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Adhesion GPCRs: from *in vitro* pharmacology to *in vivo* mechanisms

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List of non-standard abbreviations:

7TM, seven-transmembrane; aGPCR or ADGR, Adhesion G protein-coupled receptor; BAI, brain-specific angiogenesis inhibitors; CELSR, cadherin, EGF LAG seven-pass G-type receptors; CIRL, Calcium- independent receptor of α- latrotoxin; CTF, C-terminal fragment; DMR, dynamic mass redistribution; ECD, extracellular domain; ECM, extracellular matrix; ELTD1, EGF, latrophilin, and seven transmembrane domain containing 1; EMR1, EGF-like module-containing mucin-like hormone receptor-like 1; GAIN, GPCR autoproteolysis-inducing; GPCR, G protein-coupled receptor; GPS, GPCR proteolysis site; GRK, GPCR kinase; ICD, intracellular domain; IUPHAR, International Union of Basic and Clinical Pharmacology; LAT, latrophilin; NTF, N-terminal fragment; PAR, Protease-activated receptor; PKA, protein kinase A; PKC, protein kinase C; TRP, transient receptor potential; TSH, Thyroid-stimulating hormone; VEGF, vascular endothelial growth factor; VLGR1, very large GPCR 1

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Abstract

The adhesion family of G protein-coupled receptors (aGPCRs) comprises 33 human members. aGPCRs are characterized by their enormous size and complex modular structures. While the physiologic importance of many aGPCRs has been clearly demonstrated in recent years, the underlying molecular functions have only recently begun to be elucidated. In this minireview, we present an overview of our current knowledge on aGPCR activation and signal transduction with a focus on the latest findings regarding the interplay between ligand binding, mechanical force, and the tethered agonistic *Stachel* sequence, as well as implications on translational approaches that may derive from understanding aGPCR pharmacology.

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Introduction

G protein-coupled receptors (GPCRs) represent the largest superfamily of receptors in the human genome (Pierce et al., 2002). Based on phylogenetic comparison of their seven-transmembrane spanning (7TM) domain, GPCRs are classified into five families: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste, and Secretin (Lagerström and Schiöth, 2008). Their presence on every cell and responsiveness to diverse stimuli link GPCRs to a great variety of physiological processes.

The 7TM domain with some phylogenetic relation to secretin-like receptors clearly groups adhesion GPCRs (aGPCRs) into the GPCR superfamily. The large extracellular N terminus of aGPCRs is not unique to this class, but is present in all members. A unique feature of the class is a juxtamembrane GPCR proteolysis site (GPS), within the highly conserved GAIN (GPCR autoproteolysis-inducing) domain), that facilitates autocatalytic processing such that the extracellular N-terminal fragment (NTF) and the 7TM/cytoplasmic C-terminal fragment (CTF) are non-covalently associated (Lin et al., 2004; Arac et al., 2012). While the NTF comprises most of the extracellular domain (ECD), the CTF is characteristically composed of a residual ECD, the 7TM domain, and the complete intracellular domain (ICD) (Fig. 1). Historically, orphan aGPCRs have been assigned numbers on discovery, which has led to a rather unstructured assembly of this receptor class. Upon the initiative of the Adhesion GPCR Consortium and the International Union of Basic and Clinical Pharmacology (IUPHAR), a new nomenclature was recently proposed (Hamann et al., 2015). The new names composed of ADGR, a letter, and a number will be presented alongside the old names with first use within this review.

The NTF is responsible for the enormous size of most aGPCRs and presents characteristic modular protein domains. Many of the ~20 different protein domains found in aGPCR NTFs (e.g., cadherin, epidermal growth factor, immunoglobulin, leucine-rich repeat) can mediate contacts with cellular or extracellular matrix (ECM)-associated molecules. So far, about a dozen binding partners have been identified. Notably, these binding partners are structurally highly diverse and have been assigned to a relatively small number of aGPCRs, while the majority of aGPCRs remain orphan with respect to ligand binding (Hamann et al., 2015).

