CELL BIOLOGY

Sticky Signaling—Adhesion Class G Protein–Coupled Receptors Take the Stage

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Adhesion-type heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors (Adhesion-GPCRs) comprise a class of widely distributed seven-transmembrane spanning (7TM) receptors with unusual layout and properties. The key to understanding the function of Adhesion-GPCRs appears to be their hybrid architecture: They have an extracellular domain containing an extended array of protein folds fit for interactions, and structural elements of GPCRs with a 7TM and an intracellular domain. If and how these distinct protein portions interact is currently under intense investigation. Intriguingly, all Adhesion-GPCRs have a juxtamembrane GPCR autoproteolysis-inducing domain that, in many homologs, facilitates the autocatalytic processing into an N-terminal fragment (NTF) and a C-terminal fragment (CTF), which subsequently remain attached at the cell surface. The NTF provides the ability for combinatorial engagement with cellular or matrix-associated molecules facilitating cell adhesion, orientation, and positioning during development, immune responses, and tumor growth. The CTF, like in canonical GPCRs, initiates interactions with different types of signaling molecules, including heterotrimeric G proteins, small guanosine triphosphatases, and transmembrane protein partners, yet the agonistic potential of most known Adhesion-GPCR ligands is uncertain. Studies with truncated receptors suggest that the NTF and CTF of Adhesion-GPCRs may function as autonomous adhesive and signaling units, respectively, but other studies in nonvertebrates demonstrating NTF-CTF interplay challenge this view. We discuss the available data concerning the main structural elements of Adhesion-GPCRs in the context of receptor function and signaling.

Adhesion-GPCRs—A Common Denominator at Large

Seven-transmembrane spanning (7TM) receptors represent the largest branch of receptors in the human genome. Historically, these receptors were known as heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors (GPCRs) due to the ability of its founding members to signal through activation of heterotrimeric G proteins (*1*). Present on every cell and responding to a plethora of stimuli, GPCRs are involved in a great variety of physiological processes and thus provide numerous pharmacological routes into diseased cell biological conditions. According to the GRAFS classification that is based on structural comparison of the 7TM regions, GPCRs can be grouped into five classes: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste 2, and Secretin (*2*, *3*).

Discovery of Adhesion-GPCRs

The Adhesion class comprises 33 receptors in humans that are classified in nine distinct families according to the molecular signature of their 7TM domains and extracellular termini (*4*) (Fig. 1). Not recognized as a 7TM receptor at that time, the first Adhesion-GPCR widely studied was F4/80, a cell surface marker of most mouse tissue macrophages that was instrumental for exploring the mononuclear phagocyte system in the 1980s (*5*). Molecular cloning of epidermal growth factor (EGF)–like molecule containing mucin-like hormone receptor 1 (EMR1; the human paralog of F4/80) and CD97 (*6*–*9*) paved the way for the recognition of a new type of 7TM receptors on immune cells, designated EGF-TM7 molecules because of the presence of tandemly arranged EGF-like domains at the end of an extended extracellular region (*10*). Shortly after, the cloning of a calcium-independent receptor for the black widow spider neurotoxin α-latrotoxin (α-LTX) on neurons, called latrophilin, identified another 7TM receptor with a long extracellular region comprising hormone-binding (HRM), olfactomedin-like (OLF), and lectin-like (RBL) domains (*11*, *12*). Genomic organization and sequence homology in the membrane-spanning part initially placed these new 7TM receptors in proximity to the Secretin (class B)–GPCRs (*13*). However, after completion of the human genome project and deeper phylogenetic analyses, it became clear that they are part of a separate clade of 7TM receptors that became known as LNB-TM7 (TM7 receptors containing a long N-terminal extracellular region related to class B), class B2, and finally Adhesion-GPCRs (*2*, *14*–*16*). The name Adhesion-GPCRs refers to the numerous protein domains implicated in cell and matrix interactions that are found in the extracellular region of the receptors (Fig. 1).

Evolutionary origin

Adhesion-GPCRs are of ancient origin and present in vertebrates, the closest relatives to vertebrates, and also the most primitive animals. More archaic Adhesion-GPCRs comprising short extracellular regions have been identified in fungi and unicellular relatives of the metazoan lineage, implying the possibility that the Adhesion class evolved before the split of Unikonts from the common ancestor of eukaryotes about 1275 million years ago (*17*). Like other GPCRs, the Adhesion class likely evolved from the adenosine 3′,5′-monophosphate (cAMP) receptors. On the basis of the advanced mosaic structure of the N-terminal fragments (NTFs), it seemed reasonable to assume that Adhesion-GPCRs diverged from the closely related Secretin-GPCRs. Intriguingly, however, analysis of metazoan GPCR data sets indicates that the Secretin-GPCRs originate from the Adhesion-GPCRs, probably from family V (*18*). The early presence of the Adhesion-GPCRs latrophilin (LAT-1, LAT-2, CIRL) and Flamingo (FMI-1) in invertebrates has facilitated studies in *Caenorhabditis elegans* and *Drosophila melanogaster*. In contrast, family II (EGF-TM7) of Adhesion-GPCRs receptors evolved only in vertebrates and underwent major changes even in the great apes (*19*). Thus, extremely old and very young receptors coexist within the Adhesion class, which

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makes it likely that, next to evolutionarily conserved activities, these receptors also execute relatively new and potentially redundant biological functions.

A common denominator?

One of the most intriguing problems in Adhesion-GPCR biology has been the identification of a common denominator among these noncanonical 7TM receptors. Adhesion-GPCRs are widely found in normal and malignant cells of endodermal, ectodermal, and mesodermal origin. Consequently, Adhesion-GPCRs are present in almost every organ system with physiological roles in development, immunity, reproduction, epithelial and neuronal function, and tumorigenesis (Table 1). The potential consequences of loss of an Adhesion-GPCR in humans were first recognized when *GPR56* was identified as the causative mutated locus of bilateral frontoparietal polymicrogyria (BFPP), a recessively inherited genetic disorder of cerebral cortical development (*20*).

Molecular and genetic studies in several organisms show that members of the latrophilin and CELSR (cadherin EGF LAG seven-pass G-type receptor) families and very large GPCR 1 (VLGR1) are involved in planar cell polarity (PCP), ependymal, neuronal, sensory, and cilial development (*21*–*28*). Latrophilins, CELSRs (and homolog Flamingo, hereto referred to as Flamingo/CELSRs), GPR56, and members of the EGF-TM7 family are involved in cell movement and positioning of neurons, leukocytes, and tumor cells (*25*, *29*–*35*). Although knowledge of individual Adhesion-GPCRs progressed, a conclusive definition of Adhesion-GPCRs based on expression pattern, molecular function, or physiological context was still lacking. At the same time, the unique molecular structure that sets Adhesion-GPCRs apart from other 7TM receptors has been the focus of intense research to aid in identifying common principles shared by all Adhesion-GPCRs.

Adhesion-GPCR molecules can be divided into constituent components by two different schemes: a tripartite scheme based on the topology of the receptors or a bipartite scheme determined by the self-cleavage of Adhesion-GPCRs. Under the tripartite compartmentation, also used for other transmembrane protein families, Adhesion-GPCRs consist of an extracellular domain (ECD) holding adhesive folds and the entire GPCR autoproteolysis-inducing (GAIN) domain, followed by a 7TM domain and an intracellular domain (ICD; Fig. 2A). In contrast to canonical GPCRs, the ECD of many Adhesion-GPCRs is much larger and contains a variety of structural domains that facilitate cell-cell and cell-matrix interactions (Figs. 1 and 2A). Adhesion-GPCRs are present at the cell surface as noncovalently linked heterodimers consisting of an extracellular and a membrane-

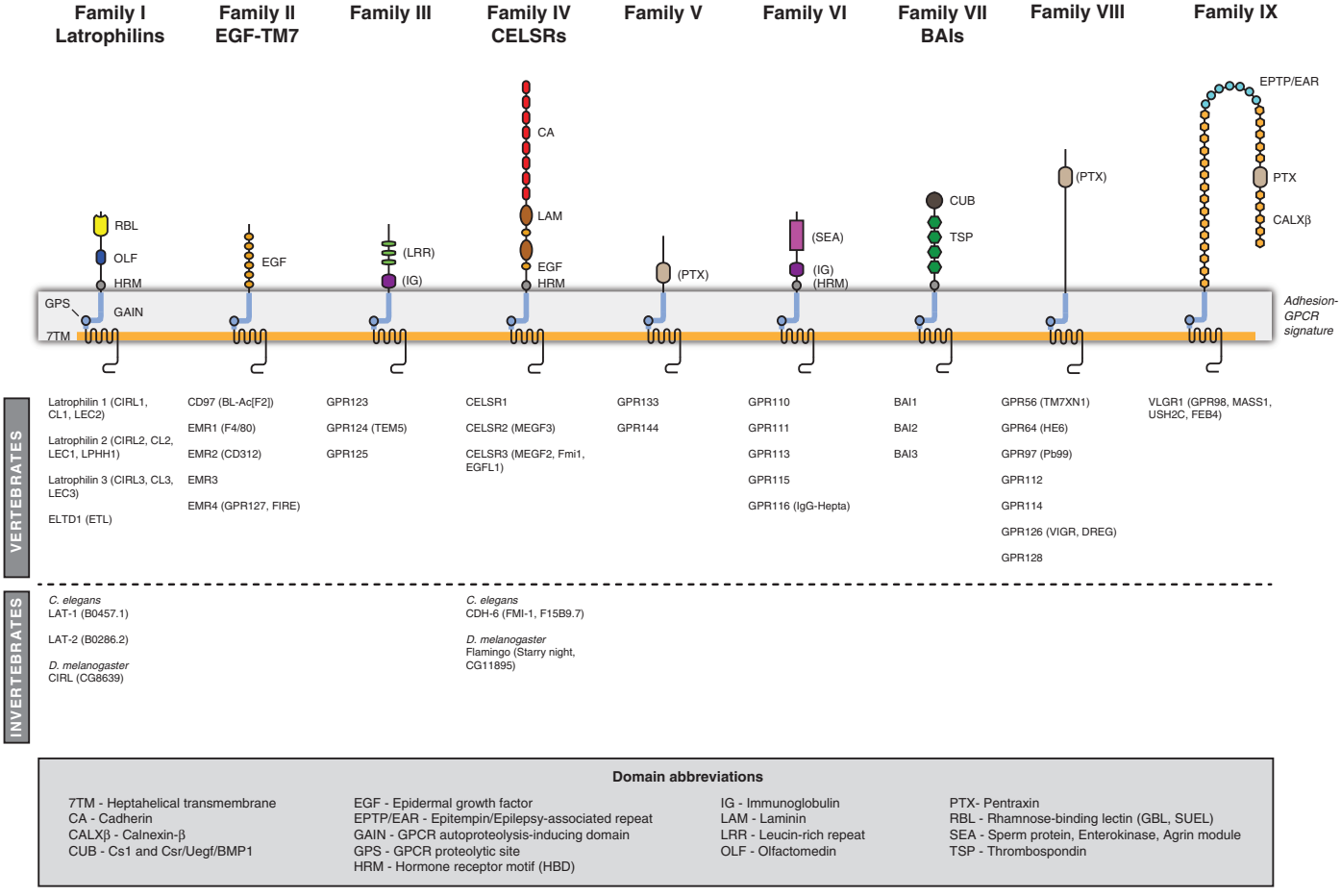

Fig. 1. The Adhesion-GPCR class. Adhesion-GPCRs can be subdivided into nine distinct families with 33 homologs in mammalian genomes. Each family is specified by a unique combination of extracellular folds within the NTF (bracketed domains are not present in every homolog of the family) and strong sequence similarity at the level of the 7TM domain. All families share a GAIN and 7TM domain (Adhesion-GPCR signature). Families I and IV are present in invertebrate species, whereas all families can be found in vertebrates. Synonymous names are given in brackets. #Note that GPR123 (family III) does not contain a GAIN domain.

