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REVIEW

Hunting for the function of orphan GPCRs – beyond the search for the endogenous ligand

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Seven transmembrane-spanning proteins (7TM), also called GPCRs, are among the most versatile and evolutionary successful protein families. Out of the 400 non-odourant members identified in the human genome, approximately 100 remain orphans that have not been matched with an endogenous ligand. Apart from the classical deorphanization strategies, several alternative strategies provided recent new insights into the function of these proteins, which hold promise for high therapeutic potential. These alternative strategies consist of the phenotypical characterization of organisms silenced or overexpressing orphan 7TM proteins, the search for constitutive receptor activity and formation of protein complexes including 7TM proteins as well as the development of synthetic, surrogate ligands. Taken together, a variety of ligand-independent functions can be attributed to orphan 7TM proteins that range from constitutive activity to complex formation with other proteins and include 'true' orphans for which no ligand exist and 'conditional' orphans that behave like orphans in the absence of ligand and as non-orphans in the presence of ligand.

LINKED ARTICLES

This article is part of a themed section on 5th BPS Focused Meeting on Cell Signalling. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-13

Abbreviations

7TM, seven transmembrane; AgRP, agouti-related protein; AMPK, AMP-activated protein kinase; CRC, colorectal cancer; CREB, cAMP response element-binding protein; ES, Ewing sarcoma; FFA, free fatty acid; GnRH, gonadotropin-releasing-hormone receptor; HCMV, human cytomegalovirus; LGR, leucine-rich repeat containing GPCR; mGluR, metabotropic glutamate receptor; Mrg, MAS-related GPCR; MSN, medium spiny neurons; Nogo, neurite outgrowth inhibitor; S1P, sphingosine 1-phosphate; SpD, surfactant protein D; TG, transglutaminase; TNBC, triple-negative breast cancer

Tables of Links

TARGETS		
GPCRs[a]	GPR21	GPR126
CCR5	GPR22	GPR161
CXCR4	GPR26	Melanocortin MC_4 receptor
Dopamine D_1 receptor	GPR27	Melatonin MT_1 receptor
Dopamine D_2 receptor	GPR34	$mGlu_6$, metabotropic glutamate receptor
FFA1, free fatty acid receptor	GPR37	MRGPRD
$GABA_{B1}$ receptor	GPR48/LGR4	MRGPRE
$GABA_{B2}$ receptor	GPR50	Taste T1R receptors (TAS1R1)
Ghrelin receptor	GPR52	**Ion channels**[b]
Glucocorticoid receptor	GPR64	TRPM1
GnRH receptor	GPR82	**Enzymes**[c]
GPR3	GPR83	AMPK, AMP-activated protein kinase
GPRC5A	GPR85	MMP1
GPRC5B	GPR88	Rac1
GPR6	GPR97	Fyn
GPR12	GPR110	GRK2, GPCR kinase
GPR15	GPR116	
GPR20	GPR124	

LIGANDS
AgRP, agouti-related protein
Amyloid β peptide
cAMP
β-Catenin
CGP7930
Cocaine
Dopamine
Leptin
LTC_4
LTD_4
Morphine
Prosaptide
S1P, sphingosine 1-phosphate

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c]Alexander *et al.*, 2013a,b,c).

Introduction

The seven transmembrane (7TM) domain GPCRs constitute the largest membrane receptor family. These proteins respond to a wide variety of extracellular molecules and play a crucial role in cell-to-cell communication by transmitting extracellular signals into cells (Rosenbaum *et al.*, 2009). Based on sequence homology, different receptor subfamilies have been defined: rhodopsin (class A), secretin, adhesion (class B), glutamate (class C), frizzled receptors and other 7TM proteins. In contrast to the latter group, all others are considered to be G-protein coupled. The involvement of GPCRs in a variety of physiological and pathophysiological processes makes this class of proteins the most common target of pharmaceutical drugs (Drews, 2000). Genome sequencing projects indicated that approximately 400 sequences belong to the non-odourant GPCR family in the human genome (Joost and Methner, 2002; Fredriksson *et al.*, 2003; Vassilatis *et al.*, 2003). Most of them have been matched with known ligands using different strategies. However, in spite of the extensive and long-standing efforts of academic and industrial research to pair 7TM proteins to potential ligands, 91 non-odourant receptors still remain orphans and another 37 are awaiting further input to be considered as deorphanized, according to IUPHAR (Davenport *et al.*, 2013). Deorphanization needs, as a minimal requirement, that two or more refereed papers from independent research groups should demonstrate activity of the ligand at the receptor, with a potency that is consistent with a physiological function. In some cases, although two independent groups have reported a pairing, others have failed to reproduce this finding and thus the deorphanization process requires further validation (Davenport *et al.*, 2013).

Although deorphanization still remains an important step towards the identification of the function of orphan 7TM proteins, other alternative strategies have become equally important over the last years to provide new insights into the function of orphan 7TM proteins (Levoye and Jockers, 2008). Among these strategies are the phenotypical characterization of animal models with modified expression of 7TM proteins of interest (overexpression or silencing), the characterization of constitutive receptor activity, the association of 7TM proteins with other proteins such as GPCRs, transporters or enzymes in heteromeric protein complexes and the identification of synthetic, surrogate ligands (Figure 1). The most recent advances in the identification of the function of 7TM proteins, using alternative strategies, will be the focus of this article.

Pathophysiological functions of orphan 7TM proteins

Because orphan 7TM proteins represent a potential resource for future drug development, various approaches, including transgenic and gene knockout approaches in mice, have been used to decipher their biological roles and their involvement in different pathophysiological conditions such as cancer, metabolism, neurodegenerative disorders and energy metabolism diseases (see Tables 1–3).

