGRL1, dCIRL (59). Neurons were made to express cleavage-deficient variants of dCIRL via alanine substitution mutations at the H or T of the GPS (HL/T). A normal response to mechanical stimulation was observed in the H to A mutant, but a null phenotype was observed in the T to A mutant, indicating that tethered agonism is the primary means of receptor activation, but the receptor exhibits contextual allosteric signaling. Ligand binding and shear force may dissociate the ADGRL NTF and CTF in other contexts, such as when a synapse breaks (Fig. 4, right). The strong tethered agonist-dependent $\text{G}_{12/13}$ signaling that would result upon synaptic breakage coincides with the known Rho/Rac-dependent signaling events that drive actin-dependent cell shape change pathways (122–127). Recent studies have also demonstrated that in migrating neurons, ADGRL1 interacts in trans with teneurin and FLRT to promote detachment and neurite retraction for repulsion processes (121). The repulsive effects seem to contrast with roles that ADGRL ligands such as teneurin have in mediating synaptogenesis (81, 113, 134). It is possible that ADGRLs have dual roles in force-dependent axonal guidance. Migrating neurons may form transient FLRT/teneurin/ADGRL complexes that experience shear forces that elicit fragment dissociation and tethered agonist $\text{G}_{12/13}$ signaling for cytoskeletal-dependent neuron repulsion. This is supported by the fact that strong $\text{G}_{12/13}$ signaling is associated with neurite retraction (122, 123). In contrast, the synaptonic effects attributed to FLRT and teneurin may be the consequence of stable complexes formed with ADGRLs that

### Table 2

| Class | Receptor | ECM components | Lipids/soluble proteins/small molecules | Trans-presented proteins | References |
|-------|----------|----------------|---------------------------------------|--------------------------|------------|
| A     | ADGRA1   | Integrin-αVβ3, glycosaminoglycans, syndecan-1,2 | Orphan | | |
|       | ADGRA2   | | | | |
|       | ADGRA3   | αVβ5 integrin | Orphan | | |
|       | ADGRB1   | Phosphatidylserine, lipopolysaccharide | Orphan | | |
|       | ADGRB2   | Glutaminase-interacting protein (GIP) | RTN4R, CD36 | | 140, 142, 145, 165, 166 |
|       | ADGRB3   | C1q1-C1q4 | | | 167 |
| C     | ADGRC1   | Orphan | | | 139, 141 |
|       | ADGRC2   | Orphan | | | |
| D     | ADGRD1   | Dystroglycan | Orphan | | 146 |
|       | ADGRD2   | Orphan | | | |
| E     | ADGRE1   | Chondroitin sulfate, Integrins-αVβ3, α5β1 | Orphan | | 168 |
|       | ADGRE2   | Unknown NK cell receptor | CD90 | | 9, 147 |
|       | ADGRE3   | Unknown ligand on macrophages and neutrophils | | | 169 |
|       | ADGRE4   | Unknown B cell ligand | CD55, CD90, lysophosphatidic acid receptor | | 170 |
|       | ADGRE5   | Chondroitin sulfate, integrins αVβ3, α5β1 | | | 38, 147, 171–173 |
| F     | ADGRF1   | Chondroitin sulfate, Integrins-αVβ3, α5β1 | Progastrin | | 155, 174 |
|       | ADGRF2   | Synaptamide | | | 50, 63, 148, 151, 153, 160, 175–177 |
|       | ADGRF3   | Orphan | | | |
|       | ADGRF4   | Orphan | | | |
|       | ADGRF5   | Surfactant protein-D | | | |
| G     | ADGRG1   | Collagen III, heparin, transglutaminase-2, laminin | | | 155, 174 |
|       | ADGRG2   | Orphan | | | 39, 154, 178 |
|       | ADGRG3   | Orphan | | | |
|       | ADGRG4   | Orphan | | | |
|       | ADGRG5   | Orphan | | | |
|       | ADGRG6   | Collagen IV, laminin-211 | Cellular prion protein | | |
|       | ADGRG7   | Orphan | | | |
| L     | ADGRL1   | Teneurin-2/4, neurexin-1α, -β2, -β3, FLRT1/3 | Orphan | | |
|       | ADGRL2   | Teneurin-2, FLRT3 | | | 78, 180 |
|       | ADGRL3   | Teneurin-3, FLRT1/3, UNC5A | | | 78, 81, 181 |
| V     | ADGRL4   | Orphan | | | |
|       | ADGRL5   | Orphan | | | |

*Predicted based on structural similarities.
allosterically signal through $G_{i/o}$ and $G_q$ to regulate axonal growth and synapse formation. This is supported by studies demonstrating that ADGRL1/teneurin-2 promote calcium-dependent signaling and axonal growth (113, 134).