Table 1. Physiological functions of Adhesion-GPCRs. Phenotypes resulting from genetic studies in whole organisms reveal the physiological roles of Adhesion-GPCRs. ADHD, attention deficit/hyperactive disorder; C+E, convergence and extension; CNS, central nervous system; DKO, double knockout; GWA, genome-wide association; LDL, low-density lipoprotein; SNP, single-nucleotide polymorphism.

Family/receptor	Species	Evidence	Phenotype	Cellular context	References
			Family I (latrophilins)		
Latrophilin 1	Mouse	Null allele	Abnormal maternal attendance to litters	Unknown	(*157*)
Latrophilin 3	Human	Genetic linkage analysis	Susceptibility to ADHD	Unknown	(*158*)
	Zebrafish	Null allele	Developmental defects of the dopaminergic system and enhanced locomotor activity	Neurogenesis, migration	(*159*)
LAT-1	*C. elegans*	Null allele	Anterior-posterior tissue polarity defect during embryogenesis	PCP, migration, differentiation	(*25*, *77*)
ELTD1	Mouse	Null allele	Cardiac hypertrophy after pressure overload	Unknown	(*160*)
			Family II (EGF-TM7)		
CD97	Mouse	Transgene overexpression (*villin* promoter)	Stronger adherens junctions in intestinal epithelia, attenuation of experimental colitis	Adhesion	(*124*)
		Transgene overexpression (*thyroglobulin* promoter)	Increased vascular invasion and lung metastasis in a model of follicular thyroid cancer	Migration	(*127*)
	Mouse	Null allele	Protection from experimental arthritis	Adhesion?	(*65*)
			Enhanced granulopoiesis	Adhesion?	(*64*)
EMR1	Mouse	Null allele	Defective peripheral immune tolerance	Migration?	(*161*)
			Family III		
GPR124	Mouse	Null allele	Embryonic lethality from CNS-specific angiogenesis arrest; defective palate and lung development	Migration	(*162*–*164*)
		Transgene overexpression (*Tie2* promoter)	CNS-specific hyperproliferative vascular malformations	Migration	(*162*)
			Family IV (CELSRs)		
FMI-1	*C. elegans*	Null or hypomorphic allele	Navigation defects of motor, pioneer, and follower axons	Migration, adhesion	(*33*, *35*)
Flamingo/FMI-1	*Drosophila*	Null allele	Distorted wing cell planar polarity	PCP	(*21*)
		Various alleles	Altered polarity of cuticular structures	PCP	(*165*)
		Transgene overexpression	Non-autonomous neuronal connectivity	Migration, adhesion	(*146*)
CELSR1	Mouse	Spontaneous nonsynonymous NTF mutations	Severe neural tube defects, disrupted inner ear hair cell planar polarity, homozygous mice embryonic lethal	PCP	(*166*)
		Conditional null allele	Defects in the orientation of hair outgrowth	PCP	(*167*)
		Spontaneous nonsynonymous NTF mutation or null allele	Defects in hindbrain neuron migration	Migration	(*168*)
	Zebrafish	Gene knockdown	Epiboly defects	PCP, C+E, migration, adhesion	(*123*)
CELSR2	Human	SNPs in GWA study	Associated with LDL cholesterol or early-onset myocardial infarction	Unknown	(*169*, *170*)*
	Mouse	*LacZ* knock-in null allele	Defective ependymal cilia development and planarity	PCP, ciliogenesis	(*171*)
CELSR3	Mouse	Null allele	Neonatal death from ventilation failure, axonal fascicle anomalies	Migration	(*22*)
		Conditional null allele in forebrain sectors	Absence of cortico-subcortical connections	Migration	(*172*)
CELSR2/3 (DKO)	Mouse	*Celsr2 LacZ* knock-in null allele, *Celsr3* conditional null allele	Lethal hydrocephalus	PCP, ciliogenesis	(*171*, *172*)
CELSR1, CELSR2, CELSR3	Mouse	*Celsr1* null or mutant allele, *Celsr2 LacZ* knock-in null allele, *Celsr3* null or conditional null allele	Defective facial branchiomotor neuron migration	Migration	(*22*, *168*, *171*, *172*)
CELSR3; CELSR2/3 (DKO)		*Celsr3* null or conditional null allele, *Celsr2/3* null allele	Defective pancreatic β cell differentiation	PCP	(*22*, *173*)

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Family/receptor	Species	Evidence	Phenotype	Cellular context	References
			Family V		
GPR133	Human	SNP in GWA study	Associated with height	Unknown	(*174*)*
	Mouse	Selective sweep in mice selected for extreme body weight	Associated with weight	Unknown	(*175*)
			Family VI		
GPR116	Mouse	Conditional null allele	Glucose intolerance, insulin resistance	Unknown	(*176*)
			Family VII (BAIs)		
BAI2	Mouse	Null allele	Antidepressant-like behavior, increased hippocampal cell proliferation	Unknown	(*177*)
BAI3	Linkage analysis, GWA confirmed		Associated with early-onset thromboembolism	Unknown	(*178*)
			Family VIII		
GPR56	Human	Mutations	BFPP	Unknown	(*20*)
	Mouse	Null allele	Neuronal ectopia in the cerebral cortex	Adhesion	(*179*)
			Malformation of the rostral cerebellum	Adhesion	(*32*)
			Reduced fertility (defective testis development)	Adhesion	(*180*)
GPR64	Mouse	Null allele	Male infertility (fluid dysregulation in the efferent ducts)	Unknown	(*181*)
GPR126	Human	SNPs in GWA study	Associated with height or pulmonary function	Unknown	(*182*, *183*)
	Zebrafish	Hypomorphic alleles	Defective Schwann cell myelination	Differentiation	(*184*)
	Mouse	Null allele	Embryonic lethality (cardiovascular failure)	Unknown	(*185*)
			Severe hypomyelinating peripheral neuropathy	Differentiation	(*186*)
			Family IX		
VLGR1	Human	Nonsense mutation (deletion of the C terminus)	Familial febrile and afebrile seizures	Unknown	(*187*)
		Various mutations	Usher syndrome type 2	Unknown	(*188*, *189*)
		SNP in GWA study	Increased risk of bone fractures	Unknown	(*190*)
	Mouse	Spontaneous nonsense mutations, NTF-only knock-in allele, Null allele	Audiogenic seizure phenotype	Unknown	(*191*–*193*)
		Null allele, NTF-only knock-in allele	Abnormal stereocilia development, impaired cochlea function, deafness	Adhesion, ciliogenesis	(*24*, *192*–*194*)
			Mild impaired visual function	Unknown	(*24*, *147*, *192*)
			Decreased bone mineral density, mechanical fragility of bones, altered osteoblast/osteoclast function	Unknown	(*190*, *193*)

*Confirmed in other GWA studies.

spanning subunit (Fig. 2A) (*8*, *11*). This two-subunit structure is the result of autocatalytic processing at a GPCR proteolysis site (GPS), which is found in close proximity to the 7TM domain (*36*, *37*). Consequently, two polypeptide chains are generated by the autoproteolytic event: an NTF (NT, α subunit, α chain) and a CTF (C-terminal fragment; CT, β subunit, β chain) contributing to the typical cleavage-based bipartite compartmentation of Adhesion-GPCRs (Fig. 2A). The GPS motif is part of a much larger (about 320-residue) GAIN domain that forms a tightly and noncovalently associated heterodimer upon proteolysis (Fig. 2B) (*38*). The universal presence of the GAIN domain in all Adhesion-GPCRs (Fig. 1) and its implications for its structure and likely also its signaling function have changed the view on the "spacer" region, which is now known to bear part of the GAIN domain. The GAIN domain connects the protein domains at the N terminus of the NTF to the membrane-spanning CTF (Fig. 2A). Currently, the GAIN domain seems to be the most common denominator specific to Adhesion class GPCRs. In the following sections, we discuss the current knowledge of the major structural elements of Adhesion-GPCRs in the context of receptor function.

The NTF—Mosaicism Aiding Combinatorial Recognition

The NTF of Adhesion-GPCRs is the receptor segment of the ECD before the GPS that comprises the adhesion domains and a large part of the GAIN domain (Fig. 2A). The NTF varies in size from about 200 amino acids in

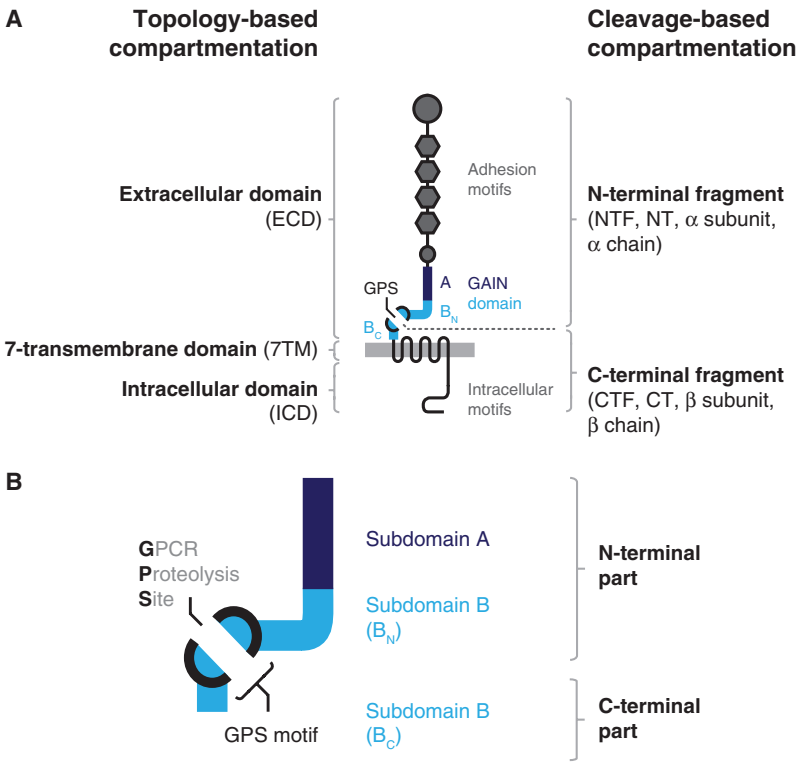

Fig. 2. Structural blueprint of Adhesion-GPCRs. (A) Topology- and cleavage-based compartmentation of Adhesion-GPCR architecture. The protein layout of all Adhesion-GPCRs is marked by a three-partite structure consisting of ECD, 7TM domain, and ICD. Alternatively, Adhesion-GPCRs that undergo autoproteolysis also display a two-partite structure containing the NTF and the CTF. (B) The GAIN domain is a complex fold that mediates autoproteolysis and subsequent attachment of the cleaved NTF and CTF fragments. The domain is divided into two subdomains (A and B), of which the latter one is cleaved at the GPS located inside the GPS motif. Hence, the GAIN domain parts N-terminal to the GPS belong to the NTF, whereas the C-terminal portion is accommodated within the CTF.

GPR114 to more than 5600 amino acids in VLGR1, the largest membrane-anchored receptor known in humans (*39*). Accordingly, the NTF is largely responsible for the unusually large size of most Adhesion-GPCRs.

Modular domain combinations facilitate various interactions

NTFs contain a wide variety of protein domains, which render them typical modular proteins (*40*) and distinguish them from other GPCR classes. Whereas several families, including family I (RBL, OLF, HRM), family II (EGF), family IV (CA, LAM, EGF, HRM), and family VI (TSP, HRM) (*7*, *21*, *38*, *41*, *42*), of Adhesion-GPCRs have characteristic domains or combinations of domains, the composition of the N terminus in other families differs between individual members (*4*) (for domain abbreviations, see Fig. 1). VLGR1 contains up to 35 CALXβ repeats, depending on alternative RNA splicing (*43*). Similarly, the EGF-TM7 family II members CD97, EMR1, and EMR2 comprise splice isoforms with different numbers of EGF-like domains (*10*).