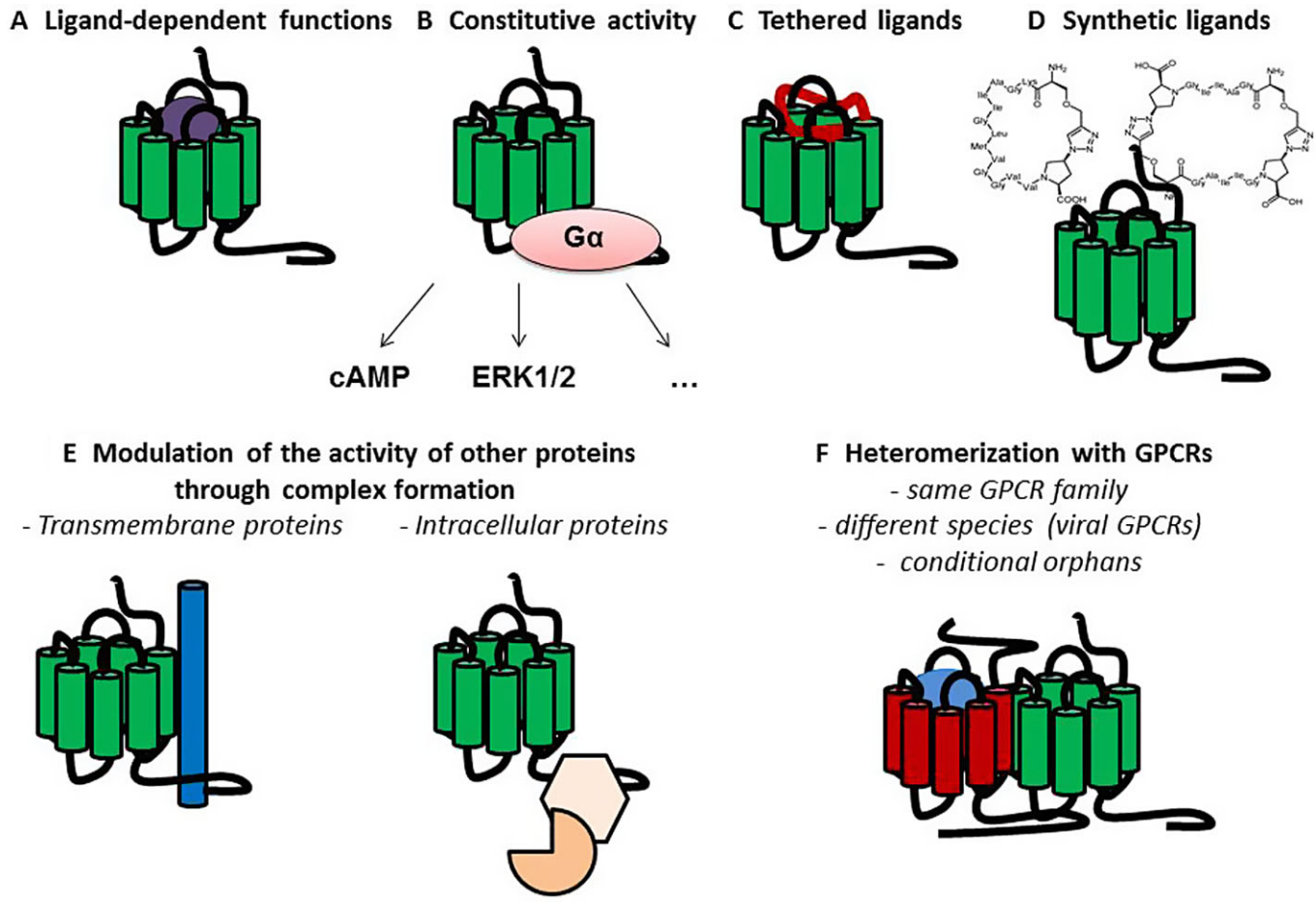

Figure 1

Functions of orphan 7TM proteins. Orphan 7TM proteins can have various cellular functions. (A) They can depend on a yet-to-be identified natural ligand(s). (B) Orphan 7TM proteins can also display constitutive activity, mainly based on constitutive coupling to G-proteins and engaging different downstream signalling pathways. (C) This constitutive activity can be maintained by the presence of intramolecular N-terminal tethered ligands. (D) Medicinal chemistry allows the design and synthesis of suitable surrogate ligands that can modulate the activity of 7TM proteins. Orphan 7TM proteins can also exert their function in complex with other proteins (E–F) with different cellular localizations as extracellular (not shown, example GPR56 and TG 2), transmembrane or intracellular proteins and modulate their function or enzymatic activity. Heteromer formation with other GPCRs is a specific complex-dependent action of orphan 7TM proteins that occurs between different cellular GPCRs and are found in different species as for heteromers between viral orphan 7TM proteins and host cell GPCRs. Additionally, deorphanized GPCRs can behave in distinct cellular contexts as conditional orphans and allosterically modulate the function of their binding partner in the absence of their natural ligands.

Orphan 7TM proteins in cancer metabolism. One of the most highlighted roles of orphan 7TM proteins is in cancer biology. Different forms of cancers such as triple-negative breast cancer (TNBC), skin cancer and lung cancer have been shown to be linked to orphan 7TM proteins (Gugger *et al.*, 2008; Perez-Gomez *et al.*, 2013; Feigin *et al.*, 2014). The foremost and the most recent among these studies are related to GPR161, which was found to be overexpressed specifically in TNBC and to correlate with poor prognosis. Overexpression of GPR161 in human mammary epithelial cells increases cell proliferation, migration, intracellular accumulation of E-cadherin and formation of multi-acinar structures in three-dimensional cultures. In contrast, knockdown of GPR161 impairs proliferation of human basal breast cancer cell lines. Therefore, GPR161 is a promising new therapeutic target for TNBC. Another orphan 7TM protein, GPR19, is frequently overexpressed in tissue samples of lung cancer patients and is therefore considered as a new potential candidate drug target for the treatment of a subset of lung cancers (Kastner *et al.*, 2012).

Recent reports are also suggesting the role of adhesion GPCRs in cancer and tumour development. GPR64, for example, was found to be highly up-regulated in Ewing sarcomas (ES) (Richter *et al.*, 2013). The study suggests that the GPR64 is able to induce invasiveness and metastasis in ES by orchestrating placental growth factor (VEGF receptor 1 ligand) and MMP1 expression. Given that GPR64 is a membrane-bound, and thus potentially druggable protein, makes it a promising candidate for the development of novel antitumour therapies in the near future. Recent studies