ADGRB receptors (ADGRB1–3) contain an N-terminal HormR domain and thrombospondin type 1 repeats (TSRs). Like ADGRLs, the ADGRB receptors are enriched in various brain tissues, depending on the receptor subtype, including the cerebral cortex, hippocampus, basal ganglia, olfactory bulb, and thalamic nuclei (135–138). ADGRBs signal through $G_{12/13}$ to activate Rho pathways to elicit downstream cytoskeletal changes (40, 64). Defined ligands for ADGRB receptors are the soluble C1q-like proteins (C1q1–4) and phosphatidylserine, which both interact with the TSRs (139, 140). ADGRB1 (BAI1) interaction with C1q-like proteins influences synapse formation in neurons (Fig. 4), whereas interactions with phosphatidylserine are thought to serve as an engulfment signal for apoptotic fibroblasts (139–141). Whereas the C1q1 proteins and phosphatidylserine are the most studied ligands for ADGRBs, the trans-presented reticulin 4 receptor (RTN4R, or Nogo 66 receptor) was recently proposed to be an ADGRB1 ligand that also interacts through the TSRs (142). RTN4R shares many functional features with ADGRB1: both are enriched in neurons and regulate axonal growth, axonal regeneration, and synaptic plasticity (143). RTN4R was characterized as a potential ADGRB1 ligand in only one study, but it may account for the findings from another report that described an unknown ligand that was linked trans-synaptically to ADGRB1 (46). The mechanism of receptor activation mediated by C1q, phosphatidylserine, or RTN4R ligands is currently unknown, and downstream signaling outcomes have not been well-characterized outside of microscopy-based synaptic growth assays that monitor synaptic densities and dendrite growth (46, 139, 141). Given that the ADGRB ligands bind to the same regions of the NTF, the binding may be mutually exclusive. For the ADGRB-RTN4R interaction, both allosteric and orthosteric tethered-agonist means of activation are plausible (Fig. 4). In contrast, ADGRB binding to soluble C1q proteins may only impart allosteric activation, as soluble proteins lack the anchoring properties that are necessary for force-mediated fragment dissociation. Interestingly, C1q proteins can form higher-order multimeric complexes (144). Multimeric C1q proteins could potentially link multiple ADGRB receptors to influence signaling activation in an unknown manner (139).

**Extracellular matrix ligands**

Select AGPCRs are involved in cell-to-ECM adhesion events through direct interactions with protein or carbohydrate components of the ECM. In many cases, the ECM ligands are insoluble, such as different subtypes of collagen, and serve as anchor points for AGPCRs in several essential cell-signaling processes (24, 39, 145–148). As with trans-cell AGPCR complexes, AGPCR-ECM complexes are most often formed by the AGPCR NTFs, depending on the specific adhesive subdomains of individual receptors. ECM and ECM-associated components that have been identified as ligands for AGPCRs include integrins, glycosaminoglycans, laminins, transglutaminase-2 (TG2), and collagen subtypes, which were identified for the ADGRA, ADGRB, ADGRE, and ADGRG receptor subfamilies (Table 2). Interactions between ADGRG-family receptors, particularly ADGRG1 and ADGRG6, and ECM components are perhaps the best-characterized examples of ECM-AGPCR
interactions and provide insight into how these ligands may activate AGPCRs.

ADGRG1 and ADGRG6 are widely expressed but best known for functions within the nervous system. ADGRG1 regulates oligodendrocyte development in the central nervous system and is also present in skeletal muscle (148–151), whereas ADGRG6 is a receptor used within Schwann cells of the peripheral nervous system to promote nerve myelination and repair (42, 152). Collagen subtypes III and I and tissue TG2 were identified as ECM interactors for ADGRG1, and collagen subtype IV and laminin-211 were found to bind to ADGRG6 (39, 148, 151, 153, 154). The addition of these ligands to both receptors resulted in signaling stimulation both in vitro and in vivo, but the mechanisms of how this occurs are not entirely known. Given the capability of multivalent interactions within the ECM, it is plausible that ECM ligands serve as an anchoring point for AGPCRs to allow for receptor fragment dissociation following a mechanical stimulus, such as cell movement that leads to signaling via tethered agonism. Supporting this, HEK293 or COS-7 cells expressing ADGRG6 exhibited increased cAMP production when exposed to collagen IV or extracellular laminin-211 under a shaking or rotating force (39, 154). ECM components may also activate AGPCR signaling via simultaneous binding of more than one ligand, as shown by the interaction of ADGRG1 with TG2 and laminin. ADGRG1 was only activated by simultaneous binding of TG2 and laminin; neither ECM component was capable of activating the receptor alone (63). Interestingly, activation by laminin and TG2 was receptor cleavage–dependent, as the H381S cleavage-deficient ADGRG1 mutant was not activated by TG2 and laminin (63). In each of these cases, details of the mechanism fell short of demonstrating ligand-dependent NTF/CTF dissociation that was commensurate with force and ligand action, but the cleavage dependence of ADGRG1 activation provides reasonable evidence to support a tethered-agonist–based activation mechanism.