Many of the 20 or so different protein domain types found in Adhesion-GPCRs have the ability to mediate contact with cellular or matrix-associated molecules. Attempts to deorphanize Adhesion-GPCRs enabled the identification of about a dozen ligands (Table 2). Notably, these binding partners have been assigned to a relatively small number of eight individual (and partly closely related) Adhesion-GPCRs from only four families, several of them engaging contacts with a few unrelated molecules. This leaves a majority of orphan Adhesion-GPCRs with unknown ligands.

The first Adhesion-GPCR ligand found was CD55, which interacts with the first two EGF domains of CD97 (*44*). Another ligand, chondroitin sulfate B, binds to the fourth EGF domain of both CD97 and EMR2 (*45*). Studies on CD97 uncovered characteristics of Adhesion-GPCR interactions including their ability to interact with different ligands in parallel, depending on alternative splicing and glycosylation (*46*–*49*). Moreover, subtle changes in domain composition can abrogate binding capacity for a ligand. For example, three amino acid changes within the first two EGF domains of human EMR2 prevent its binding to CD55 (*47*, *50*). These principles might also apply to other Adhesion-GPCRs and create the ability to interact with many different ligands through their various domains (*51*–*60*), probably in a regulated, cell type–specific manner.

Are Adhesion-GPCRs context recognizers?

The role of protein domains in the engagement with other molecules suggests that Adhesion-GPCRs recognize other cells, extracellular matrix structures, or pathogens through their NTF. For example, BAI1 enhances the uptake of apoptotic cells and bacteria through binding the "eat-me" signal phosphatidylserine and the Gram-negative cell wall constituent LPS (lipopolysaccharide), respectively (*52*, *61*). Moreover, an extensive panel of in vivo data obtained in invertebrate and vertebrate models implies that Adhesion-GPCRs facilitate cell adhesion, orientation, migration, and positioning during development, immune responses, and tumor progression (Table 1). Studies in *C. elegans* and *D. melanogaster* clearly indicate an essential role for latrophilins and Flamingo/CELSR in planar cell and tissue polarity and in neuronal development (*21*, *25*, *28*). Studies in vertebrates provided evidence for the roles of CD97, EMR1, GPR56, GPR64, and VLGR1 in various developmental processes and immunity. Several parallels exist between CD97 and GPR56. Both receptors bind some seemingly unrelated molecules (Table 2) that individually are widely distributed but together are coexpressed on the surface and extracellular matrix of stromal cells at certain tissue sites, such as the intimal lining in the synovium (CD97) (*29*) and the pial membrane in the brain (GPR56) (*62*). It seems possible that these ligands are part of address codes provided by stromal cells to direct recruitment of developing cells such as immune cells to the lymphoid tissue. When presented aberrantly, stromal address codes can drive persistent accumulation of the same cells within peripheral tissue, resulting in inflammatory diseases (*63*). Notably, mice lacking either CD97 or its ligand CD55 had increased granulopoietic activity and reduced disease phenotypes in experimental models of arthritis (*64*, *65*). Luo and co-workers showed that presentation of the GPR56 ligand collagen III by meningeal fibroblasts in the pial membrane controls cortical development and lamination by restricting the migration of developing neurons (*62*). Mice lacking GPR56 and collagen III develop reciprocal defects in brain development similar to the cortical BFPP malformation caused by dysfunctional GPR56 in humans (*54*). Most identified BFPP-associated missense mutations are located within the NTF of GPR56, where they effect ligand binding (*20*, *54*, *66*, *67*). This suggests that the loss of functional GPR56 to facilitate adhesion of developing neurons to

Table 2. Adhesion-GPCR ligands. The currently known nonhomophilic ligands of Adhesion-GPCRs are listed. Homophilic ligands (not listed) have been reported for Flamingo/CELSR, latrophilin 1, and GPR56 (*21*, *26*, *59*, *60*, *77*). FLRT, fibronectin leucine-rich transmembrane protein.

Family/receptor	Species	Ligand	References
Family I (latrophilins)			
Latrophilin 1	Rat	Teneurin-2 (Lasso)	(*57*)
		FLRT1, 3	(*195*)
		Neurexin-1α, -1β, -2β, -3β	(*51*)
Latrophilin 2		FLRT3	(*195*)
Latrophilin 3		FLRT1, 3	(*195*)
		Teneurin-3	(*195*)
HC110-R	*Haemonchus contortus*	FMRFamide-like neuropeptides AF1, AF10, PF2	(*196*)
Family II (EGF-TM7)			
CD97	Human	CD55 (decay-accelerating factor)	(*44*)
		Chondroitin sulfate B (dermatan sulfate)	(*45*)
		Integrin $\alpha_5\beta_1$, $a_v\beta_3$	(*101*)
		CD90 (Thy-1)	(*56*)
	Mouse	CD55 (decay-accelerating factor)	(*197*)
EMR2	Human	Chondroitin sulfate B (dermatan sulfate)	(*45*)
Family VII (BAIs)			
BAI1	Mouse	Phosphatidylserine	(*61*)
BAI3	Mouse	C1q-like proteins	(*53*)
Family VIII			
GPR56	Human	Tissue transglutaminase 2	(*30*)
	Human	Collagen III (α-1)	(*54*)
	Mouse	Collagen III (α-1)	(*62*)

the pial membrane causes BFPP. Moreover, GPR56 restricts tumor cell migration possibly by binding to tissue transglutaminase 2, which is abundantly present in tissue and tumor stroma (*30*). In the context of cell migration control but as opposed to GPR56, the presence of CD97 has been linked with invasive behavior of tumor cells (*68*, *69*).

The GAIN Domain—One Domain to Rule Them All?

Although the assortment of protein domains within the NTF of Adhesion-GPCRs generates an astonishing variety of putative interactions, the juxtamembrane region of the NTFs has long been considered as a spacer that exposes the adhesive modules at a distance from the plasma membrane. Sequence comparison indicates an astonishing structural similarity between paralogous and orthologous Adhesion-GPCRs (*55*). The underlying structural reason for this high conservation is the presence of a GAIN domain holding the GPS motif at its C-terminal end (*38*, *55*). The GAIN-GPS region is also found in another protein context, where it also features close to the first transmembrane segment of polycystin-1/polycystic kidney disease protein 1 (PKD-1) and its homologs (*70*). This suggests that proximity of the GAIN domain to the plasma membrane figures prominently in its biological function.

GAIN domain structure

To date, the most intriguing property of the GAIN-GPS region is its capacity to promote self-cleavage of Adhesion-GPCRs and PKD-1–like molecules, which has been a focal point in Adhesion-GPCR research. Adhesion-GPCR cleavage was first recognized in CD97 (*8*), followed shortly after in latrophilin 1. The exact cleavage site was determined using N-terminal amino acid sequencing (*11*), and this peculiar 40–amino acid–long motif was named the GPS (*55*). Recently, the GPS motif has been placed in a domain context by means of crystallographic determination of the GAIN fold of cleaved rat latrophilin 1 and uncleaved mouse BAI3, respectively (*38*). The late discovery of the GAIN domain as an individual fold shows that primary sequence similarity searches, unlike for the GPS motif, do not readily identify GAIN domains in different proteins. Secondary structure-based search algorithms, however, could be used only after the structural properties of the GAIN domain had become available. These algorithms detected the presence of the GAIN fold in all Adhesion-GPCRs and PKD-1–like proteins (*38*), suggesting that the steric properties, but not necessarily the amino acid makeup, of the GAIN domain are evolutionarily highly valuable.

The crystal structures revealed that the GPS motif itself is part of the larger GAIN fold that consists of two subdomains, A and B, stretching about 320 residues in mammalian Adhesion-GPCR (Fig. 2). The GPS motif is located in the most C-terminal five β strands of subdomain B, protected within a sandwich of additional β strands, and associated with subdomain A comprising six α helices. Although the GPS motif is necessary for autoproteolysis (*71*), it is the GAIN domain that comprises the minimal structural element sufficient for autoproteolysis (*38*).

The unique structural properties of the GAIN domain, its singularity in occurrence only in Adhesion-GPCRs and PKD-1–like proteins, and the functional potency conferred by its presence render it a useful criterion of Adhesion-GPCR identity. Several 7TM receptors found in ancient organisms, such as Fungi, Amoebozoa, and Alveolata, have sequence similarity to Adhesion-GPCRs at the level of the 7TM domain (*17*). However, they lack extended NTFs including the GAIN domain and thus likely constitute evolutionary intermediates before acquisition of Adhesion-GPCR functionality by inclusion of a GAIN domain in a fashion reminiscent of mosaic exon shuffling (*72*).

Also among "true" Adhesion-GPCRs, not all exhibit strict secondary sequence conservation across the entire GAIN domain, indicating that functional diversity among the receptor class spans from their highly variable adhesion domain outfits to different flavors of the GAIN domain. For example, EGF-TM7 family II Adhesion-GPCRs lack the six α helices of GAIN subdomain A, whereas EGF, latrophilin, and seven-transmembrane domain–containing protein 1 (ELTD1; family I) contains a full subdomain A structure, supporting its phylogenetic distance to family II despite the presence of extracellular EGF motifs (*38*). GPR123, a family III member, lacks a GPS motif altogether (*73*).

GAIN domain cleavage

The scissile bond in Adhesion-GPCR GAIN domains is located at a kinked interstrand loop where the catalytic triad [consisting of a residue serving as a charge relay system due to its pK_a (where K_a is the acid dissociation constant) close to the physiological pH (−2) positioned in close proximity to an aliphatic (−1) and a small polar (+1) amino acid] is assembled. The consensus of this cleavage triad is present in most, if not all, cleavable Adhesion-GPCRs and comprises the residues His/Arg_{-2} − $Leu/Met/Ile_{-1}$ ↓ $Thr/Ser/Cys_{+1}$. The chain of reactions resulting in cleavage encompasses deprotonation of the nucleophilic amino acid in position +1 by the −2 residue; subsequently, a nucleophilic attack in cis on the −1 residue forms an ester intermediate via an acyl shift, which is finally hydrolyzed and gives rise to two separate

protein fragments. This autoproteolytic mechanism was first established in EMR2 (*37*), confirmed in a non–Adhesion-GPCR layout for the GPS of PKD-1 (*74*), and corroborated by the structures of the GAIN domains of latrophilin 1 and BAI3 (*38*). The mechanism of GAIN domain autoproteolysis bears similarities with the self-cleavage of the thyroid-stimulating hormone receptor (TSHR), a canonical GPCR, as well as with N-terminal nucleophile hydrolases such as inteins and hedgehog ligands (*37*, *75*), and it can be controlled by site-specific N-glycosylation at the GAIN-GPS region (*76*).

Clearly, the self-cleavage of Adhesion-GPCRs has drawn much attention because of its implicit possibilities for receptor biology: maturation, interactions, signaling, and protection of membrane or receptor integrity during mechanical stress. Whereas these realms are attractive research foci in relation to Adhesion-GPCR autoproteolysis, it is important to consider that GPS cleavage can be lost in individual Adhesion-GPCRs including EGF-TM7 (family II), CELSRs (family IV), and BAIs (family VII) receptors, without noticeably affecting function (*77*, *78*). This indicates that the evolutionary requirement for cleavage is less stringent than the preservation of GAIN domain structure itself.

Because GPS cleavage occurs in the endoplasmic reticulum soon after translation and before receptor transit to the Golgi (*8*, *36*), initial studies tested the requirement of GPS cleavage for receptor biosynthesis and trafficking. Although some studies show that GPS cleavage is necessary for membrane targeting of some Adhesion-GPCRs (*36*, *79*), others find no membrane localization defects in cleavage-deficient mutants in vitro (*37*, *71*, *80*) and in vivo (*77*). This indicates that Adhesion-GPCRs do not generally rely on autoproteolysis for normal membrane trafficking and that additional factors, such as structural irregularities introduced into the GAIN domain by the mutations, might impair surface presentation of cleavage-incompetent receptors.

Adhesion-GPCRs—Receptors with split personality?