Table 1

Identified functions of class A orphan GPCRs

GPCRs	Identified functions	Assay	Expression system	Reference
GPR3	Modulate early phases of cocaine reinforcement	GPR3-/- mice	–	Tourino *et al.*, 2012
	Protect neurons from apoptotic stimuli	GPR3-/- mice	–	Tanaka *et al.*, 2014
GPR6	Alters striatal cAMP and dopaminergic system	GPR6-/- mice	–	Oeckl *et al.*, 2014
GPR12	Increase neurite extension	IF, immunoblot	PC12 cell line	Lu *et al.*, 2012a
	Cell proliferation	MTT assay, immunoblot	HEK293 (transient)	Lu *et al.*, 2012b
GPR15	Mediate murine skin lymphocyte homing	GPR15-/- mice FACS, histology	Mouse fetal thymic lymphocytes	Lahl *et al.*, 2014
	Up-regulated in human rheumatoid arthritis patients	FACS, IF, RT-PCR	Human PBMC	Cartwright *et al.*, 2014
	TLR3 induced up-regulation in HIV patients	HIV patients blood, FACS, IHC	Human PBMC	Kiene *et al.*, 2014
	Controls T-cell homing in intestinal inflammation	GPR15-/- mice, FACS, IHC	Mouse primary intestinal cells	Kim *et al.*, 2013
GPR20	Constitutive inhibition of cAMP production through $G\alpha_i$	cAMP	HEK293 (stable)	Hase *et al.*, 2008
GPR21	Improves insulin sensitivity in diet-induced obesity	GPR21-/- mice, BMT studies, *in vivo* metabolic studies	–	Osborn *et al.*, 2012
GPR22	Protective role in response to haemodynamic stress	GPR22-deficient mice	HEK293 (stable)	Adams *et al.*, 2008
GPR26	Regulates energy homeostasis through AMPK in hypothalamus	GPR26-/- mice	–	Chen *et al.*, 2012
	Constitutive stimulation of cAMP production	cAMP	HEK293 (transient)	Jones *et al.*, 2007
GPR27	Regulation of insulin production	IP1, cAMP, qPCR, luciferase assay	Mouse primary islets, MIN6 cells	Ku *et al.*, 2012
GPR34	Affects cellular response against immune challenges	GPR34-deficient mice, FACS, behavioural tests	COS-7 (transient)	Liebscher *et al.*, 2011
	Role in lymphoma cell growth via ERK activation	Gene expression profiling analysis, FACS, ISH, confocal microscopy, qPCR	HeLa (transient) OCI-Ly19 (transient)	Ansell *et al.*, 2012
GPR37	Regulates dopaminergic transmission and implicated in pathophysiology of Parkinson's disease	cAMP, luciferase reporter assay	HEK293 (transient)	Low and Aebischer 2012, Gandía *et al.*, 2013
	Receptors for neuroprotective factors prosaptide and prosaposin	cAMP, microscopy, GTPγS binding assay	Mice cortical astrocytes, HEK293T, COS-7	Meyer *et al.*, 2013
GPR50	Regulates leptin signalling, thermogenesis and torpor	GPR50-/- mice	–	Bechtold *et al*, 2012
	Heterodimerization with MT_1 receptor inhibits melatonin binding and MT_1 receptor signalling (G-protein and β-arrestin recruitment)	BRET, co-IP, ligand binding, cAMP, GPR50 siRNA	HEK293 (transient), hCMEC/D3 (endogenous)	Levoye *et al.*, 2006
	Regulates energy metabolism	GPR50-deficient mice	-	Ivanova *et al.*, 2008
GPR52	Alters dopaminergic/glutamatergic system in psychiatric disorders	GPR52-/- and transgenic mice, cAMP, IHC	HEK293 (transient) CHO (stable)	Komatsu *et al.*, 2014
GPR82	Influences food intake, body weight and energy balance	GPR82-deficient mice, genotyping of human population, glucose and insulin tolerance test	–	Engel *et al.*, 2011

Table 1

Continued

GPCRs	Identified functions	Assay	Expression system	Reference
GPR83	Regulates systemic energy metabolism	GPR83-/- mice, gene expression profiling in mouse tissues, IP3, ISH	COS-7 (transient) N41 (transient) HEK293 (transient)	Muller *et al.*, 2013
GPR85 (SREB2)	Influences brain size, behaviour and vulnerability to schizophrenia	GPR85 transgenic mice GPR85 deficient mice	–	Matsumoto *et al.*, 2008
GPR88	Alters striatal GABAergic/glutamatergic signalling	GPR88-/- mice, microarray analysis, behavioural parameters, *in vivo* electrophysiology,	–	Quintana *et al.*, 2012
	Modulates striatal dopamine functions, implicated in schizophrenia	GPR88-/- mice, ISH, Northern blotting, histology	–	Logue *et al.*, 2009
GPR161	Overexpressed and play a role in pathogenesis of TNBC and promotes proliferation, invasion	Profiling of human cancer samples, IP, MTT assay, IF	MDA-MB361 (transient) MDA-MB436 (transient) HEK293T (transient) MCF-10A	Feigin *et al.*, 2014
	Negatively regulates sonic hedgehog signalling during neural tube development	GPR161-/- mice, histology, HTRF-cAMP assay, IP, ISH	–	Mukhopadhyay *et al.*, 2013
	Control right left patterning during embryo development	GPR161 knockdown in transgenic zebrafish line	–	Leung *et al.*, 2008
	Controls neurulation and lens development, TNBC	Vacuolated lens mouse model	–	Matteson *et al.*, 2008
GPR48/LGR4	Promotes invasion and tumour metastasis in colorectal cancer	Human cancer tissue samples, histology, IHC	HEK293T (transient) HCT116 (transient)	Wu *et al.*, 2013
	Regulation of postnatal epididymal morphogenesis via maintenance of extracellular matrix	*Lgr*4 hypomorphic mutant mice	–	Hoshii *et al.*, 2007

AMPK, AMP-activated protein kinase; BMT, bone marrow transplantation; COS-7, fibroblast-like kidney cell line; co-IP, co-immunopreciptation; hCMEC/D3, human brain capillary endothelial cell line; HCT116, human colon adenocarcinoma cell line; HeLa, human cervical cancer cell line; HIV, human immunodeficiency virus; HTRF, homogeneous time resolved fluorescence; IF, immunofluorescence; IHC, immunohistochemistry; IP, immunopreciptation; IP_1, inositol-1-phosphate; IP_3, inositol 1,4,5-trisphosphate; ISH, *in situ* hybridization; *Lgr*4: leucine-rich repeat containing GPCR 4; MCF-10A, human mammary epithelial cell line; MDA-MB, human breast cancer cell line; MIN6, mouse pancreatic beta cell line; MTT, tetrazolium dye (MTT) colorimetric assay; MT_1, melatonin receptor type 1; N41, embryonic mouse hypothalamic cell line; OCI-Ly19, human B-cell lymphoma cell line; PBMC, peripheral blood mononuclear cell; PC12, rat adrenal pheochromocytoma cell line; qPCR, quantitative PCR; RT-PCR, reverse transcriptase PCR; siRNA, small interfering RNA; TLR3, Toll-like receptor3; TNBC, triple-negative breast cancer.

showed that GPR48, also known as leucine-rich repeat containing GPCR (LGR)4, plays an important role in the development of various organs, cancer development and progression such as gastric cancer and colorectal cancer (CRC; Gao *et al.*, 2006; Steffen *et al.*, 2012). Overexpression of GPR48 in primary CRC and metastatic lymph nodes correlated with tumour invasion and metastasis. Further, GPR48 increased nuclear β-catenin accumulation, T-cell factor 4 transcriptional activity and expression of its target genes including cyclin D1 and c-Myc in CRC cells. Correlation analysis showed that GPR48 expression in CRC tissues was positively associated with β-catenin expression (Wu *et al.*, 2013).