aGPCR family members facilitate cell adhesion, orientation, migration, and positioning in various organ systems, a concept that is based on *in vivo* data obtained in invertebrate and vertebrate models (Hamann et al., 2015). Studies in *Caenorhabditis elegans* and *Drosophila melanogaster* clearly indicate essential roles for the Latrophilin (*ADGRL*) homolog CIRL (Calcium- independent receptor of α- latrotoxin) and the CELSR (cadherin, EGF LAG seven-pass G-type receptors) (*ADGRC*) homolog Flamingo in planar cell and tissue polarity and in neuronal development. Studies in vertebrates unraveled roles of many

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aGPCRs including ELTD1 (EGF, latrophilin, and seven transmembrane domain containing 1) (*ADGRL4*); CD97 (*ADGRE5*), EMR1 (EGF-like module-containing mucin-like hormone receptor-like 1) (*ADGRE1*), GPR124 (*ADGRA2*), CELSRs (*ADGRCs*), BAIs (brain-specific angiogenesis inhibitors) (*ADGRBs*), GPR56 (*ADGRG1*), GPR64 (*ADGRG2*), GPR126 (*ADGRG6*), and GPR98/VLGR1 (very large GPCR 1) (*ADGRV1*) in various developmental processes, immunity, and tumor progression (Hamann et al., 2015).

Despite their wide distribution and remarkable phenotypes associated with receptor dysfunction, aGPCRs remained ‘functional orphans’ for a long time (Hamann et al., 2015). Only recently have concepts emerged describing how these non-canonical GPCRs are activated. These models were discussed at the 2014 Lorentz Center Workshop on *Exploring the biology of GPCRs - from in vitro to in vivo*.

In vitro **pharmacology of aGPCRs**

Demonstration of G-protein coupling as one signaling mode of aGPCRs was a central issue in previous investigations. Early experiments showed that LPHN1 can be co-purified with $G\alpha_o$ (Lelianova et al., 1997) while stimulation with alpha-latrotoxin, a known LPHN1 ligand, evokes neurotransmitter release in the presence of extracellular calcium in a phospholipase C-dependent manner (Rahman et al., 1999). Other indirect functional evidence came from second messenger assays (Mogha et al., 2013; Liebscher et al., 2014b Bohnekamp and Schöneberg, 2011; Gupte et al., 2012) and activated downstream components of G protein-signaling cascades (Iguchi et al., 2008; Yang et al., 2011; Giera et al., 2015; Ward et al., 2011; Bohnekamp and Schöneberg, 2011).

Knock-down and overexpression of G proteins caused a depletion and increase, respectively, of second messenger accumulation (Bohnekamp and Schöneberg, 2011; Liebscher et al., 2014b), strongly supporting the concept of G protein/aGPCR interaction. Final proof of G protein-coupling was provided through [^{35}S]GTPγS incorporation assays performed on GPR97 (*ADGRG3*) (Gupte et al., 2012) and GPR126 (Liebscher et al., 2014b) under basal and stimulated conditions.

It is important to differentiate between binding partners and agonists for aGPCRs, as ligand-induced receptor activation has been demonstrated in only a few cases. For example, type III collagen can activate RhoA downstream of GPR56 (Luo et al., 2014) and type IV collagen can elevate cAMP via GPR126 (Paavola et al., 2014). A different ECM molecule, Laminin-211, appears to act in a more complex way on GPR126. Under static cell culture conditions, Laminin-211 inhibits cAMP accumulation, while dynamic conditions activate the G_s pathway (Petersen et al., 2015) (Fig. 2).

Another intriguing observation on ligand-mediated aGPCR activation was reported by Luo and colleagues (Luo et al., 2014). Here, interaction between collagen III and GPR56

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leads to the removal of the NTF followed by RhoA activation. This finding is consistent with earlier observations on other aGPCRs where NTF removal activates the receptor (Okajima et al., 2010; Paavola et al., 2011; Ward et al., 2011; Stephenson et al., 2013; Liebscher et al., 2014b; Paavola et al., 2014). An activation scenario in which the release of the large ECD triggers receptor activation combines all proposed mechanisms. In this model, an abundant, but likely tissue-specific, ligand binds to the adhesive domains of the NTF, which then abrogates its inhibitory function, facilitated by a natural breakpoint at the cleavage site within the conserved GPS. However, the question remains whether this activation is due to the removal of an inhibitor and/or through the exposition of an agonist. The answer(s) to this question is key, as it will guide future approaches to externally activate or inactive these receptors.