A most intriguing finding on GPS-cleaved Adhesion-GPCRs (and PKD-1–like proteins) concerns their ability to "reassociate" (*11*, *80*) by means of hydrophobic interactions arising between the split parts of the GAIN domain (*38*). This finding raises the question of why biological effort is invested into cracking up a contiguous polypeptide chain when subsequently its overall integrity is maintained in an almost unchanged appearance, shown through crystallography of the cleaved latrophilin 1 GAIN domain (*38*). One answer is proposed by Volynski and colleagues with their "split personality" receptor hypothesis (*81*). Their findings on latrophilin 1 suggested that GPS cleavage is necessary to generate Adhesion-GPCR fragments (NTF and CTF) that can behave independently and reassociate dynamically at the cell membrane upon presentation of extracellular NTF interaction partners. This makes reassociation a key step in receptor signaling (*81*). The ligation-induced heterodimerization observed for EMR2 supports this model (*82*). Additional studies surprisingly show the NTF and CTF from different Adhesion-GPCRs (for example, between latrophilin 1 and EMR2 or GPR56) can cross-complex and form chimeric receptors (Fig. 5C) (*82*, *83*). The biological implications of this potential split personality mechanism are vast, combining signal input through the NTF of one Adhesion-GPCR with varying signal outputs through the CTF of another.

In contrast to these results, the structure of GAIN domain suggests that the site of cleavage is buried within a rigid domain structure, and the resulting subunits cannot be easily detached or recombined unless the protein is substantially denatured (*84*). This also accounts for the high structural similarity between GAIN domains of noncleavable and cleavable Adhesion-GPCRs (*38*). Further, functional experiments using invertebrate latrophilins could not substantiate the split personality receptor model in *C. elegans*, in which cleavage-deficient constructs of the LAT-1 or LAT-2 homologs show full restoration of function and no apparent gain-of-function phenotypes (*77*). This might be because either mammalian Adhesion-GPCRs have evolved novel modes of interactions or in vivo assays under physiological conditions lack the sensitivity required to detect the functional outcome of heterogeneric receptor formation. Further experiments are required to test the fascinating "split receptor" hypothesis and its physiological relevance. In particular, the conceptional nucleus of the model, namely, that formation of heterogeneric Adhesion-GPCR hybrids is abolished by disabling GPS proteolysis in either partner, remains to be tested.

Adhesion-GPCRs—Made to break?

Not the least explanation for a biological demand for cleaved GAIN domains, but certainly the most underresearched thus far, is that cleavage of the GAIN domain confers a protective mechanism during mechanical stress. The long, adhesive N termini of Adhesion-GPCRs bind to cellular or matrix components, thereby anchoring the NTF firmly to substrates that move relative to the receptor-presenting cell, resulting in mechanical strain. An autoproteolyzed GAIN domain could function as a molecular mechanical fracture device with a predetermined dissociation threshold, above which the NTF is released from the CTF. This is a similar scenario to juxtamembrane ectodomains of membrane-anchored mucins, which are also autocatalytically cleaved and noncovalently reassociated at an extracellular sea urchin sperm protein–enterokinase–agrin (SEA) module (*85*). SEA cleavage is thought to have a predetermined breaking point in the heterodimer to prevent harm to the cell membrane upon mucin-dependent mechanical strain, such as rupture of cell membrane integrity, which can occur when mucus is transported tangentially to the epithelial surface (*86*). GAIN domain cleavage could serve a similar function, indicating that Adhesion-GPCRs might function in mechanically challenged environments.

Investigation of the CD97-CD55 interaction enabled direct detection of the interaction between an Adhesion-GPCR and its binding partner in vivo, and the effect this has on NTF-CTF dissociation. Circulating leukocytes from CD55-deficient mice had higher amounts of CD97, which rapidly normalized after adoptive transfer into wild-type mice due to contacts with CD55 on both leukocytes and stromal cells. Reduction of CD97 required shear stress, involved reduction of both the NTF and CTF, and correlated with an increase in plasma levels of soluble CD97, suggesting that dissociation of the NTF triggers degradation of the CTF (*87*). As a consequence, CD97-mediated cellular contacts may be restricted to specific tissue sites, thereby preventing uncontrolled clustering of blood cells in the circulation.

Signaling of Adhesion-GPCRs—NTF-Dependent Activities

The NTF with the GAIN domain as interaction interfaces

Proteolysis of the GAIN domain might serve different functions than just simple separation of NTF and CTF. The GPS cleavage event could also impinge on the structure of the GAIN domain surface (*38*), which inevitably determines the scope of protein interactions that might occur at the GAIN domain interface, and consequently alter the cellular mechanisms controlled by Adhesion-GPCRs. This is underscored by the location of mutations in Adhesion-GPCRs (such as *LPHN1*, *LPHN3*, *BAI3*, and *GPR56*) and *PKD-1*–like genes isolated from cancer, BFPP, and autosomal dominant polycystic kidney disease patient samples. Mutations accumulate in GAIN domain–encoding exons, with increased frequency at the transition between both constituent GAIN subdomains A and B and in the middle of subdomain B (Fig. 2B) (*20*, *80*, *88*). Whereas some mutants affect folding, proteolysis, or membrane delivery of gene products, other mutants do not affect these functions but potentially result in defective interaction between the GAIN domain and binding partners, either within the receptor protein itself or

with other proteins (*38*). Studies of chimeric versions of LAT-1 in *C. elegans* indicate that exchange of the endogenous LAT-1 GPS motif for a related one from LAT-2 (the second worm latrophilin homolog) is sufficient to abolish activity of the receptor during development (*77*). This indicates that the function of LAT-1 is sensitive to the structural integrity of the GAIN domain, which could determine interaction interfaces with signaling partners.

Putative partners at GAIN domains comprise endogenous interactors of canonical GPCRs, such as receptor activity–modifying proteins (RAMPs) (*89*) and tetraspanins, of which the latter stabilize the association between GPR56 and $G\alpha_{q/11}$ (*90*). Signals can be relayed forwardly in either a cell-autonomous manner (Fig. 3A) or a non–cell-autonomous manner by engaging with molecules presented on a neighboring cell (Fig. 3B). An example of non–cell-autonomous Adhesion-GPCR signaling is axon navigation in *C. elegans*, in which the NTF of FMI-1 (the worm's Flamingo/CELSR homolog) executes a function separate from the signaling unit of the CTF. FMI-1 is found both in pioneer axons, which explore and pave a migratory route during neuronal development, and in follower axons, which orientate at pioneer axons. *Fmi-1* mutants display guidance defects in both axon classes. Intriguingly, follower axon defects in the mutant are rescued by FMI-1 constructs that lack the ICD and 7TM domain, indicating that FMI-1 directs axon navigation in follower axons via the NTF (*33*). Such non–cell-autonomous actions of FMI-1 in axonal migration have been described for other neuron classes as well (*35*).

Soluble ectodomains of Adhesion-GPCRs

A direct consequence of GAIN domain cleavage and release of the NTF from the receptor heterodimer is that soluble NTFs could engage with interactors over large distances, another mode of non–cell-autonomous activity (Fig. 3C). The existence of circulating soluble ectodomains is reported for several Adhesion-GPCRs, including latrophilin 1, CD97, GPR124, GPR116, BAI1, BAI2, and GPR126 (*8*, *42*, *91*–*95*). Experimental evidence supports two scenarios of how NTF release might come about: (i) the NTF can be dislocated from the NTF-CTF heterodimer by means of receptor ligation, or (ii) shedding requires an independent proteolytic cleavage event. The latter is supported by the fact that the soluble ectodomain of BAI1 closely matches the predicted size of the NTF and requires cleavage at the GPS for its formation (*42*). The former is supported by the observation that soluble CD97 is absent in the circulation of CD55-deficient mice, indicating a ligation-mediated release mechanism that nevertheless might require proteolytic cleavage (*87*). Additionally, several investigations demonstrate that Adhesion-GPCRs can also undergo GPS-independent proteolysis. In rat latrophilin 1 and latrophilin 2, a second cleavage occurs, perhaps mediated by a membrane-bound protease, such as a sheddase, at a site between the GPS motif and the first transmembrane stretch of the 7TM domain (*94*). The cleavage product releases the NTF—still bound to the CTF fragment through the GAIN domain—off the membrane. Under denaturing conditions, the noncovalent GAIN association of NTF and CTF is broken, and a 15–amino acid peptide can be detected by mass spectrometry. Intriguingly, this second cleavage is more efficient if GPS cleavage is abolished (*94*). Another example, CELSR1, lacks the canonical GPS; yet, in mouse embryo extracts, next to the 400-kD full-length receptor, a 85-kD CTF fragment could be detected, indicating an alternative cleavage event in close proximity to TM1 but toward the C terminus of the crippled GPS (*96*).

For GPR124, BAI1, BAI2, GPR116, and GPR126, an additional cleavage toward the N terminus of the GPS results in the release of subfragments of the NTF (*91*–*95*, *97*). Inhibitor and coexpression studies revealed that BAI2, GPR116, and GPR126 are processed intracellularly in the trans-Golgi network by the pro-protein convertase furin (*92*, *95*, *98*). For human BAI2, GPS-independent cleavage also releases the NTF in the absence of GPS cleavage. This suggests that alternative cleavage events prevalent in Adhesion-GPCRs occur nonhierarchically, in contrast to proteolytic cascades that govern regulated intramembrane proteolysis (RIP) of membrane-bound substrates like Notch or amyloid precursor protein (*99*). Finally, GPR124 is cleaved at the cell surface by thrombin-induced shedding promoted by the cell surface protein disulfide isomerase (*100*).

Release of Adhesion-GPCR NTFs could abrogate receptor activity (Fig. 4A). For example, the NTF and CTF of CELSR2 are both required for dendritic arborization in organotypic brain slice cultures (*58*). NTF shedding could also initiate non–cell-autonomous

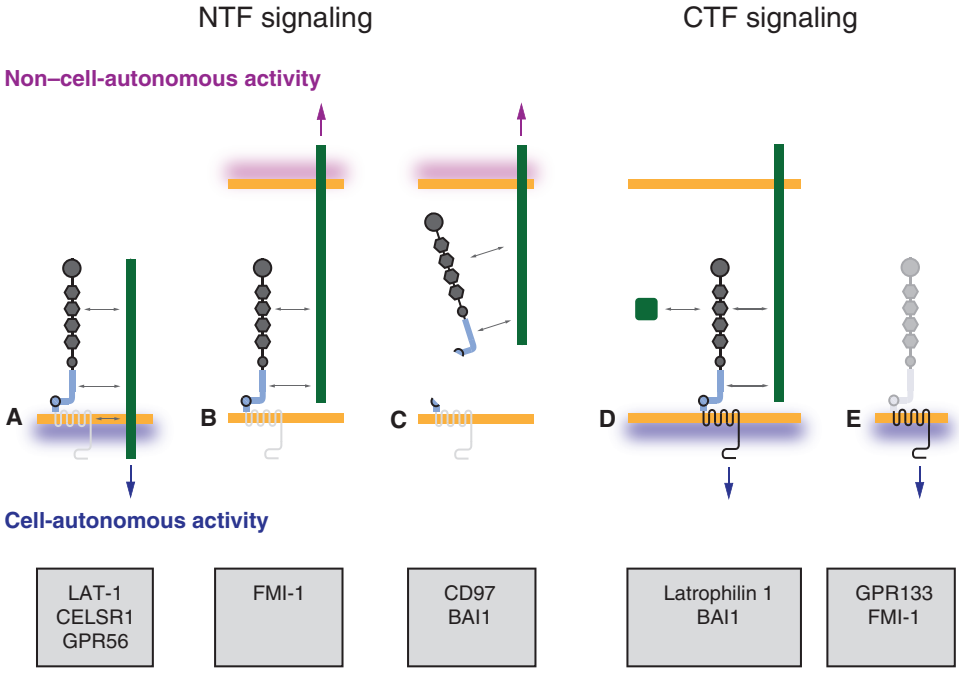

Fig. 3. Principal signaling scenarios of Adhesion-GPCRs. (A and B) The NTF can engage partner structures (dark green) that are located either at the surface of (A) the receptor-carrying (*26*, *77*, *90*) or (B) a neighboring cell (*33*, *58*, *123*). The latter includes interactors in the extracellular matrix. (C) NTF shedding can result from ligand interaction, mechanical strain, or secondary cleavage. Shed NTFs might consequently induce non–cell-autonomous signals at neighboring cells or more distant locations (magenta) (*42*, *101*, *103*). (D and E) CTF-dependent signals (blue) are elicited either by (D) classical agonist interactions at the NTF in cis or trans (*12*, *52*, *57*, *61*) or (E) the CTF independent of an interaction relayed through the NTF. This interaction can rely on the presence of the 7TM domain and intracellular tail (*113*), although in some cases, the 7TM appears dispensable for signaling (*33*). Receptor parts not implicated in signaling are in gray.