LGR5 and LGR6 (having ~50% homology at the amino acid level) have also been found implicated in cancer stem cells and other forms of cancers (Gong *et al.*, 2012; Nakata *et al.*, 2014). Furthermore, these receptors have been reported to act as a receptor for R-spondins (R-spondins 1 and 3), which are secreted proteins particularly involved in development and stem cell growth. LGR5/6 is also able to regulate Wnt/β-catenin signalling in stem cells during malignant growth (Carmon *et al.*, 2011; Gong *et al.*, 2012) and therefore they are emerging as potential targets for different forms of cancers. With further studies, this receptor family could be utilized as a prognostic biomarker of a broad range of cancers in patients in the near future. There are other studies suggesting the involvement of different orphan 7TM proteins in cancer metabolism, including GPRC5a in breast cancer (Sokolenko *et al.*, 2014) and GPR34 in lymphoma cell growth (Ansell *et al.*, 2012). Taking into account all these studies, we believe some orphan 7TM proteins could be biomarkers for different cancer types and could become therapeutic targets

Table 2

Identified functions of class B (Adhesion) orphan GPCRs

GPCRs	Identified functions	Assay	Expression system	Reference
GPR64	Promotes invasiveness and metastasis in Ewing sarcomas	Immune-deficient Rag2$^{-/-}\gamma_c^{-/-}$ mice, RNA interference	Human ES cell line and osteosarcoma lines	Richter *et al.*, 2013
GPR97	Mediate development and function of the lymphatic vascular system	Taqman GPCR array, siRNA knockdown of GPR97, wound healing assay, FACS, IF, confocal microscopy	Human and mouse LECs	Valtcheva *et al.*, 2013
GPR110	As oncogene overexpressed in lung and prostate cancer	qPCR, IHC, immunoblot	Human lung and prostate cancer cell lines, HEK293T/17,	Lum *et al.*, 2010
GPR116	Regulation of lung surfactant level through surfactant D	Gene-targeted deletion of GPR116 in mice	–	Fukuzawa *et al.*, 2013
	Lung surfactant homeostasis	GPR116 conditional knockout in mice	–	Yang *et al.*, 2013
	Regulates lung surfactant pool size	Targeted mutation of GPR116 in mice	–	Bridges *et al.*, 2013
GPR124	Required for VEGF-induced tumour angiogenesis	GPR124 silencing, mouse xenograft model	Human endothelial cells	Wang *et al.*, 2014
	Regulator of angiogenesis and barrier genesis of the developing CNS	GPR124 deletion in mice, histology, FACS, ISH	Primary human and mouse ECs	Cullen *et al.*, 2011
	Required for proper angiogenic sprouting into the developing neural tube	Targeted deletion of GPR124	Primary human and mouse ECs	Anderson *et al.*, 2011
	Regulates CNS-specific angiogenesis	GPR124-/- mice	Mouse brain endothelial cells	Kuhnert *et al.*, 2010
GPR126	Regulates myelination and heart phenotype	GPR126-/- mice and zebrafish, genotyping, ISH, histology	–	Patra *et al.*, 2013
	Modulates cAMP in Schwann cells to control differentiation and myelination	GPR126-/- mice and conditional knock out, ISH, IHC, PCR, microscopy	COS-7 (transient)	Mogha *et al.*, 2013
	Associated with adolescent idiopathic scoliosis disease	Human subjects genotyping	–	Kou *et al.*, 2013
	Required for Schwann cell myelination in mammals	GPR126-/- mice, morphometric analysis, IHC, microscopy	–	Monk *et al.*, 2011

bEND3, mouse brain endothelial cell line; COS-7, fibroblast-like kidney cell line; ECs, endothelial cells; ES, embryonic stem cells; IF, immunofluorescence; IHC, immunohistochemistry; ISH, *in situ* hybridization; LECs, cultured lymphatic endothelial cells; qPCR, quantitative PCR; Rag2: Recombination-activating genes; siRNA, small interfering RNA.

for the treatment of cancer-related disorders in the near future, justifying the development of synthetic, surrogate ligands.

Orphan 7TM proteins in neurodegenerative and psychiatric disorders. Most orphan 7TM proteins are abundantly expressed in the brain, implying a possible role in brain physiology and neurodegenerative and psychiatric disorders. Two orphan GPCRs that have attracted particular interest over the past 15 years in neurophysiology are GPR37 and GPR37L1, a pair of closely related receptors that exhibit distant similarity to endothelin receptors and other peptide-activated GPCRs (Marazziti *et al.*, 1997; Zeng *et al.*, 1997). They are found exclusively in the nervous system and are known to be expressed in both neurons and glia (Marazziti *et al.*, 1998; Valdenaire *et al.*, 1998; Imai *et al.*, 2001). GPR37 was identified as a substrate of the E3 ubiquitin ligase parkin, earning it the alternative name 'parkin-associated endothelin-like receptor' (Valdenaire *et al.*, 1998), and is associated with autosomal recessive juvenile Parkinson's disease (Yang *et al.*, 2003; Obeso *et al.*, 2010). The connection between GPR37 and parkin has led to a focus on the dopaminergic system in GPR37 knockout mice, which exhibited a reduced dopaminergic tone and various subtle perturbations in dopaminergic signalling in the brain (Imai *et al.*, 2007; Marazziti *et al.*, 2007). Recently, Meyer *et al.* (2013) have reported prosaposin and prosaptide (a peptide derived from prosaposin) as cognate endogenous ligands for GPR37 and GPR37L1. These ligands bind to GPR37 and GPR37L1, induce receptor internalization and stimulate GPR37- and GPR37L1-mediated signalling through *Pertussis* toxin-sensitive G-proteins. Furthermore, they are also necessary for mediating endogenous

Table 3

Identified functions of class C orphan GPCRs

GPCRs	Identified functions	Assay	Expression system	Reference
GPRC5A	Modifier of breast cancer risk in BRCA1 mutation carriers	Whole exome sequencing, qPCR, gene knockdown	MDA-MB231	Sokolenko *et al.*, 2014
	Constitutive inhibition of $G\alpha_s$ mRNA and cAMP production increases cell proliferation	cAMP, real-time PCR, proliferation assay, GPRC5A siRNA	Human thyroid follicular epithelial cells (endogenous)	Hirano *et al.*, 2006
GPRC5B	Modulates neurogenesis in mouse cortex	ISH, immunostaining, RT-PCR	HEK293 (transient), mouse cortical progenitor cells	Kurabayashi *et al.*, 2013
	Modulation of insulin secretion	Immunoblot, confocal microscopy, qPCR	Human and mouse pancreatic islets	Soni *et al.*, 2013