While the idea of an inhibitory NTF function has been widely discussed (Okajima et al., 2010; Paavola and Hall, 2012; Langenhan et al., 2013; Liebscher et al., 2013), examples among rhodopsin-like GPCRs provide a second model, in which a tethered agonist activates a GPCR upon NTF removal. Protease-activated receptors (PARs) are probably the best known example for this scenario (Vu et al., 1991a; Adams et al., 2011; Hollenberg et al., 2014) (Fig. 2). Similarly, the deletion of the ECD of the (thyrotropin) TSH receptor was shown to lead to constitutive activity of the residual receptor, which was discussed to be either caused by the release of an activating or an inactivating fragment (Zhang et al., 1995; Zhang et al., 2000). However, they concluded that the release of an inhibitory element accounted for their observations. *In vivo* structure-function studies of the *C. elegans* latrophilin receptor LAT-1 initially suggested that the GPS motif can interact with the 7TM domain. *lat-1* is a maternal gene and dynamically expressed during early embryonic development. Analysis of *lat-1* null mutants showed that the receptor governs the establishment of tissue polarity across the developing embryo, and that loss of *lat-1* function results in embryonic lethality (Langenhan et al., 2009). When receptor variants that lacked the 7TM domain but harbored an intact GPS motif, or alternatively contained a chimeric GPS motif but normal 7TM domain were expressed, neither receptor type was able to rescue the polarity defects of *lat-1* mutants individually. However, expression of both defective receptor fragments restored polarity, indicating that the GPS motif and 7TM domain cooperate during receptor activity (Prömel et al., 2012a).

A recent study has now provided compelling evidence that the concept of a tethered peptide agonist is valid for aGPCRs (Liebscher et al., 2014b). Using GPR126 and GPR133 (*ADGRD1*) mutants and receptor-derived synthetic peptide libraries, tethered agonists for these receptors were identified spanning 16 and 13 amino acids, respectively, between the natural GPS cleavage site and TM1 (Fig. 1). Referring to the protruding nature of this agonistic region after exposure, we termed this region the *Stachel* sequence (German word

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for stinger). Due to the tethered nature of the agonist and its resulting 1:1 stoichiometry, high concentrations of the synthetic peptide are required to elicit an intracellular second messenger response. An estimated EC_{50} value of more than 400 µM was found to elicit G_s-induced cAMP elevation in GPR126- and GPR133-overexpressing cells. *In vivo,* however, a peptide concentration of 100 µM was sufficient to suppress nervous system defects in *gpr126* mutant zebrafish (Liebscher et al., 2014b). Even though high peptide concentrations were used to stimulate significant receptor activation, each peptide was highly specific for the aGPCR from which it originated. Future studies will focus on the discovery of agonists, antagonists, or inverse agonists that can bind aGPCRs with higher affinity and that can be easily diluted in physiologically inert solutions. This can further promote aGPCR studies in animal models and help to make aGPCRs feasible targets for pharmaceutical therapies. Starting points could be to optimize *Stachel* sequence-derived peptides or to identify small molecule agonists. The first and so far only successfully identified small molecule agonistic compound for an aGPCR is beclomethasone dipropionate for GPR97 (Gupte et al., 2012).

Stachel-induced activation of aGPCRs follows rhodopsin-like GPCR kinetics. Label-free analysis of dynamic mass redistribution (DMR) within the cell allows for real-time monitoring of agonist-induced intracellular signaling. An immediate DMR signal was observed for peptide-induced activation of GPR126, comparable to DMR signals induced by isoprenaline on endogenously expressed β adrenergic receptors (Liebscher et al., 2014b).