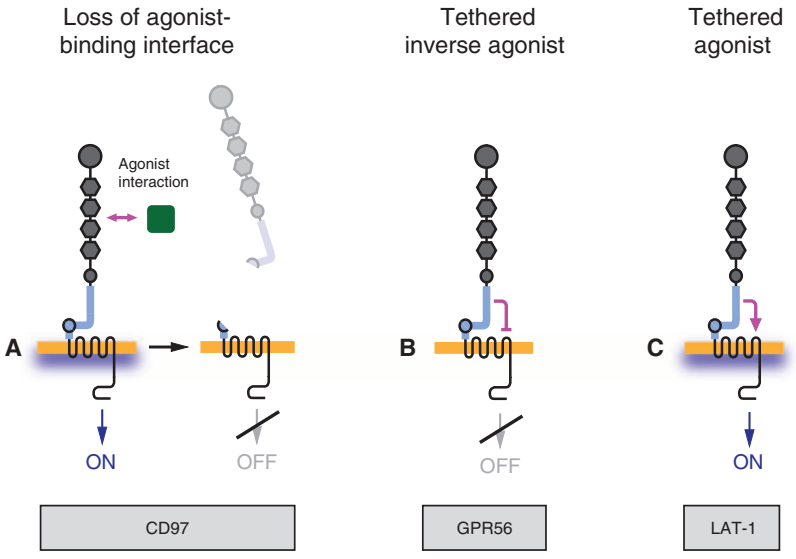

Fig. 4. Activation/deactivation of Adhesion-GPCRs by NTF-CTF interactions. (A) Simple GPS proteolysis–mediated shedding of the NTF abrogates receptor signaling through loss of agonist binding (*91*). (B) In a tethered inverse agonist model, a part of the NTF, such as the GAIN domain, represses CTF signaling. Dislodgement of the NTF relieves CTF repression, and signaling ensues (*60*, *138*). (C) In an opposing model, the NTF has activating capacity of the CTF and receptor cleavage terminates signaling (*77*). Note that for scenarios in (B) and (C), loss of NTF would invert signaling outcome of the receptor. This might result from interaction with a ligand.

activities at distant locations (Fig. 3C). Indeed, soluble CD97 stimulates angiogenesis through binding to the integrins $\alpha_5\beta_1$ and $\alpha_v\beta_3$ (*101*). Whereas both the NTFs of the shortest and longest CD97 isoform increase endothelial cell chemotaxis, only the NTF of the longest isoform stimulates their invasion through Matrigel, suggesting that NTFs of the various isoforms of individual Adhesion-GPCRs may potentially have different physiological effects by engaging different ligands. As further examples, soluble GPR124 has an exposed cryptic RGD (Arg-Gly-Asp) motif that binds $\alpha_v\beta_3$ and supports the survival of endothelial cells (*93*), whereas BAI1 has an RGD motif that inhibits angiogenesis by engaging with integrins (*102*). Consequently, in contrast to that of GPR124, the NTF of BAI1 (vasculostatin) has antiangiogenic and antitumorigenic activities (*42*, *103*).

Mechanisms that control the release of soluble Adhesion-GPCR ectodomains are likely to exist. One example of this is the soluble ectodomain of CD97, which is found at sites of inflammation (*8*, *104*, *105*) but not in CD97-positive tumors (*68*). These findings suggest that proteolysis is a recurring theme in the regulation of Adhesion-GPCRs and a putative pharmacological handle to modulate their activity.

Signaling of Adhesion-GPCRs—CTF-Dependent Activities

The CTF of Adhesion-GPCRs comprises the receptor fragment that is C-terminal to the GPS within the GAIN domain and thus contains a nibble of the GAIN domain itself, the 7TM domain, and the ICD (Fig. 2). The 7TM domains of Adhesion-GPCRs are most homologous to Secretin class GPCRs and have been used to distinguish nine families within the Adhesion class (*4*) (Fig. 1). Adhesion-GPCRs harness well-known intracellular signal pathways by virtue of their 7TM domain, an interface that is highly suited for interactions with various different types of molecules: heterotrimeric G proteins, nonheterotrimeric G proteins, and transmembrane protein partners (Table 3).

Adhesion-GPCR signaling through G proteins

Adhesion-GPCRs exhibit molecular features in their protein architecture that strongly imply that they function as receptors in pharmacological terms. They likely work as receptive conduits that (i) recognize and are activated by native agonists, (ii) transduce the message carried by the agonistic stimulus through conformational means within the receptor molecule across the plasma membrane, and (iii) elicit an intracellular signal (*106*). Whereas some Adhesion-GPCRs ligands, such as compounds that physically interact with the receptor molecules in a biochemical sense, have been determined (Table 2), the agonistic potential of a given ligand and therefore the impact of ligand interaction on Adhesion-GPCR signal transduction have remained untested or unclear in many cases (*107*). This situation is additionally aggravated by the limited number of identified intracellular signaling pathways into which Adhesion-GPCRs couple and which could be used to test the activating potential of individual ligands. Thus, here, we distinguish ligands (interactors with potential activating function) from agonists (interactors with demonstrated activating function) of Adhesion-GPCRs, especially because ligand interaction at Adhesion-GPCR NTFs might solely serve adhesive rather than signaling functions.

Binding to G proteins qualifies a 7TM receptor to be regarded as a GPCR. For a long time, this term was avoided for the Adhesion class because no direct evidence for G protein binding and signaling was known. Although our knowledge about these signaling routes is still rudimentary for Adhesion-GPCRs compared to Rhodopsin-, Glutamate-, and Secretin-type GPCRs (*1*, *108*–*111*), substantial progress has been made to illuminate the signaling of Adhesion-GPCRs.

Coupling of a 7TM receptor to a specific G protein specifies the quality of downstream signaling. Thus, pathway-specific second messengers can be quantified under constitutive or agonist-induced activation (if an agonist is known) to identify the coupled G protein. Latrophilin 1 was the first Adhesion-GPCR for which specific binding and functional coupling to $G\alpha_0$ were demonstrated, which depended on the binding of α-LTX, an exogenous latrophilin 1 agonist derived from the venom of black widow spiders. Exposure to α-LTX correlated with an elevation of both cAMP and IP_3 (inositol 1,4,5-trisphosphate) (*12*) reminiscent of classical GPCR activation mechanisms (Fig. 3D). Recently, teneurin-2 (also known as Lasso) was identified as an endogenous ligand for latrophilin 1, which causes an increase of intracellular calcium concentration when applied to latrophilin 1–positive cells or hippocampal neurons (*57*). Teneurins are single transmembrane molecules, which could be copresented in cis by latrophilin-positive cells (*77*) or in trans on opposite cell membranes (*57*) to effect signaling (Fig. 3D). It is not clear whether teneurin-induced latrophilin signals are relayed through G proteins. Together, this example illustrates the potentially different cellular recognition capacities of Adhesion-GPCRs (Table 3).

A larger-scale screen for specific G protein coupling used assays that monitor cAMP and IP_3 accumulation in cells coexpressing Adhesion-GPCRs and individual Gα proteins ($G\alpha_{s,q,i,o,15,16}$). Using this strategy, 13 Adhesion-GPCRs failed to show increased basal receptor activity (*112*). However, EMR2 and GPR115 as well as GPR114 and GPR133 displayed constitutive coupling to $G\alpha_{15}$ or $G\alpha_s$, respectively (*112*, *113*). Whether these are the natural signal modes remains to be shown, because GPR114, GPR115, and GPR133 are functional orphans, and the presence of an unknown agonist in the assay could not be excluded.

Table 3. Intracellular signaling of Adhesion-GPCRs. The signaling interactions and functions for Adhesion-GPCRs are listed, along with a brief insight to the methods used to identify them. ADCY6, adenylyl cyclase 6; cAMP, adenosine 3′,5′-monophosphate; CHO, Chinese hamster ovary; DAAM, Dishevelled-associated activator of morphogenesis; ELMO1, engulfment and cell motility protein 1; ERK, extracellular signal–regulated kinase; GST, glutathione *S*-transferase; GTP-γ-S, guanosine 5′-*O*-(3′-triphosphate); IF, immunofluorescence; IHC, immunohistochemistry; IP, immunoprecipitation; IP_3, inositol 1,4,5-trisphosphate; LPAR, lysophosphatidic acid receptor; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PAI-1, plasminogen activator inhibitor-1; pAkt, phosphorylated Akt; PKC, protein kinase C; PLC, phospholipase C; qPCR, quantitative polymerase chain reaction; RhoGEF, Rho-specific guanine nucleotide exchange factor; SRE, serum response element; TCF, T cell factor; VEGF, vascular endothelial growth factor; WB, Western blot analysis; Y2H, yeast-two hybrid screen.

Family/receptor	Coupling/signaling shown for	Evidence/setting	References
		Family I (latrophilins)	
Latrophilin 1	Binding to $G\alpha_0$	Affinity chromatography	(*12*, *198*, *199*)
	Binding to $G\alpha_{q/11}$	Affinity chromatography	(*198*)
	↑ cAMP, ↑ IP_3	IP_3 accumulation assay, cAMP assay of latrophilin 1 overexpressing COS7 cells	(*12*)
	Stimulation of PLC	Stimulation of synaptosomes with α-LTX and PLC inhibitor	(*198*)
	↑ intracellular Ca^{2+},stimulation of PLC	Ca^{2+} imaging of NB2A cells overexpressing latrophilin 1 with mutant α-LTX^{N4C}, IF of PLC translocation	(*81*)
		Family II (EGF-TM7)	
CD97	↑ nonphosphorylated membrane-bound or cytosolic β-catenin, ↑ pAkt	WB	(*124*)
	RhoA-dependent binding to $Ga_{12/13}$; ↑ pERK, ↑ pAkt through LPAR1 signaling	SRE-luciferase reporter assay of COS7 cells transfected with CD97, RhoA, $G\alpha_{12}$, and RGS domain of p115 Rho-GEF, WB	(*126*)
	↑ pERK, ↑ Ki-67 through LPAR1 signaling	WB, IHC	(*127*)
EMR2	Binding to $G\alpha_{15}$	IP_3 accumulation assay of HEK 293 cells overexpressing EMR2 and individual G proteins	(*112*)
		Family III	
GPR124	Cdc42-dependent directional migration	Migration assay of GPR124-overexpressing bEND3 cells with dominant-negative Cdc42N17	(*162*)
		Family IV (CELSRs)	
CELSR1	Homophilic interaction: activation of Rho kinase	Co-IP, GST pull-down, IF colocalization, RhoGEF exchange assay	(*26*)
CELSR2, CELSR3	Homophilic interaction: ↑ intracellular Ca^{2+}	Ca^{2+} imaging of primary neurons overexpressing CELSR2 or CELSR3, inhibition by thapsigargin or U73122	(*200*)
		Family V	
GPR133	Binding to $G\alpha_s$	cAMP assay of COS7 cells overexpressing GPR133 and G proteins	(*113*)
GPR133	Binding to $G\alpha_s$	cAMP assay of HEK293 cells overexpressing GPR133 and G proteins	(*112*)
		Family VI	
GPR115	Binding to $G\alpha_{15}$	IP_3 accumulation assay of HEK293 cells overexpressing GPR115 and G proteins	(*112*)
		Family VII (BAIs)	
BAI1	Binding to the complex ELMO1/Dock180/Rac-1	Y2H, co-IP of LR73 cells overexpressing BAI1	(*61*)
	ELMO1-dependent activation of Rac-1	Rac-1 pull-down assay of CHO cells overexpressing BAI1 in combination with ELMO1 knockdown	(*52*)
BAI2	Stimulation of NFAT signaling, ↑ IP_3, binding to Ga_{16}	NFAT reporter assay of HEK293 overexpressing BAI2 or BAI2-CTF	(*95*)