Multivalent ligand binding may also account for the ability of collagen to activate AGPCRs. Select subtypes of collagen activate both ADGRG1 and ADGRG6 receptors in vitro and in vivo by binding to the NTFs: collagen III interacts with the ADGRG1 PLL domain (also referred to as its collagen-binding domain), whereas ADGRG6 interacts with collagen IV through its C1r/C1s/Uegf/Bmp1 (CUB) and pentraxin (PTX) domains (39, 58, 148). These interactions are vital for function of the receptors in neurons, as disruption of the collagen binding sites by point mutation or deletion resulted in myelination defects (39, 150). ADGRG1 loss-of-function mutations also cause the neurological disease bilateral frontoparietal polymicrogyria, a cortical brain malformation disorder (57). Fibrillar collagen cross-linked within the ECM could have the capacity to serve as an anchored, multivalent ligand for both ADGRG1 and ADGRG6 and bind tightly to allow force-mediated NTF/CTF dissociation and tethered agonism. The addition of collagen III to HEK293 cells overexpressing ADGRG1 reduced the amount of NTF at the cell surface while enriching it in conditioned medium, suggesting that collagen was capable of allowing or inducing AGPCR fragment dissociation (58). Not all AGPCRs that interact with ECM components are self-cleaved, such as ADGRA2, which interacts with integrins and glycosaminoglycans (Table 2). These ligands may instead form a cell-to-ECM tethered complex that regulates AGPCR signaling via allosteric modulation.

**Soluble extracellular ligands**

Compared with trans-cell and ECM ligands, there is a markedly lower number of known soluble ligands that regulate AGPCR signaling, which is unconventional for most other GPCR classes. The ligands that have been identified consist of a few small molecules, peptides, and soluble proteins. This is perhaps indicative of how AGPCR signaling is manifested. Whereas most GPCRs are activated by orthosteric binding of a soluble ligand, AGPCRs are activated by their own tethered ligand. Thus, it makes sense that there are virtually no known endogenous AGPCR ligands that bind to the 7TM domain at sites akin to the orthosteric site of Class A GPCRs. The soluble ligands that have been identified for AGPCRs instead target the NTF, similar to the other two classes of AGPCR ligands. However, given that diffusible ligands are not anchored, it is probable that they regulate activity by allosteric modulation rather than tethered agonism. Two receptors that illustrate this well are ADGRF1 and ADGRF5.

ADGRF5 is a Gq/11–coupled receptor enriched in the lungs and kidneys that regulates the levels of pulmonary surfactants, which are lipid-protein complexes produced by alveolar type II (AT-II) cells that reduce surface tension at the air-liquid interface in the alveoli (43, 155). Pulmonary surfactants are essential to prevent lung collapse, and impairments to their production are associated with neonatal respiratory distress syndrome, acute lung injury, and acute respiratory distress syndrome (156). The proposed function of ADGRF5 is to suppress production and stimulate uptake of pulmonary surfactants by binding surfactant protein D (Sp-D) (155). Deletion of ADGRF5 in mice resulted in enlarged alveoli that contain an excess of surfactant, leading to hypertrophy of AT-II cells. Sp-D was proposed to be an ADGRF5 ligand by demonstration that the two proteins could be co-immunoprecipitated. The interaction between ADGRF5 and Sp-D is not fully characterized beyond the knowledge that Sp-D binds the NTF of ADGRF5, which contains several Ig-like repeats. However, a separate study showed that overexpression of Sp-D did not decrease alveolar surfactant pools or cause respiratory distress, which would be expected if Sp-D were indeed a ligand for ADGRF5 (43, 157). Given that alveolar cells undergo consistent expansion and compression in the ventilatory cycle, it was proposed that ADGRF5 may be activated by mechanical stretching of AT-II cells (43). Whereas mechanical stimulation in this context is reasonable, there have been no proposed ECM or trans-presented ligands that could serve as the anchoring point. It is also plausible that mechanical stimulation activates ADGRF5 via an unknown receptor through Sp-D, where Sp-D acts to link two receptors that may allow for a transmission of a mechanical signal. In this instance, Sp-D would instead influence the mechanical activation of ADGRF5 rather than serving as an activating ligand itself. ADGRF5 can also be activated by synthetic peptide agonists, leaving open the probability of...
tethered agonism (43). A thorough ADGRF5 ligand deorphanization effort may clear up this ambiguity.