Most GPCRs couple to more than one G-protein family, and aGPCRs are no exception. For example, GPR126 and GPR133 have been shown to interact with both G_s and G_i proteins (Liebscher et al., 2013; Mogha et al., 2013). Similarly, GPR64 interacts with G_s and G_q proteins (Kirchhoff et al., 2006), GPR56 can couple to $G_{q/11}$ (Little et al., 2004) as well as $G_{12/13}$ (Iguchi et al., 2008), and VLGR1 can couple to G_i (Hu et al., 2014), G_q, and G_s proteins (Shin et al., 2013). While these studies measured basal G-protein signaling, it will be interesting to examine G protein-coupling specificities upon agonist activation. As aGPCRs interact with β-arrestin 2 (see below), biased signaling, as described for canonical GPCRs, is also very likely for aGPCRs and needs further elucidation.

Classical GPCR signaling can be terminated by homologous (receptor-specific) desensitization or heterologous (via external stimuli) desensitization (Lohse, 1993). The result of both events is receptor internalization followed by either degradation or recycling. Homologous desensitization can be achieved through ubiquitination or phosphorylation of the receptor via second messenger kinases (e.g., protein kinase A and C (PKA, PKC)) or a distinct family of GPCR kinases (GRKs), the latter acting in concert with arrestins (Pierce et al., 2002). There are hints that aGPCRs follow these patterns of signal inactivation. A constitutively active GPR56 mutant enhances interactions with β-arrestin 2 and ubiquitination of the receptor (Paavola et al., 2011). Further, ligand-induced downregulation has been

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demonstrated for CD97 in circulating leukocytes (Karpus et al., 2013). This downregulation of CD97 required shear stress and correlated with an increase in plasma levels of soluble CD97, suggesting that dissociation of the NTF triggers degradation of the CTF of the receptor. Whether this downregulation of CD97 is playing a role in aGPCR activation through revelation of the *Stachel* sequence remains to be examined. Another study shows that GPR56 is efficiently downregulated by stimulation with phorbol 12-myristate 13-acetate (PMA), a PKC activator (Little et al., 2004). These data provide evidence of aGPCR signal termination mechanisms comparable to rhodopsin-like GPCRs. Excitingly, the recently identified peptide agonists allow us to systematically investigate desensitization and downregulation kinetics of aGPCRs.

In vivo **mechanisms of aGPCR activation**

As noted in the minireview on ‘Model organisms in GPCR research’ (Langenhan et al., this issue), the first experiments suggesting that GPR126 can couple to G proteins derived from work in zebrafish. Addition of forskolin, an adenylyl cyclase activator, suppresses nervous system defects in *gpr126* zebrafish mutants (Monk et al., 2009; Glenn and Talbot, 2013). Subsequent studies demonstrated that cAMP elevation could also suppress *gpr126* zebrafish mutant ear defects (Geng et al., 2013) and mouse mutant defects (Mogha et al., 2013). Critically, these suggestive *in vivo* studies were complemented by *in vitro* approaches (Mogha et al., 2013; Liebscher et al., 2014b) clearly demonstrating the G_s-coupling ability of GPR126.

Recent studies on GPR126 in zebrafish also shed light on the *in vivo* relevance of *Stachel*-mediated aGPCR activation. Liebscher et al. described a new zebrafish mutant in which two key amino acids in the *Stachel* domain are deleted. Critically, this mutant receptor is cleaved and traffics appropriately to the cell membrane, but the nervous system defect is identical to previously published strong loss-of-function mutants that lack the entire CTF (Liebscher et al., 2014b). Namely, Schwann cells (glial cells in the peripheral nervous system) cannot generate the myelin sheath. Thus, tethered agonist-mediated activation of GPR126 is essential for myelination *in vivo*. Interestingly, the GAIN domain crystal structures of other aGPCRs demonstrate that the tethered agonist is embedded between two beta-sheets of the GAIN domain (Arac et al., 2012). This sequence is deeply buried and likely requires significant structural changes, perhaps mechanical removal of the NTF, to mediate aGPCR activation. At the Lorentz Center workshop in Leiden, a potential mechanism for mechanical NTF removal *in vivo* was proposed (Fig. 2).