continued on next page

Family/receptor	Coupling/signaling shown for	Evidence/setting	References
		Family VIII	
GPR56	Binding to $G\alpha_{q/11}$ and tetraspanins CD81 and CD9	Co-IP, mass spectrometry of HEK293 cells overexpressing GPR56, CD81 antibody–induced complex dissociation	(*90*)
	Stimulation of PAI-1, TCF, NF-κB response elements	Reporter assays in HEK293 cells overexpressing GPR56	(*201*)
	Stimulation of $G\alpha_{12/13}$ and Rho	SRE-luciferase reporter assay, RhoA pull-down, F-actin formation assay in HEK293 cells overexpressing GPR56	(*128*)
	Binding of $G\alpha_{12/13}$ and β-arrestin 2, ↑ RhoA-GTP, ↑ ubiquitination	WB, co-IP, RhoA pull-down, IF colocalization of HEK293 cells overexpressing GPR56 or GPR56ΔNTF	(*60*)
	Binding to $G\alpha_{12/13}$, ↑ RhoA-GTP	GTP-Rho pull-down blunted by dominant-negative $G\alpha_{13}$	(*62*)
	Activation of PKCα	VEGF assay of MC-1 cells overexpressing GPR56 CTF or ΔGPS with PKC inhibitors and dominant-negative PKCα; WB	(*138*)
GPR97	Binding to $G\alpha_o$	IP_3 accumulation assay of HEK293 cells overexpressing GPR97 and G proteins, aequorin assay, [^{35}S]GTP-γ-S binding	(*112*)
GPR114	Binding to $G\alpha_s$	cAMP assay in HEK293 cells overexpressing GPR114 and G proteins	(*112*)
GPR126	Binding to G protein(s)	Restoration of myelination by treatment of *gpr126*-deficient zebrafish mutants with forskolin	(*184*)
		Family IX	
VLGR1	Activation and redistribution of ADCY6	qPCR, IF colocalization	(*202*)

Adhesion-GPCR signaling through small guanosine triphosphatases

Apart from canonical G protein coupling, many GPCRs engage nonheterotrimeric G proteins with guanosine triphosphatase (GTPase) activity to convey signals into the cell (*114*) (Table 3). This interaction can be mediated through specific G proteins, GEFs (guanine nucleotide exchange factors), GDIs (guanine nucleotide dissociation inhibitor), and GAPs (GTPase-activating proteins) (*115*).

Mounting evidence shows that Adhesion-GPCRs also couple into pathways that use small GTPases, RhoGAPs (Rho GTPase-activating proteins), or kinases that modify the activity of those factors. Indirect interplay between BAI1 and Rac-1, a small GTPase of the Rho family, is observed during phagocytic engulfment of apoptotic cells (*61*). Upon recognition of phosphatidylserine (the endogenous BAI1 ligand) on apoptotic cells, BAI1 promotes the recruitment of a two-component GEF consisting of ELMO1 (engulfment and cell motility protein 1) and Dock180 (dedicator of cytokinesis). In complex, ELMO1-Dock180 functions as a GEF for Rac-1, which promotes actin polymerization at the protruding edges of engulfing or migrating cells (*116*). In a similar fashion to phosphatidylserine, surface LPS of Gram-negative bacteria is also recognized by macrophage-resident BAI1, resulting in the recruitment of the ELMO1/Dock180/Rac-1 complex, terminating bacterial phagocytosis (*52*) (Fig. 3D).

Whereas BAI1 directly recruits small GTPases, several other Adhesion-GPCRs cooperate with nonheterotrimeric G proteins indirectly to regulate cell polarity and migration. Therefore, there collectively appears to be a common physiological context for functional Adhesion-GPCR requirements: the control of cell motility by polarized regulation of the cytoskeleton at cell-cell junctions. For example, Flamingo and CELSR1 (invertebrate and vertebrate homologs of family IV CELSRs, respectively) form a core component of the PCP pathway (Fig. 2) (*117*). First recognized in *Drosophila*, this pathway controls the patterned polarization of cells in the plane of an epithelium or tissue and is essential for shaping planar and tubular epithelia (*118*–*120*). Both in invertebrates and vertebrates, Flamingo/CELSR1 physically associates with Frizzled (*26*, *59*), another GPCR upstream of a well-defined cascade that regulates the contractility of the actomyosin cytoskeleton mediated by the small GTPase RhoA (*121*). By means of homotypic interaction of its NTF at intercellular contacts of neighboring cells (Fig. 5B), Flamingo/CELSR1 directs different amounts of the Frizzled apparatus to opposite sides of cell boundaries, thereby relaying polarity information across the entire extent of an epithelium (*26*, *59*, *122*, *123*). This results in local contraction of actomyosin in each cell within the polarized tissue to coordinate cell motility in developmental bulk movements such as convergent extension, intercalation, epiboly, and neural tube closure. Similar to Flamingo/CELSR1, although their downstream effectors are still unclear, latrophilins are also involved in cell polarity and directed migration. In a *C. elegans* mutant that lacks *lat-1*, severe defects in the anterior-posterior orientation of embryonic cell divisions are apparent. In addition, the migration of seam cells, epidermoblasts required to form the hull of the worm and to initiate epidermal morphogenesis, is severely impaired (*25*). It will be interesting to investigate whether LAT-1 exerts polarized control over the cytoskeleton similar to Flamingo/CELSR1.

Further evidence suggests that Adhesion-GPCRs are key factors in controlling the behavior of cell-cell contacts, such as adherens junctions, through negotiating the local activity of small GTPases. CD97 is located at adherens junctions between enterocytes, and elevated CD97 content correlates with increased migration and invasive capacity of colorectal tumor cells (*68*, *124*). Transgenic mice overexpressing CD97 exhibited strengthened lateral enterocytic cell-cell contacts, whereas removal of CD97 in a knockout strain caused the reverse effect (*124*). CD97 activity is also a founding factor in the dedifferentiation and invasiveness of other epithelial cancer cell lineages, including thyroid and prostate cancer (*125*, *126*). CD97 heterodimerizes with LPAR1 (lysophosphatidic acid receptor 1) in prostate cancer cells; LPAR1 controls the invasiveness of these cells in a RhoA-dependent manner in response to chemoattractants, whereas CD97 signals through $G\alpha_{12/13}$ in this model. The mutual interaction between CD97 and LPAR1 results in increased motility of prostate adenocarcinoma cells, and the abundance of CD97 correlated positively with this (*126*). Similar results were obtained in a mouse model for thyroid cancer progression (*127*). In another example, GPR56 directly couples to $G\alpha_{12/13}$ (*60*, *62*) and controls the migration of neural progenitor cells in a RhoA-dependent manner, whereby GPR56 protein abundance is inversely correlated with the migration

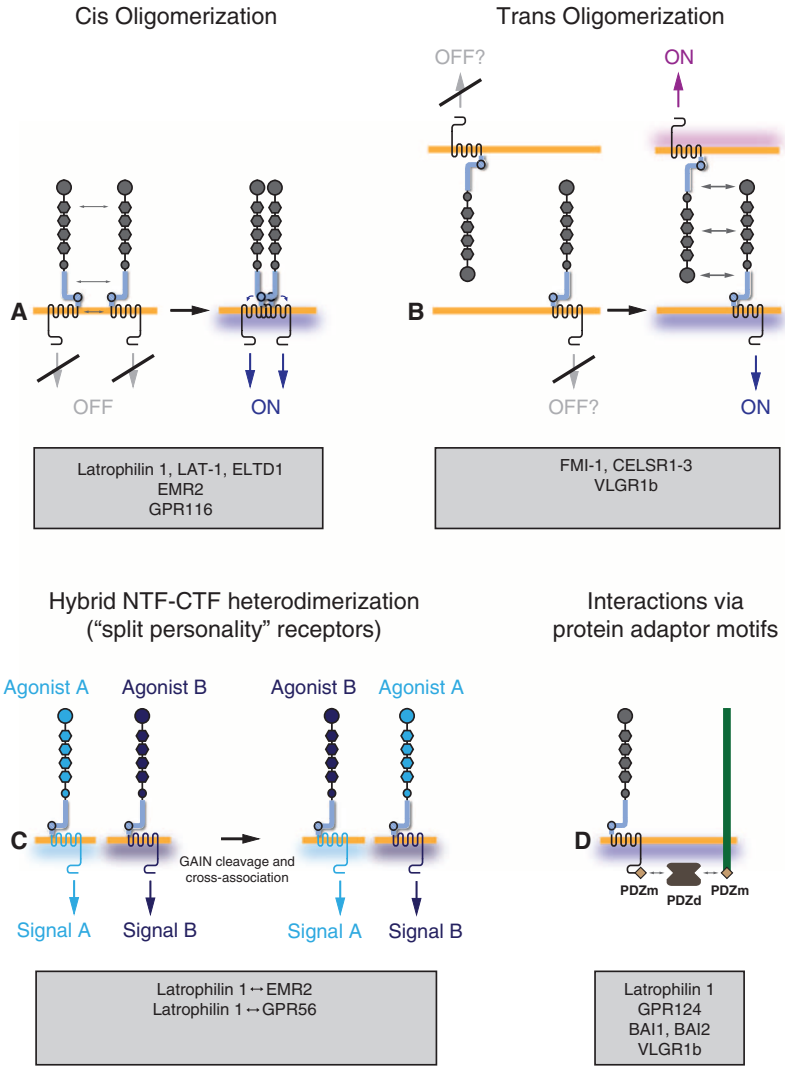

Fig. 5. Molecular interactions of Adhesion-GPCRs. (**A**) Cis oligomerization can lead to cross-activation of receptors, likely through an interaction of the GAIN and 7TM domains (compare with signaling model in Fig. 4C) (*77*, *81*, *143*–*145*). (**B**) Other Adhesion-GPCRs engage across cell borders through their NTFs forming intercellular complexes. This could be a permissive or instructive condition required for signaling, or alternatively only play a role in adhesion (*24*, *26*, *58*, *59*, *147*, *214*). For clarity, only homophilic interactions are depicted, but heterophilic interactions are equally feasible. (**C**) The split personality receptor model suggests that NTFs and CTFs of different Adhesion-GPCRs can be interchanged after GAIN domain cleavage and form functional hybrid receptors with inverted ligand or signaling specificity, respectively (*82*, *83*). (**D**) Assembly of multicomponent protein networks is permitted through the presence of protein adaptors such as a PDZ-binding motif (PDZm) at the C terminus of the intracellular tails of several Adhesion-GPCRs enabling the interaction with PDZ domain (PDZd)–containing proteins (*23*, *148*, *150*, *151*, *154*, *155*).

competence of these cells (*128*). It will be crucial to determine whether these Adhesion-GPCR–mediated effects on cell motility depend on their activation by extracellular agonists, or whether homotypic or heterotypic interactions at the level of the NTF and 7TM domains suffice to control the biological activity of Adhesion-GPCRs.

Last, similar to NTF-specific activities, results from *Drosophila* studies indicate that the CTF of Adhesion-GPCRs could function independently from the NTF. Fly mutants lacking Flamingo show defects in controlling dendrite arborization resulting in overgrown dendritic fields. This defect was rescued by Flamingo versions lacking all adhesion domains in the NTF but not variants without the ICD (*129*). Comparably, studies on the *C. elegans* Flamingo homolog FMI-1 revealed that FMI-1–dependent navigation of pioneer axons, apart from the exclusive role of the FMI-1 NTF in follower axon migration, requires the presence of the CTF (*33*). Here, the ICD was essential to rescue pioneer axon navigation in a cell-autonomous fashion, but the 7TM domain was dispensable, demonstrating that Adhesion-GPCRs might relay intracellular signals differently than other GPCR classes (Fig. 3E) (*33*).