GPR12 GPR12BRCA1, breast cancer1, early onset gene; IHC, immunohistochemistry; ISH, *in situ* hybridization; MDA-MB: human breast cancer cell line; qPCR, quantitative PCR; RT-PCR, reverse transcriptase PCR.

responses in primary cortical astrocytes. This topic gains significance as both prosaposin and prosaptide exerted neuroprotective and glioprotective effects (O'Brien *et al.*, 1994; 1995; Campana *et al.*, 1998; Li *et al.*, 2010) via the stimulation of G-protein-mediated pathways (Hiraiwa *et al.*, 1997; Campana *et al.*, 1998; Yan *et al.*, 2000). Thus, future studies aiming at the neuroprotective and glioprotective actions of prosaposin and prosaptide, and the development of low MW ligands for these receptors, may provide new therapeutic possibilities for the treatment of Parkinson's disease and other neurodegenerative disorders. More recent findings from Gandía *et al.* (2013) have highlighted the role of the cysteine-rich domain in this GPR for receptor-mediated cytotoxicity and improved our understanding of its involvement in the pathophysiology of Parkinson's disease.

Other promising orphan 7TM proteins reported to have a role in psychiatric disorders are GPR50 (Thomson *et al.*, 2005), GPR88 (Logue *et al.*, 2009; Del Zompo *et al.*, 2014), GPR6 (Oeckl *et al.*, 2014) and GPR52 (Komatsu *et al.*, 2014). Based on genotyping study in human subjects, Thomson *et al.* (2005) reported a link between polymorphisms in the GPR50 gene and major mental illness while other studies targeted the dopaminergic system in the striatum and found a possible role of GPR6, GPR52 and GPR88 in related neurodegenerative/psychiatric disorders. For instance, GPR6 was abundantly expressed in striatopallidal neurons and its depletion reduces cAMP concentrations in the striatum and alters the striatal dopaminergic system. Furthermore, knockdown of GPR6 caused an interesting behavioural phenotype in the form of enhanced motor activity combined with reduced abnormal involuntary movements. These findings could offer an opportunity for the treatment of Parkinson's disease beyond dopamine replacement (Oeckl *et al.*, 2014). In the same line, another study based on detailed histological investigation suggests that GPR52 may modulate dopaminergic and glutamatergic transmission in neuronal circuits responsible for cognitive function and emotion (Komatsu *et al.*, 2014). GPR52 knockout and transgenic mice exhibited psychosis-related and antipsychotic-like behaviours respectively. Similarly, GPR88 has also been the centre of attraction especially in the research of neuropsychiatric diseases. In rodents, GPR88 is highly expressed in the striatum, with its expression being limited to dopamine D_1 and D_2 receptor-containing medium spiny neurons (MSN) implicated in the pathophysiology of and being modulated by treatments for schizophrenia. The modulatory role of GPR88 in striatal dopamine function suggests it may be a new target for the treatment of psychiatric disorders (Logue *et al.*, 2009). In the same line, targeted viral expression of GPR88 in MSNs rescued the molecular as well as electrophysiological properties and normalized the behaviour (Quintana *et al.*, 2012).

There are a couple of other orphan 7TM proteins, which have been observed to modulate neurogenesis, neurite outgrowth and differentiation in the CNS and its associated pathophysiologies. GPRC5B is predominantly expressed in neural progenitors in the developing mouse brain and its depletion in progenitors results in a failure to adopt a neuronal fate. Further, GPRC5B-mediated signalling affects β-catenin signalling, which is important for the neuronal differentiation of progenitors during the neurogenic phase (Kurabayashi *et al.*, 2013).

Interestingly, among those orphan 7TM proteins that constitutively activate G_s proteins, GPR3, GPR6 and GPR12 have been found to mediate various neurological functions. They are able to promote neurite outgrowth by constitutively up-regulating the cAMP/PKA pathway (Tanaka *et al.*, 2007). Recently, GPR3 has been reported to inhibit the proliferation of cerebellar granule cells *in vitro* and to promote survival of neurons by inhibiting their apoptosis in various physiological conditions (Tanaka *et al.*, 2009; 2014). GPR12 was shown to enhance neurite outgrowth and increase cAMP levels during neurite extension. Moreover, GPR12 knockout mice showed impaired locomotion, motor function and learning (swimming) in the Morris water maze, suggesting its potential involvement in learning and memory functions. GPR3 has also been reported to regulate the expression and development of neuropathic pain and in the analgesia induced by morphine. The genetic deletion of GPR3 produced hypersensitivity to thermal non-noxious and noxious stimuli without affecting the spinal inflammatory response associated with sciatic nerve injury and reduced morphine antinociception (Ruiz-Medina *et al.*, 2011). Furthermore, these mice showed

differences in the locomotor, rewarding and reinforcing effects of cocaine mainly after acute administration of cocaine, compared with wild-type mice. Taken together, GPR3 is emerging as a new molecular target in neuropathic pain therapy as well as a new component of the pro-opioid receptor system. With all this information in hand, we feel that orphan 7TM proteins are developing as potential therapeutic targets for CNS-related diseases.

Orphan 7TM proteins in energy metabolism and diabetes. Orphan 7TM proteins have been proposed to play a noteworthy role in modulating energy expenditure and metabolism as well as energy homeostasis and related physiological functions. GPR50 plays a role in the regulation of energy metabolism (Ivanova *et al.*, 2008) and GPR50$^{-/-}$ mice show reduced weight and partial resistance to diet-induced obesity. Furthermore, GPR50 seems to play an important role in leptin-dependent adaptive thermogenesis (Bechtold *et al.*, 2012). GPR83 could be involved in the central regulation of energy metabolism as a potential modulator of the hypothalamus–pituitary–adrenal axis (Muller *et al.*, 2013). In the arcuate nucleus, GPR83 colocalizes with the ghrelin receptor and the agouti-related protein (AgRP). The orexigenic and adipogenic effect of ghrelin is accordingly potentiated in GPR83-deficient mice. GPR83 modulates ghrelin action and regulates systemic metabolism through other ghrelin-independent pathways. Several other orphan 7TM proteins have also been linked to lipid metabolism and type 2 diabetes (Bhattacharyya *et al.*, 2006; Engel *et al.*, 2011; Chen *et al.*, 2012).