During peripheral nerve development, Schwann cells synthesize and secrete ECM proteins that form a basal lamina. ECM proteins in the basal lamina include collagen IV and Laminin-211, known binding partners for GPR126 (Paavola et al., 2014; Petersen et al.,

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2015). Critically, during basal lamina maturation, Laminin-211 polymerizes and this is essential for proper Schwann cell development and myelination (McKee et al., 2012). Petersen et al. reasoned that Laminin-211 polymerization could facilitate GPR126-NTF removal, thereby exposing the tethered agonist to drive myelination. In line with this model, overexpression of wild-type but not non-polymerizing *lama2* (the gene encoding the alpha chain of Laminin-211) could suppress myelin defects in *gpr126* hypomorphic mutants. This, coupled with the dynamic culture assays described above support the notion that Laminin-211 polymerization could be one mechanism that facilitates GPR126-NTF removal *in vivo* (see also Langenhan et al., this issue).

Complementary evidence for mechanical activation of aGPCR signals *in vivo* comes from recent studies on the ADGRL homolog Latrophilin/CIRL of the vinegar fly *D. melanogaster* (Scholz et al., 2015). Here, the aGPCR is resident in mechanosensory neurons, which – upon mechanical stimulation through sound, touch or stretch – respond adequately with an increase in electrical activity. Removal of Latrophilin/CIRL results in a dramatic drop of this input-output relationship leading to profound insensitivity towards mechanical stimulation and blurred signal-to-noise discrimination. Genetic experiments have further indicated that Latrophilin/CIRL may act by modulating ionotropic mechanosensors of the transient receptor potential (TRP) channel family and may impinge on their properties in a mechanostimulus-dependent manner (see also Langenhan et al., this issue). Whether this effect of Latrophilin/CIRL is dependent on NTF, CTF, or both aGPCR fragments acting together, remains to be determined.

How autoproteolysis as a prominent biochemical asset of aGPCRs and prerequisite for NTF removal is linked to signaling is still a conundrum, and may vary for different aGPCRs or even for the same aGPCR in different developmental or cellular contexts. The existence of cleavage-deficient aGPCR homologs due to lack of the consensus GPS site, like GPR123 (*ADGRA1*) or the conserved cleavage motif as in GPR111 (*ADGRF2*) and GPR115 (*ADGRF4*) (Prömel et al., 2012b) suggests that not all aGPCRs rely on a releasable NTF. Further, studies on LAT-1 in *C. elegans* have directly studied this phenomenon *in vivo* using the transgenic complementation assay described above. Intriguingly, autoproteolysis-resistant *lat-1* variants perform indistinguishably from wild-type versions of the receptor, suggesting that separation of NTF and CTF is not necessary for full receptor function (Prömel et al., 2012a). This indicates that not all aGPCRs require a *Stachel sequence* to signal and/or that accessibility of the *Stachel sequence* may not require NTF release.

Additionally, metabotropic function may not be the sole biological signal controlled by aGPCRs. Many aGPCR layouts exhibit large interaction interfaces through their extracellular adhesion domains including the GAIN domain, through which they can form receptor complexes with other transmembrane signaling proteins such as frizzled GPCRs and the

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tetraspanning polarity protein Van Gogh that execute their own signals (Chen et al., 2008; Nishimura et al., 2012). Secondly, aGPCR ECDs may even engage with signaling proteins located on neighboring cells and govern non-cell autonomous signals (Shima et al., 2007; Steimel et al., 2010). In this scenario, aGPCRs act as a ligand rather than a receptor. Finally, emerging evidence also suggests that the NTF and CTF of aGPCRs can have distinct biological functions (Prömel et al., 2012; Patra et al., 2013, Petersen et al., 2015). Continued work is required to more fully understand these non-metabotropic functions of aGPCRs and whether these properties can be modulated in future pharmacological strategies.