ADGRF1 is enriched in neural stem cells and was initially recognized as an oncogene such as gliomas, osteosarcomas, and lung and prostate cancers. (11, 158, 159). Recently, the brain-enriched lipid metabolite N-docosahexaenoylethanolamine (synaptamide) was found as an endogenous ADGRF1 ligand that mediates neurodevelopment (68–70). ADGRF1 interacts with synaptamide at nanomolar affinity and may increase neurite growth and neurogenesis in cortical neurons and neural stem cells (70). Synaptamide binds at the GAIN_A and GAIN_B subdomain junction and was predicted from cross-linking studies to induce conformational changes to the ECR and TM6 (69). ADGRF1 is self-cleaved at its GPS and responds to synthetic agonist peptides, and urea treatment of the full-length receptor results in increased G_	ext{q} signaling, showing that the receptor can be activated by tethered agonism (21, 47). As with other soluble AGPCR ligands, synaptamide is unanchored, which leaves open the question of whether it can induce NTF/CTF dissociation. It seems probable that synaptamide may work through allosteric means to activate ADGRF1.

Besides endogenous soluble ligands, small-molecule AGPCR probe compounds were identified from high-throughput screens based on the ability to activate or inhibit select receptors (e.g. for the ADGRG subfamily). Cell-based gene reporter assays showed that ADGRG1 could be activated by the small-molecule partial agonist 3-α-acetoxydihydrodeoxygedunin (3-α-DOG) and beclomethasone dipropionate (50, 160). A calcium signaling–based reporter screen for ADGRG3 identified beclomethasone dipropionate as a potential agonist (161). Interestingly, both of these probes for similar ADGRG receptors share a four-ring steroid-like structure. The shared molecular skeletons of 3-α-DOG and beclomethasone suggest that ADGRG1 and ADGRG3 may have conserved binding pockets for these similar small molecules, and they probably lie within the 7TM as 3-α-DOG activated an ADGRG1 ΔNTF variant (50). A screen for small molecules that influenced ADGRG6 signaling in zebrafish recently identified apomorphine as a potential activator of ADGRG6 (162). Outside of these examples, AGPCRs remain relatively untapped as targets for small-molecule probe development. Discovery of high-affinity agonists or antagonists could aid AGPCR-targeted therapeutic design and in stabilizing the receptors for much needed structural studies.

Conclusions

AGPCRs are a widely expressed and diverse group of receptors that have dramatic variations of organization of their ECRs. Many mechanisms have been proposed for how the ECRs mediate signaling by the 7TM. Given the evidence reviewed here, we propose that AGPCRs primarily engage in ligand– and shear force–dependent tethered-peptide agonist or allosteric modes of activation. A fuller understanding of the modes of activation is desirable as two fundamental research questions linger: 1) there is little clarity as to how endogenous ligands influence AGPCR activity, and 2) there exists no solved structure of a full-length AGPCR.

Consequently, AGPCRs have few pharmacological tools and remain untapped as therapeutic targets. A thorough understanding of how AGPCRs are activated in physiological contexts could pave the way to initiate drug screening strategies to treat the many pathologies that are linked to AGPCR dysfunction. The activation mechanisms discussed in this review are not confirmed entirely, but we have provided a distilled layout of the most probable theories with reference to supporting data in the literature.

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Abbreviations—The abbreviations used are: GPCR/GPR, G protein–coupled receptor; AGPCR/ADGR, adhesion G protein–coupled receptor; BAI, brain-specific angiogenesis inhibitor; CRD, cysteine-rich domain; CTF, C-terminal fragment; CL, C/Cl urinary epidermal growth factor and bone morphogenetic domain; 3-α-DOG, 3-α-acetoxydihydrodeoxygedunin; ECL, extracellular loop; ECM, extracellular matrix; ECR, extracellular region; ECD, extracellular domain; FLRT, fibronectin leucine-rich-repeat transmembrane protein; GAIN, GPCR autoproteolysis–inducing (domain); GPS, GPCR proteolysis site; HormR, hormone receptor (domain); Lec, lectin-like (domain); NTF, N-terminal fragment; Olf, olfactomedin (domain); PAR, protease-activated receptor; PLL, pentraxin/amin/orphin/sex hormone–binding globulin-like (domain); RTN4R, reticulon 4 receptor; SEA, sperm protein, enterokinase, and agrin (domain); Sp-D, surfactant protein D; TG2, transglutaminase-2; TM1, first transmembrane span; 7TM, seven-transmembrane; TSR, thrombospondin type 1 repeat; VFT, Venus flytrap (domain); AT-II alveolar type II; PDB, Protein Data Bank.

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