Phosphorylation of the CTF

The activity of canonical GPCRs is tightly controlled by GPCR kinases (GRKs), which phosphorylate activated receptors and promote high-affinity binding of arrestins, precluding further G protein coupling and inducing internalization of the receptor through a mechanism that involves ubiquitination (*1*). Whereas the latter mechanisms await concrete investigations with respect to Adhesion-GPCRs, there is mounting evidence that Adhesion-GPCRs might be regulated by phosphorylation (Table 4). GRKs phosphorylate GPCRs usually at serine, threonine, or tyrosine residues contained in the third intracellular loop of the 7TM domain and the ICD. Bioinformatic analyses predict phosphorylation for two-thirds of the Adhesion-GPCRs (http://www.phosphosite.org; http://www.phosphonet.ca). Recent phosphoproteomic screens have confirmed phosphorylation mainly in the C-terminal tail for the vast majority of Adhesion-GPCRs, but sites located in the NTF have also been identified through these large-scale efforts (Table 4) (*130*–*135*). Whether these phospho-ectodomains represent soluble or membrane-bound NTFs is currently unclear, and associating functional consequences to NTF phosphorylation could be a promising topic of future Adhesion-GPCR studies. Phosphorylation of GPR116, GPR112, and GPR128 also revealed signaling functions of family members that are not in the current spotlight of Adhesion-GPCR research (Table 4) (*130*, *136*, *137*).

Phosphorylation of Adhesion-GPCRs that couple to G proteins such as latrophilin 1 and GPR56 is demonstrated (*60*, *84*, *130*, *134*). The CTF of latrophilin 1 is phosphorylated in rat brain but not in cultured cells, suggesting that phosphorylation occurs in vivo, perhaps as a result of physiological brain function that cannot be recapitulated in vitro (*84*). Ectopic presence of CD97 and GPR56 on tumor cells is efficiently decreased by phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C (PKC) (*90*, *125*). Phosphorylation by PKC, which also activates GRKs, is involved in the rapid internalization and desensitization of canonical GPCRs (*1*). Curiously, despite the rapid removal of GPR56 from the cell surface upon PMA treatment, no phosphorylation of GPR56 could be detected (*90*). A GPR56 with a truncated NTF signals through PKCα (*138*), indicating that PKC activation downstream of Adhesion-GPCRs may mediate receptor desensitization through an unknown feedback mechanism.

Table 4. Phosphorylation of Adhesion-GPCRs. The phosphorylation sites for Adhesion-GPCRs are listed. These were identified in proteomic screens and require validation by further experimental work.

Family/receptor	Phosphorylation site	Location	References
	Family I (latrophilins)		
Latrophilin 1	$Ser^{1032, 1033, 1040, 1041}$	7TM	(*134*)
	Tyr^{1451}	ICD	(*134*)
	Tyr^{1385}	ICD	(*135*)
	Tyr^{1440}	ICD	(*203*)
Latrophilin 2	$Tyr^{1421, 1406}$	ICD	(*135*)
Latrophilin 3	Thr^{1445}	ICD	(*131*)
	$Thr^{455, 456}$	ECD	(*204*)
	Thr^{610}	ECD	(*205*)
	$Tyr^{1411, 1412}$	ICD	(*206*)
	Tyr^{170}	ECD	(*132*)
	Family II (EGF-TM7)		
CD97	Ser^{815}	ICD	(*207*)
	Ser^{818}	ICD	(*208*)
	Ser^{831}	ICD	(*133*, *208*)
	Ser^{833}	ICD	(*133*)
	Thr^{820}	ICD	(*134*)
	Family III		
GPR124	Ser^{253}	ECD	(*209*)
	Family IV (CELSRs)		
CELSR1	$Ser^{2761, 2764}$	ICD	(*133*)
CELSR2	$Ser^{2642, 2648, 2868, 2869, 2871}$, Tyr^{2650}	ICD	(*133*)
CELSR3	Ser^{805}, Thr^{806}, Tyr^{821}	ECD	(*133*)
	Family VI		
GPR116	Thr^{221}	ECD	(*136*)
	Family VII (BAIs)		
BAI2	Tyr^{267}	ECD	(*132*)
BAI3	Ser^{619}	ECD	(*133*)
	Family VIII		
GPR56	$Ser^{689, 690, 691}$	ICD	(*130*)
GPR64	Ser^{1007}	ICD	(*210*)
	Ser^{1010}	ICD	(*130*, *133*, *206*)
GPR112	Thr^{2678}	ECD	(*137*)
GPR126	$Ser^{1160, 1162, 1164, 1165, 1168}$	ICD	(*133*)
	$Tyr^{1171, 1172, 1195, 1196}$	ICD	(*211*)
GPR128	Tyr^{760}	ICD	(*130*)

Interplay of NTF and CTF—Functioning Apart Together

Recent studies suggest that the versatility of Adhesion-GPCRs' two-component structure (Fig. 2) is mirrored by their signal spectrum, demonstrating that the NTF and CTF can serve independent functions related to adhesion and signaling, but also interact with each other to tune signaling (compare to Fig. 3).

Tethered interactions

GPR56 is a prime example for this interplay. Pioneering studies measured the signaling capacity of full-length and CTF-only GPR56 variants, which behaved differently in several experimental systems (*60*, *138*). The presence of full-length GPR56 in melanoma cell lines resulted in inhibition of vascular endothelial growth factor (VEGF) production, angiogenesis, and tumor growth. These effects were dependent on the presence of a serine/threonine/proline-rich segment within the NTF, which contains the N-terminal part of the subdomain A of the GPR56 GAIN domain (helices 1 to 4) and a signaling route involving PKCα. In contrast, in the same assays, the CTF-only variant of GPR56 further increased the secretion of VEGF through enhanced PKCα signaling. This provided evidence that an NTF-CTF interplay exists in GPR56. Further, the enhanced activity in the absence of the NTF implies that in the full-length receptor, the NTF might function as a repressor (an inverse agonist) of the constitutive activity of the CTF (Fig. 4B), providing parallels with canonical glycoprotein hormone-binding GPCRs like TSHR (*139*). This is supported by findings that CTF-only variants of GPR56 and CD97 display a marked increase of reporter activity compared to the full-length receptor (*60*, *126*). Accordingly, naturally occurring splice variants of GPR56 with a shorter NTF further increase reporter gene activity compared to wild-type GPR56 (*140*). Similarly, a CTF-only variant of BAI2 results in a potently increased reporter activity compared with a full-length BAI2 receptor (*95*).

The previous examples may expose a potentially general mode of Adhesion-GPCR signaling. This model could serve as a blueprint to grasp effects of naturally occurring receptor variants that might have lost NTF inhibition, such as by inherited or acquired somatic mutations within Adhesion-GPCR genes resulting in CTF-only splice variants. These mutants could turn out to be constitutively active, if the NTF (or its suppressive capacity) was lost, leaving the CTF activity free-running (Fig. 4B), and promote angiogenesis, tumor growth, and invasion under pathological conditions. It is important to recognize that some Adhesion-GPCRs, such as latrophilins and BAIs, have indeed already been identified as a class of highly mutated genes in human cancers, suggesting that they suppress malignant activity under wild-type conditions (*88*). Further, the GAIN domain as a natural interface between the NTF and CTF appears to be a hotspot for Adhesion-GPCR mutations in neoplasias (*38*).

The possibility that NTF truncation or depletion may enhance signaling by affecting protein amount of the receptors needs to be carefully investigated. Contrary to the tethered inverse agonist model, basal activities of an NTF of human GPR133 replaced by the N terminus of bovine rhodopsin are lower compared to those of the full-length receptor (Fig. 4C), challenging the notion that suppression of CTF signaling by the NTF is a common mechanism present in all Adhesion-GPCRs (*113*), and further supporting the notion that the CTF might be able to signal independently of the NTF (Fig. 3E). Primarily, the working model poses a biologically unfavorable situation, because the quenched but constitutive CTF would require constant surveillance by the presence of the NTF or other mechanisms. It can be further speculated that, once NTF removal unleashes CTF signaling, termination of receptor activity would require reliable modes of CTF inactivation or removal, such as by internalization.

Receptor activity modulation by the GAIN domain

The suppressive effect of NTF presence on CTF activity, together with the position of the GAIN domain, renders it likely that the GAIN domain functions as an intramolecular coordinator of divergent NTF and CTF signals, but detailed studies on a potential GAIN-7TM domain interface (for example, by mutagenesis mapping) are necessary to corroborate this model. Studies in *C. elegans* and zebrafish have started to dissect NTF and CTF interplay in more detail. In nematodes, LAT-1 is required for fertility and embryonic development by maintaining fidelity of tissue polarity (*25*, *77*). Complementation of a *lat-1* null mutant with truncated receptor transgenes revealed that LAT-1 relays at least two different signals by

using different parts of the receptor molecule (*77*). One activity requires the presence of the CTF and probably operates in a fashion that is comparable to classical GPCRs (7TM-dependent signaling) during development (Fig. 3, D to F). The other activity, however, is independent of the CTF and only requires the NTF (7TM-independent signaling) for restoring fertility in *lat-1* mutant animals (Fig. 3, A to C). The GPS motif located within the GAIN domain, but not its proteolysis site, was required for both activities. Further, the 7TM-dependent activity of LAT-1 was highly sensitive to GPS motif sequence changes, indicating that GAIN and 7TM domains physically interact and that the GAIN domain functions as an endogenous ligand for the CTF (*77*) (Fig. 4C).

Supramolecular Interactions—Teaming Up for Action

Receptor oligomerization

Assembly of GPCRs into homo- and heterodimers as well as higher-order oligomers can change agonist recognition, signaling, and trafficking of the participating receptors through allosteric mechanisms reported for many GPCRs (*1*, *141*, *142*). Evidence for dimerization of Adhesion-GPCRs in cis (Fig. 5A) has been obtained for latrophilins, ELTD1, EMR2, and GPR116 (*77*, *81*, *143–145*). Most of these studies rely on transient overexpression of the receptors in conjunction with biochemical and fluorescence resonance energy transfer techniques confirming dimer formation and are supported by findings in primary cells or in vivo models. For example, the molecular size of GPR116 in rat lung tissue suggests its homodimerization in vivo (*143*). Whether dimerization is exclusively mediated by the 7TM region and is independent of GPS autoproteolysis, as shown for EMR2 (*145*), needs to be clarified. EMR2 also forms heterodimers with the related family II members EMR3 and CD97, and GPR56-CTF has been detected in immunoprecipitates of latrophilin 1–NTF from rat forebrain (*83*). Homophilic trans-interactions, a requirement for PCP signaling modulation (*26*, *59*, *146*), occur between the NTFs of CELSR homologs (Fig. 5B) and potentially exist between VLGR1, which is speculated to contribute to the ankle link structure present between the base of stereocilia of inner ear hair cells (*24*, *147*). VLGR1b might also engage with its NTF in trans-interactions with other transmembrane proteins, such as Usherin (USH2A) (*147*).

Functional complementation, such as transactivation, of two defective or chimeric receptors that restores receptor functionality is an elegant technique to show that heterodimerization is required for signal transduction. The chimeric receptor latrophilin 1–NTF:neurexin-CTF binds the latrophilin agonist α-LTX but cannot engage with G proteins, whereas EMR2-NTF:latrophilin 1–CTF does not bind α-LTX but can signal through G proteins. Upon reassembly after GPS proteolysis, the cross-complex of latrophilin 1 derived from the complementary chimeric fragments was functionally active, indicating that Adhesion-GPCR hybrid heterodimers may couple NTF interactions of one receptor molecule to CTF signaling of an adjacent receptor molecule, a hypothesis termed split personality receptor model (*83*), discussed earlier (Fig. 5C). One immanent assumption of this model requires that GAIN cleavage is absolutely essential for the hybrid interaction to occur. A follow-up analysis scrutinizing the split personality receptor model in *C. elegans* also used intermolecular complementation experiments of a *lat-1* null mutant by transgenes encoding nonfunctional variants of the latrophilin homolog LAT-1 (*77*). Similar to the split personality scenario (*83*), combination of an NTF-TM1 receptor version and a dysfunctional full-length version—both incapable of restoring wild-type receptor activity on their own—rescued the null phenotype when coexpressed. In contrast to previous results (*83*), GPS cleavage was not required to effect the complementation because proteolysis-deficient versions of the combinations returned similar results in complementing the null phenotype. Biochemical analysis of LAT-1 helped in refining an alternative model for Adhesion-GPCR oligomerization by showing that the NTF of LAT-1 can homodimerize. This refined model suggests that homodimerization of Adhesion-GPCRs, possibly via adhesion domains, results in cross-activation of pairs of receptors. In this model, the GPS motif of the GAIN domain interacts with the 7TM domain of the partner receptors to initiate signaling (Fig. 5A), rather than effected by an exchange and hybridization of heterogeneric NTF::CTF combinations into functional signaling complexes after GPS cleavage (Fig. 5C) (*77*).