GPR82, GPR26, GPR21, GPR27 and GPRC5B have recently been found to be involved in regulation of diet-induced obesity and insulin sensitivity. As reported by Engel *et al.* (2011), GPR82$^{-/-}$ mice show reduced body weight, food intake and triglyceride levels, and increased insulin sensitivity and glucose tolerance. Similarly, GPR26 was a potent regulator of energy homeostasis through controlling hypothalamic AMP-activated protein kinase (AMPK) activation and its targeted deletion caused hyperphagia and hypometabolism, which leads to early onset of diet-induced obesity (Chen *et al.*, 2012). Similarly, GPR21$^{-/-}$ animals are protected from high-fat diet-induced inflammation and reduced insulin sensitivity. GPR21 is highly expressed in the hypothalamus and macrophages of mice and targeted deletion of GPR21 in the whole body led to a robust improvement in glucose tolerance and systemic insulin sensitivity and a modest lean phenotype (Osborn *et al.*, 2012). A study by Ku *et al.* (2012) showed that GPR27 modulated pancreatic beta-cell function, insulin sensitivity and its knockdown in these cells reduced endogenous mouse insulin promoter activity and glucose-stimulated insulin secretion. Similarly, GPRC5B also regulated beta-cell viability and insulin secretion and its silencing was associated with increased glucose- and glutamate-induced insulin secretion. The hyperactivation of GPRC5B contributed to impaired insulin secretion, a characteristic feature of type 2 diabetes. Antagonizing GPRC5B activity might represent a means of restoring normal insulin secretory function in diabetic patients (Soni *et al.*, 2013).

Taken together, several orphan 7TM proteins appear to be involved in glucose and lipid metabolism and might in the future emerge as new drug targets for type 2 diabetes and other metabolic disorders.

Adhesion orphan 7TM proteins and their physiological aspects

Adhesion GPCRs have been neglected for a long time but have more recently become the subject of intense research. In some cases, the involvement of adhesion GPCRs in diseases such as cancer has been established, as described earlier, but the majority of studies have concentrated on elucidating their involvement in various physiological processes as discussed here (Table 2). Adhesion GPCRs are unique in several aspects. They have a long N-terminus containing multiple domains, which are linked to the 7TM region through the GPCR proteolytic site domain, which has autocatalytic properties (Baud *et al.*, 1995; Krasnoperov *et al.*, 1997). Most adhesion GPCRs are orphan 7TM proteins and only for some of them has a natural ligand and a clearly defined function been proposed. In general, adhesion GPCRs are involved in immunological function, synaptic function, planar cell polarity, tumour progression and fertility (Usui *et al.*, 1999; Steinert *et al.*, 2002; Davies *et al.*, 2004; Lin *et al.*, 2005).

Recently, GPR124 and GPR126 have been shown to regulate the development of different tissues in mammals. GPR124 affects CNS-specific angiogenesis, vascularization (Kuhnert *et al.*, 2010; Anderson *et al.*, 2011; Cullen *et al.*, 2011) and is also required for VEGF-induced tumour angiogenesis (Wang *et al.*, 2014). GPR126 is an important regulator of embryonic development in mammals and studies on GPR126$^{-/-}$ mice have shown that its disruption leads to fully penetrant embryonic lethality with cardiac abnormality (Waller-Evans *et al.*, 2010). In another study using a GPR126 knockout mouse line from Taconic (Rensselaer, NY, USA) (T-GPR126$^{-/-}$) (Monk *et al.*, 2011), most mutants die *in utero*, although a few mice survive to postnatal stages. T-GPR126$^{-/-}$ mice are characterized both by a lack of myelination in the peripheral nervous system and by multiple defects in peripheral nerves. GPR126 is thought to function in Schwann cells through G-proteins to control myelination and differentiation (Mogha *et al.*, 2013). Apart from acting at the CNS, GPR126 is also expressed in the endocardium during early mouse heart development and its knockout in mice and knockdown in zebrafish caused hypotrabeculation and affected mitochondrial functions (Patra *et al.*, 2013). The link with cardiovascular development is further supported by a recent genetic study by Kou *et al.* (2013) revealing its association with the skeletal disease called adolescent idiopathic scoliosis. Taken together, orphan 7TM proteins such as GPR126 could be a potent target for the CNS-related vascular pathologies and developmental functions.

Another member of this class, GPR97, is highly conserved among species and has been shown to be a regulator of B-lymphocyte population and to regulate constitutive cAMP response element-binding protein (CREB) and NF-κB activity in mice (Wang *et al.*, 2013). Deletion of GPR97 in mice caused disorganized spleen architecture with modified primary humoral and secondary immune responses. Further, Valtcheva *et al.* (2013) found GPR97 expressed by lymphatic endothelium and postulated a role in the development and function of the lymphatic vascular system under pathological conditions. Therefore, GPR97 as a lymphatic adhesion

orphan GPCR might open new possibilities for the future pharmacological manipulation of lymphangiogenesis.

A very interesting role of GPR116 has been reported in respiratory and lung physiology. Disruption of the GPR116 gene in mice resulted in progressive accumulation of surfactant lipids and proteins in the alveolar space, which lead to laboured breathing, and reduced lifespan. Indeed, GPR116 expression in alveolar type II cells is required for maintaining normal surfactant levels (Yang *et al.*, 2013). GPR116 appears to function as molecular sensor of alveolar surfactant lipid pool by regulating surfactant secretion (Bridges *et al.*, 2013). Consistent with this finding, Fukuzawa *et al.* (2013) found that GPR116 acts specifically on surfactant protein D (SpD) in order to regulate synthesis and secretion of surfactant lipids and proteins and to stimulate recycling (uptake), in response to elevated levels of SpD in the alveolar space. Thus, GPR116 plays an indispensable role in lung surfactant homeostasis with important ramifications for the understanding and treatment of lung surfactant disorders.

Constitutively active orphan 7TM proteins

Some of the remaining orphan 7TM proteins (more than 100) might have ligand-independent functions, expressed as a significant degree of constitutive activity triggering functional responses in the absence of ligands (Figure 1B). The molecular explanation for constitutive activity is often based on specific receptor sequences that stabilize the active receptor conformation, thus allowing the interaction with various proteins that induce cellular signalling events (Lowther *et al.*, 2013).