Translational implications of aGPCR function

aGPCRs are expressed in various tissues in the human body and play crucial roles in cellular and developmental processes (Hamann et al., 2015). In a time when knowledge on aGPCR structure and function was still limited, aGPCR mRNA variants and/or levels were correlated to biological phenotypes, and animal models have convincingly shown the importance of aGPCRs in development. Two well-known examples are GPR56 and GPR98/VLGR1, where gene mutations are causative for brain malformation (bilateral frontoparietal polymicrogyria) (Piao et al., 2004) and a form of Usher syndrome (Weston et al., 2004), respectively. Moreover, altered aGPCR gene expression is observed in several cancers (Aust et al., 1997; Fukushima et al., 1998; Carson-Walter et al., 2001; Kaur et al., 2003; Kee et al., 2004; Shashidhar et al., 2005; Aust, 2010; Lum et al., 2010; Davies et al., 2011; Favara et al., 2014; Liebscher et al., 2014a), suggest a promising role for these receptors as biomarkers for tumour recognition and possibly even targeting.

With the recent elucidation and engineering of specific aGPCR ligands/agonists as well as antibodies, it became possible to dissect protein function and signaling properties of this receptor family and consequently to associate them with (patho)physiological conditions (e.g. cancer-related processes such as angiogenesis, adhesion, migration, and proliferation). In line with this, expression of GPR56 can inhibit VEGF (vascular endothelial growth factor) production in melanoma cell lines, thereby inhibiting melanoma angiogenesis and growth, a process involving the serine-threonine-proline-rich region in the ECD of GPR56, which leads to a PKCα-dependent signalling cascade (Yang et al., 2011). Similarly, expression of GPR116 (*ADGRF5*) has been shown to promote breast cancer metastasis via activation of $G\alpha_q$-p63RhoGEF-Rho GTPase pathway (Tang et al., 2013).

It was recently stressed that aGPCRs are frequently mutated in multiple human cancers (O'Hayre et al., 2013). Our improved understanding of receptor function will help to unravel the consequences of these mutations for aGPCR function. Coding region mutations range from the ECD and GAIN domain to the 7TM in aGPCRs. In CELSR1 and CELSR3, cancer-associated mutations in the GAIN domain did not alter receptor processing or

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localization (Arac et al., 2012). It remains open whether these mutations contribute to tumor formation or are just a reflection of the higher tumor mutation rates. Perhaps ECD-mutations hamper cell-cell or cell-matrix interactions due to decreased adhesive capacity (Lagerström and Schiöth, 2008; Paavola and Hall, 2012). It is reasonable to assume that mutations in the ECD region could also affect potential ligand-binding properties or prevent NTF modulation and *Stachel*-mediated receptor activation.

Conclusions

Until recently it was unclear whether aGPCRs signal via G proteins at all. There is now mounting evidence from conventional pharmacological assays (e.g. [^{35}S]GTPγS and second messenger detection) and *in vivo* studies that aGPCRs activate classic G protein-signaling cascades. aGPCRs display similar signaling kinetics and coupling specificity upon activation when compared to rhodopsin-like GPCRs. β-arrestin interaction and ubiquitination of aGPCRs suggests that desensitization mechanisms are comparable to canonical GPCRs. Along these lines, it is likely that aGPCRs can also exhibit biased signaling, although this notion has not yet been formally tested.

Despite these key similarities, aGPCRs are distinct from rhodopsin-like GPCRs in how receptor activation can be initiated (Fig. 2). While the generation of a tethered agonist is an accepted concept for some other GPCRs (e.g., PARs), generation of such an agonist in aGPCRs is not achieved by protease action. Rather, a unique and complex activation mechanism accounts for their stimulation, and we have only started to understand the requirements for this process. This includes binding of an extracellular ligand and, at least for some aGPCRs, potentially mechanical forces that expose a tethered agonist to the 7TM.

GPCRs in general have consistently been of interest to the pharmaceutical world. Their ideal localization at the cell membrane combined with well characterized signaling properties make them excellent drug targets. Due to the plethora of aGPCR-signaling aspects, it is tempting to speculate that there are multiple manners to interfere with aGPCR function. The modulation of ECD-ligand interactions for example could prevent the interaction of the *Stachel* peptide with the 7TM region. Moreover, the 7TM could also be targeted in an allosteric manner to directly modulate receptor activation. Future works will focus on using these principles in *ex vivo*- and *in vivo* settings to elucidate their implications in the development and potential treatment of human diseases.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Monk, Hamann, Langenhan, Nijmeijer, Schöneberg, and Liebscher