Notably, Adhesion-GPCRs can also dimerize with other transmembrane molecules including unrelated GPCRs (Figs. 3, A and D, and 5, A and B). CD97 forms heterodimers with the canonical GPCR LPAR1 in tumor cells, resulting in enhanced invasiveness (*126*). CD97-LPAR1 heterodimerization amplified ligation-dependent activation of LPAR1. CD97 by itself did not require ligation to potentiate LPAR signaling to the downstream effector RhoA. Moreover, GPR56 forms complexes with the tetraspanins CD9 and CD81, which mediate coupling to $G\alpha_{q/11}$ (*90*).

Adhesion-GPCR interactions through PDZ-binding motifs

Heterophilic oligomers between Adhesion-GPCRs and other proteins cannot only be formed extracellularly or at the level of the 7TM domain. Also, several intracellular adaptor motifs exist to arrange proteins into multicomponent supramolecular complexes (Fig. 5D). The latrophilins, CD97, GPR123, GPR124, the BAIs, and VLGR1 contain at their C terminus a PDZ (PSD-95, discs large, ZO-1)–binding motif. This motif can interact with a PDZ domain, a structural fold of 80 to 90 amino acids in scaffold proteins, which anchors transmembrane receptors via their PDZ-binding motifs to the cytoskeleton and thereby forms signaling complexes. For several Adhesion-GPCRs, direct interaction with scaffold proteins with their PDZ-binding motif has been demonstrated (Table 5).

VLGR1b is part of a complex protein network of hair cell and photoreceptors (*147*). This Usher protein interactome in both the inner ear and the retina is organized around harmonin, a scaffold protein with three PDZ domains, which binds to VLGR1b at its PDZ-binding motif (*23*). The PDZ domain structure of harmonin is highly homologous to that of whirlin, which also directly interacts with VLGR1b (*148*). Supramolecular Usher protein networks containing VLGR1 contribute in photoreceptors to the regulation of cargo transfer and in inner ear hair cells to sterocilia development and function, as well as synaptic maturation (*24*, *147*, *149*). Dysfunction of any individual interactome member leads to failure of the Usher protein network and manifests by clinical symptoms characteristic of Usher disease (*24*).

BAI1 interacts with BAIAPs (BAI-associated proteins, also BAPs), members of the MAGUK (membrane-associated guanylate kinase homolog) family containing five PDZ domains. MAGUKs play a pivotal role in localization of proteins to cellular junctions. Indeed, BAI1 and BAP1 colocalize in cell junctions of cotransfected cells. The PDZ-binding motif QTEV (Gln-Thr-Glu-Val) in BAI1 is indispensable for the interaction with BAP1 (*150*). BAI2 interacts with glutaminase-interacting protein (GIP), a 13.7-kD key PDZ domain–containing scaffold protein in the mammalian brain (*151*). In family II (EGF-TM7), only CD97 contains a PDZ-binding motif. CD97 is the single family member that is not restricted to immune cells and has been found at cell contacts of epithelial cells (*124*). Interaction with PDZ domain–containing proteins probably mediates CD97 localization in the complex protein network of adherens junctions. Mammalian latrophilins interact with the PDZ domain of ProSAP (proline-rich synapse-associated protein) [also known as SSTRIP (somatostatin receptor–interacting protein) or Shank] family members at their C terminus (*152–154*). These proline-rich postsynaptic scaffold proteins are likely involved in the synaptic

Table 5. Intracellular binding partners of Adhesion-GPCRs. The structural interactions between Adhesion-GPCRs and proteins and their functional outcomes are outlined. Proteins with synonymous names are noted parenthetically. AP, adaptor protein; BiFC, bimolecular fluorescent complementation; CD, circular dichroism; DLG1, discs large homolog 1; DTHL, Asp-Thr-His-Leu; ETTV, Glu-Thr-Thr-Val; GIP3, glutaminase-interacting protein 3; Gogo, Golden goal; GST, glutathione-S-transferase; MAGI1, membrane-associated guanylate kinase1; ND, not determined; NMR, nuclear magnetic resonance; PAHX-AP1, phytanoyl-CoA α-hydroxylase–associated protein 1; PLA, proximity ligation assay; QTEV, Gln-Thr-Glu-Val; TAX1BP3, Tax1-binding protein 3; TIP-1, Tax-interacting protein-1; TRIP8b, tetratricopeptide repeat–containing Rab8b-interacting protein; VTSL, Val-Thr-Ser-Leu.

Family/receptor	Interacting region of the Adhesion-GPCR	Interacting protein	Interacting domain of the interacting protein	Function of the interacting protein	Method	References
			Family I (latrophilins)			
Latrophilin 1	Cytoplasmic tail, PDZ-binding motif VTSL?	Shank	PDZ?	Postsynaptic scaffold	Y2H	(*154*)
	Cytoplasmic tail	TRIP8b	ND	Brain-specific hydrophilic cytosolic protein that binds clathrin and AP	Y2H, GST pull-down, mass spectrometry	(*212*, *213*)
			Family III			
GPR124	PDZ-binding motif ETTV	DLG1	PDZ	Junctional scaffold	Y2H, GST pull-down, IF colocalization	(*155*)
			Family IV (CELSRs)			
FMI-1	ECD or 7TM	Gogo, indirect physical interaction	Ectodomain	Synaptic layer targeting of R-photoreceptors	IF colocalization; failed: PLA, BiFC, IP	(*214*)
			Family VII (BAIs)			
BAI1	PDZ-binding motif QTEV	BAIAP1 (BAP1, MAGI1)	ND	Scaffold in cell junctions	Y2H, GST pull-down, IF colocalization	(*150*)
	Proline-rich region of cytoplasmic tail	BAIAP2 (BAP2 or IRSp53)	SH3	Adapter that links membrane-bound small G to cytoplasmic effector proteins	Y2H, GST pull-down, IF colocalization	(*215*)
	Cytoplasmic tail	BAIAP3 (BAP3)	ND	ND	Y2H, GST pull-down, IF colocalization	(*150*)
	Cytoplasmic tail	PAHX-AP1 (BAP4)	ND	ND	Y2H, GST pull-down, IF colocalization	(*216*)
	α-Helical region of cytoplasmic tail	ELMO1	ND	Engulfment of apoptotic cells, cell migration	Y2H, GST pull-down, co-IP	(*61*)
BAI2	PDZ-binding motif QTEV?	GIP3 (TIP1, TAX1BP3)	PDZ?	ND	Y2H, CD, fluorescence, and NMR spectroscopy	(*151*)
			Family IX			
VLGR1	PDZ-binding motif DTHL	Harmonin	PDZ	Scaffold of the Usher protein interactome	Y2H, GST pull-down, co-IP	(*23*)
	PDZ-binding motif DTHL	Whirlin	PDZ	Scaffold involved in pre- and/or postsynaptic photoreceptor and hair cell receptor signaling	Y2H, GST pull-down, co-IP, IF colocalization	(*148*)

targeting of latrophilins. Finally, GPR124 binds to DLG1 (discs large homolog 1) in endothelial cells during tumor angiogenesis and neoangiogenesis (*155*). Notably, similar cytoskeletal associations have been described for Rhodopsin-type GPCRs (*156*).

Perspectives

Adhesion-GPCRs are a neglected receptor family with many opportunities to unlock yet uncharted mechanisms of biological and physiological reach. Since the initial description of the first Adhesion-GPCR structures in the mid-1990s, research on Adhesion-GPCRs has come a long way transcending biochemical, genetic, and pharmacological investigations. With the application of structural biological acumen to salient problems of Adhesion-GPCR protein architecture and the advent of assays to discern signaling routes, in which these receptors are involved, piecemeal progress on the missing links between receptor biology and physiological relevance of receptor functions has been made.

Adhesion-GPCRs combine key characteristics of canonical GPCRs at the structural (7TM domain), signaling (G protein coupling, phosphorylation), and interaction (oligomerization, PDZ-binding motif) levels with class-specific properties (extended modular NTF, GAIN domain, GPS autoproteolysis) that define them as a separate class of GPCRs, which is in line with the GRAFS classification that is based on structural comparison of the 7TM regions. The elucidation of the GAIN domain structure and its

biochemical potency to proteolyze and reattach Adhesion-GPCR fragments has been a long-sought achievement. The GAIN domain appears as a common structural denominator by virtue of which Adhesion-GPCRs can be reliably identified. Future work will need to concentrate on the functional spectrum that is laid down in the GAIN domain structure. It has already become clear that beyond its proteolytic duty, the domain also serves a key role in mediating the interplay of the two principal Adhesion-GPCR components: the NTF and the CTF.

Adhesion-GPCR NTFs are hallmark signs of the receptor class. They assume very long sizes and accommodate numerous adhesion domains. This structural mosaicism generates a broad combinatorial repertoire for tissue-specific interactions, which is even enlarged by mechanisms of pre- and post-translational modifications including alternative splicing and glycosylation.

Interactions with cellular or matrix partners are thought to initiate signaling through Adhesion-GPCRs. Although disputed for some time, increasing evidence suggests that the CTF of Adhesion-GPCRs can use GPCR-mediated signaling pathways transducing the extracellular signal to intracellular messengers such as G proteins and small GTPases. The centerpieces of these interactions are the 7TM domains and the ICDs of Adhesion-GPCRs. The presence of ways to modify CTF signals, such as phosphorylation, further supports the notion that Adhesion-GPCRs engage classical second messenger pathways.

Adhesion-GPCRs harbor additional faculty to mediate signals through their NTFs, as a separately secreted or membrane-anchored form independently from the CTF. Most intriguingly, however, in several Adhesion-GPCRs, CTF-mediated signals appear to be controlled by the presence of the NTF, suggesting that a direct interaction between both fragments exists, perhaps relayed through the GAIN domain. This provides an exciting explanation for why the adhesion and signaling functions of Adhesion-GPCRs are united within single genetic units and have been evolutionarily conserved through hundreds of millions of years.

Future work on Adhesion-GPCRs faces daunting challenges. It is still unclear how Adhesion-GPCRs are activated on a molecular level and whether they perceive stimuli of common modality despite their highly different domain outfits and expression profile. Answering this question is essential to pair the emerging structural and pharmacological properties they share with their biological and physiological functions. This quest to identify a general activation principle as a common functional denominator in Adhesion-GPCRs is aided both by initiatives to deorphanize the large remainder of class members, which lack information on specific and productive ligand-agonist interactions, and by continued efforts to link them with intracellular signaling modules. Once input and output routes of individual Adhesion-GPCRs are known, their pharmacological properties such as the kinetics and dynamics of receptor activation can be studied in greater detail.

Because the clinical context of Adhesion-GPCR function remains largely unknown to date, studies of pharmacological intervention are currently very limited. Hence, future studies will evaluate the clinical significance of the entire receptor class. They will also reveal the extent that soluble NTFs exist and have pathological relevance. The excellent pharmacological tractability of other GPCR classes and their successful clinical applications inspire hope that progress in understanding the function of Adhesion-GPCRs will return equally potent strategies for treating maladies associated with their dysfunction.

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