Constitutive activity of orphan 7TM proteins improves binding to G-proteins even in the absence of ligand. The constitutive activity of GPR3 (Mehlmann *et al.*, 2004; Ledent *et al.*, 2005), GPR6 and GPR12 (Tanaka *et al.*, 2007) induces cAMP production resulting from constitutive coupling to $G\alpha_s$ proteins. For GPR6, it has also been shown *in vivo* that its ablation leads to decreased cAMP levels in the striatum (Oeckl *et al.*, 2014). A constitutive coupling to $G\alpha_i$ proteins has been demonstrated for GPR20 (Hase *et al.*, 2008). The constant G-protein coupling results in different downstream signalling activities as shown for the $G\alpha_s$ coupled GPR26 (Jones *et al.*, 2007), which inhibits AMPK activity at the hypothalamic level in order to prevent adiposity (Chen *et al.*, 2012) and increases CREB phosphorylation in the amygdala, revealing its possible regulatory role in anxiety (Zhang *et al.*, 2011). GPR12 promotes ERK1/2 phosphorylation and the expression of the anti-apoptotic protein Bcl2, which is important for its role in supporting proliferation, cell survival (Lu *et al.*, 2012b) and neurite outgrowth (Lu *et al.*, 2012a).

The degree of constitutive activity of an orphan 7TM protein not only depends on its expression level, but also on regulatory proteins such as β-arrestin 2 and the GPCR kinase 2 that may be implicated in receptor desensitization as shown for GPR3 (Lowther *et al.*, 2013). An interesting example is GPR17, which has two constitutively active splice variants, GPR17-L and GPR17-S, in humans that are expressed in a tissue-specific manner and that differ in the length of their N-termini and activate $G\alpha_i$ proteins independently from the putative GPR17 ligands, the leukotrienes, LTC_4 and LTD_4 (Benned-Jensen and Rosenkilde, 2010).

Constitutive activity has also been observed for orphan adhesion GPCRs, mainly on the level of the small Rho GTPases RhoA, Cdc42 and Rac1, which are important players in the regulation of cellular motility (Gupte *et al.*, 2012). Indeed, GPR97, which is expressed in lymphatic epithelium, is coupled to $G\alpha_o$ and regulates the balance of the active Rho GTPases Cdc42 and RhoA (Valtcheva *et al.*, 2013; Wang *et al.*, 2013), and GPR56 interferes with cellular migration of neural cells via the Rho GTPase pathway (Iguchi *et al.*, 2008).

In an effort to provide a molecular explanation for the constitutive activity of orphan 7TM proteins, deletion of the N-terminal part of GPR61 resulted in the loss of constitutive activity leading to the hypothesis that constitutive activity of some orphan receptors might be based on the existence of an N-terminal-tethered ligand (Toyooka *et al.*, 2009) (Figure 1C). Similarly, the melanocortin MC_4 receptor, besides its ligand-induced activation, displays a constitutive activity, which is dependent on its N-terminus (Ersoy *et al.*, 2012). This constitutive activity can be counteracted by AgRP, a natural inverse agonist, thus suggesting the interesting possibility that some constitutively active 7TM proteins might be regulated by natural inverse agonists rather than agonists.

Retroviruses are known to encode proteins with a 7TM structure with sequence homology to cellular chemokine receptors (Rosenkilde *et al.*, 2008). Several of these proteins show constitutive activity. Most recent examples are UL33, encoded by the human cytomegalovirus (HCMV), and BILF, encoded by the Epstein-Barr virus, which both couple promiscuously to different Gα proteins (Vischer *et al.*, 2014). Their constitutive activity modulates the signalling in the host cell beneficially for virus-triggered promotion of cellular proliferation and transformation (Lyngaa *et al.*, 2010). The high level of constitutive activity of virally encoded GPCRs might be the result of a less conserved DRY motif, which is responsible for an inhibitory, inactive conformation of class A GPCRs (Jensen *et al.*, 2012). Thus, some viruses developed a strategy based on constitutively active 7TM proteins that modify the function of the host cell in a ligand-independent manner.

Although many GPCRs exhibit a significant level of constitutive activity, it should be mentioned that in the case of orphan 7TM proteins, the possibility always remains that the apparent constitutive activity might actually reflect the presence of an endogenous ligand that either is difficult to remove or which is produced by the cell. A study on the former orphan GPR40 (now free fatty acid receptor 1; FFA1) revealed that its apparent constitutive activity is due to a permanent occupation of the receptor binding site by its endogenous FFA ligand (Stoddart *et al.*, 2007). The detection of constitutive activity can sometimes go hand in hand with the disclosure of the identification of a ligand, as recently shown for GPR174 (Sugita *et al.*, 2013). Constitutively active 7TM proteins present new opportunities for the design of suitable inverse agonists.

Function of orphan 7TM proteins in heterodimeric complexes with GPCRs

Another function of orphan 7TM proteins relies on their ability to interact with other proteins, leading to allosteric modulation of their activity. At the beginning of the 21st century, the emerging concept of the formation of

heteromeric complexes of GPCRs was extended to orphan 7TM proteins (Levoye *et al.*, 2006) (Figure 1F). These heteromers are formed typically between a ligand-dependent GPCR and an orphan 7TM protein that are often members of the same subfamily. The case of an obligatory heteromeric complex is found in $GABA_{B1}$ and $GABA_{B2}$ receptors. Both proteins constitute a functional complex, with each one having a specific task: $GABA_{B1}$ binds the ligand and $GABA_{B2}$ is responsible for transducing the signal to the G-protein. In this case, $GABA_{B2}$ lacks the GABA-binding domain and behaves as the orphan receptor (Kniazeff *et al.*, 2002). Recently, this has also been demonstrated for GPR179, another orphan 7TM protein, which can form a heteromer with the metabotropic glutamate receptor ($mGlu_6$) in the retina and which is involved in dim-light vision signal transmission from photoreceptors to ON-bipolar cells (Orlandi *et al.*, 2013).

Another possibility is a conditional heteromerization of related GPCRs, where heteromer formation can generate a novel functional unit through allosterism between the two protomers compared with the corresponding homomers. The pioneer work in this field demonstrated the heteromer formation of the melatonin MT_1 receptor and its orphan family mate GPR50. In this case, the orphan GPR50 negatively interferes with melatonin-dependent signal transduction in a manner that is dependent on its long cytosolic C-tail (Levoye *et al.*, 2006). Another example is the β-alanine binding MAS-related receptor Mrg_D, which can form a complex with its orphan relative, Mrg_E. This interaction is associated with potentiation of signalling and inhibition of internalization of the receptor (Milasta *et al.*, 2006).

The protochordate *Ciona intestinalis* possesses four isoforms of the gonadotropin-releasing-hormone (GnRH) receptor, of which the GnRH R4 is an orphan subtype. The R4 can heteromerize with R1 and potentiate R1-induced ERK1/2 activation and calcium mobilization. In contrast, heteromers composed of R2 and R4 lead to a shift from $G\alpha_s$ to $G\alpha_i$ coupling, resulting in a decrease in cAMP production (Sakai *et al.*, 2010; 2012). These results demonstrate that the orphan R4 functions as an allosteric modulator of GnRH receptors and it will be interesting to see whether this can be also found in species other than *C. intestinalis*.