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Footnotes

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FIGURE LEGENDS

Fig. 1 Architecture of a prototypic aGPCR

Cartoon depicting structural components of a generic aGPCR. aGPCRs undergo autoproteolytic cleavage in the endoplasmatic reticulum at a highly conserved cleavage site that lies within the GPCR proteolysis site (GPS) motif, which is encompassed by the larger GPCR autoproteolysis-inducing (GAIN) domain. This cleavage event divides the receptor into an N-terminal fragment (NTF) and a C-terminal fragment (CTF). The NTF often contains conserved domains found in other proteins (LRR, Ig, EGF) and, along with most of the GAIN domain, comprises the majority of the extracellular domain (ECD). The CTF consists of a residual part of the GAIN domain/ECD, the 7TM domain, and the intracellular domain (ICD). The activating *Stachel* sequence is located within the residual ECD. Figure was adapted to include the *Stachel* sequence from (Liebscher et al., 2014a): New functions and signaling mechanisms for the class of adhesion G protein-coupled receptors, Ines Liebscher, Annals of the New York Academy of Sciences, 2014 Dec;1333:43-64, Copyright © 2014, Copyright owner: Wiley-Blackwell.

Fig. 2 Proposed activation mechanisms of GPCRs

(A) Canonical modes of GPCR activation include the classic binding of an agonist with high affinity to its cognate binding pocket for classic rhodopsin-like GPCRs. An exception lies with the protease-activated receptors (PARs) that expose a cryptic tethered agonistic region upon cleavage by a protease, which then activates the receptor. Synthetic peptides that mimic the tethered peptide sequence can also activate PARs (Vu et al., 1991b; Vu et al., 1991a). (B) Different activation mechanisms are proposed for aGPCRs. Similar to PARs, they possess a cryptic tethered agonist region, the *Stachel* sequence (S). Synthetic peptides derived from the *Stachel* amino acid sequence can activate aGPCRs. The activating *Stachel* sequence could be exposed upon NTF removal, which can then allow for CTF-mediated intracellular function(s) and independent NTF function(s). For GPR126 in peripheral nervous system development, *Stachel* exposure may require interaction with the ECM molecule Laminin-211, which is proposed to direct subsequent mechanical activation (Petersen et al., 2015). Mechanical stimulation of aGPCR has also been suggested for Latrophilin/CIRL in sensory neurons (Scholz et al. 2015), while other studies have shown a direct activation of aGPCRs through interactions with collagens (Luo et al., 2014; Paavola et al., 2014).

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Figure 1

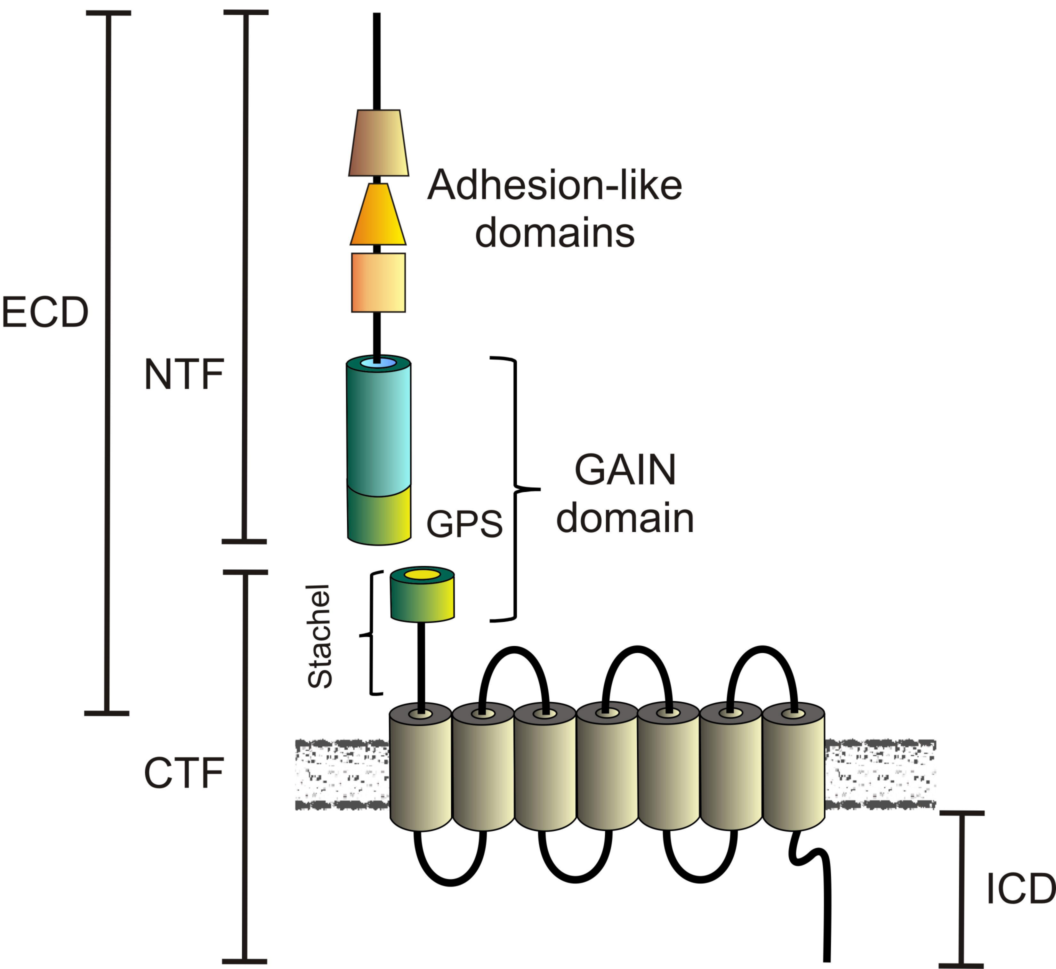
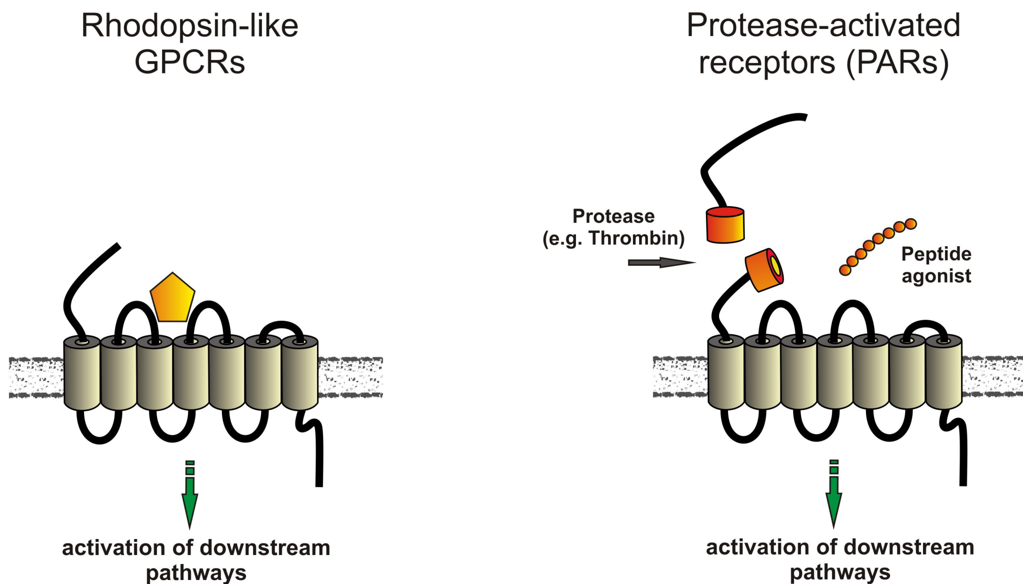


Figure 2

A



B

Adhesion-GPCR

Peptide-induced activation

Ligand/Force-induced activation

Ligand-induced activation

NTF-mediated function

Collagen

Mechanical forces

Laminin-211

Peptide agonist

S

S

S

CTF-mediated function

CTF-mediated function

CTF-mediated function

G protein-coupling
ß-Arrestin interaction
Ubiquitination