Virally encoded GPCRs can also form GPCR/orphan 7TM protein complexes as shown for HCMV-encoded proteins such as US28 that promiscuously binds several host cell chemokines, forming a complex with the orphan 7TM proteins US27, UL33 and UL78. The latter two complexes have a silencing effect on the NF-κB signalling activity of US28, providing several possibilities to adapt the signalling in the host cell depending on the expression pattern of the orphan 7TM proteins (Tschische *et al.*, 2011). Interestingly, complex formation can also be seen between virally encoded 7TM proteins and GPCRs expressed by the host cell. Indeed, UL27, UL33 and UL78 can interact with the cellular chemokine receptors CCR5 and CXCR4 and modulate their function including their HIV co-receptor properties (Tadagaki *et al.*, 2012; Arnolds *et al.*, 2013).

The constitutively active Epstein-Barr virus 7TM protein BILF1 forms heteromers with various human chemokine receptors (Vischer *et al.*, 2008). The BILF1-CXCR4 complex modulates host cell signalling in a competitive mode of action: both receptors signal via $G\alpha_i$ proteins and the complex formation induces a competition of both receptors for the cellular $G\alpha_i$ pool leading to an impairment of the CXCR4-induced $G\alpha_i$ signalling cascade (Nijmeijer *et al.*, 2010). These examples demonstrate that viruses have developed interesting strategies to use their own orphan 7TM proteins to modulate the signalling of host cell receptors in their favour.

The emerging concept of *conditional orphan receptors* is further expanding the idea of ligand-independent functions of 7TM proteins. Conditional orphan receptors can be defined as proteins with an identified ligand, which can in the absence of ligand behave as orphan receptors, as recently proposed for the ghrelin receptor. This receptor forms heteromers with the D_2 dopamine receptor in the hypothalamus, a tissue where the ghrelin peptide produced in the stomach is not supposed to localize. The conditional orphan ghrelin receptor modulates D_2 receptor activity and is responsible for the anorexigenic properties of dopamine stimulation. The independence of this interaction of the ghrelin ligand was additionally demonstrated with the use of ghrelin knockout mice (Kern *et al.*, 2012). The modulating activity of ghrelin receptors on dopamine signalling can be a consequence of the constitutive ligand-independent activity of the ghrelin receptor and it will be interesting to see whether this concept is also transferable to other ghrelin heteromers (Schellekens *et al.*, 2013) or other constitutively active GPCRs.

Function of orphan 7TM proteins in heterodimeric complexes with transporters, enzymes and other cellular proteins

The formation of functional multiprotein complexes is an emerging concept and work in recent years demonstrated that orphan 7TM proteins can be part of functional complexes between different protein families and thereby regulate their function or enzymatic activity (Figure 1E).

Proteomic techniques have been used for uncovering the existence of protein interactions. GPR50, the orphan member of the melatonin receptor subfamily, has a characteristic long cytosolic C-tail. Yeast-2-hybrid screens using the C-tail of GPR50 as bait revealed several cytosolic interactors such as neurite outgrowth inhibitor (Nogo)-A and the transcription factor TIP60. Binding of the Nogo-A to GPR50 was shown to counteract for its negative influence on neurite outgrowth (Grunewald *et al.*, 2009). Complex formation of GPR50 with TIP60 results in the mutual nuclear translocation of the cleaved C-tail of GPR50 and TIP60 and enhances the nuclear glucocorticoid receptor-dependent gene expression (Li *et al.*, 2011).

The constitutively active GPR3 is involved in the progression of Alzheimer's pathology (Thathiah *et al.*, 2009). Elucidating the mechanism has shown the influence of the GPR3 C-tail and β-arrestin 2 binding as intermediates for the increase of production of cleaved amyloid-β peptide that can lead to amyloid plaque formation. Both GPR3 and β-arrestin 2 enhance γ-secretase activity, probably through a direct interaction (Thathiah *et al.*, 2013). Demonstration of the *in vitro* interaction between GPR3 and the amyloid precursor protein suggests the formation of a multiprotein complex that is responsible for the generation of cleaved peptides in the presence of GPR3 (Nelson and Sheng, 2013).

The class C orphan GPR179, which forms a heteromer with $mGlu_6$ receptors, seems to be part of a macromolecular complex at dendrites of ON bipolar neurons in the retina together with the TRPM1 cation channels and the RGS proteins 7 and 11. The complex is required for appropriate metabotropic signal transmission from photoreceptors to ON bipolar neurons (Orlandi *et al.*, 2012; 2013; Ray *et al.*, 2014) and depletion of GPR179 leads to stationary night blindness (Peachey *et al.*, 2012; Orhan *et al.*, 2013).

The adhesion GPCR GPR56 forms a functional complex with the transmembrane tetraspanins CD81 and CD9 (Little *et al.*, 2004) and binds to the extracellular enzyme transglutaminase (TG)2 (Xu *et al.*, 2006). GPR56 can negatively influence the enzymic activity of TG2 by promoting its internalization and degradation, which blocks TG2-dependent melanoma formation (Yang *et al.*, 2014).

The cytosolic part of the class C orphan GPRC5B contains several tyrosine residues that can be phosphorylated by the Fyn kinase. GPRC5B and Fyn together form a complex, which induces NF-κB signalling and inflammatory signalling activity in adipocytes possibly in a G-protein-independent manner (Kim *et al.*, 2012).

These examples demonstrate that the function of orphan 7TM proteins need not be limited to classical GPCR signalling activity but can be expanded to other functions related to the formation of multiprotein complexes. Regulation of the formation and activity of such complexes is expected to be tissue-specific, as the expression of its components will depend on their specific expression patterns. The many techniques for identifying protein complexes (Daulat *et al.*, 2013) and their application *in vivo* will surely enable the identification of more protein complexes and binding partners of orphan 7TM proteins in the future.

Identification of synthetic, surrogate ligands for orphan 7TM proteins

Identification of synthetic surrogate ligands for orphan 7TM proteins presents an interesting alternative to classical deorphanization approaches to obtain pharmacological and therapeutic tools for these 7TM proteins (Figure 1D). It is worth mentioning that low MW synthetic molecules may not necessarily have effects identical to those of endogenous ligands and may act outside the orthosteric binding site and may modulate the receptor by binding to an allosteric binding site. The action of allosteric ligands is generally considered to depend on the presence of the orthosteric ligand. However, several examples demonstrate that allosteric agonists may have also an effect, which is independent of the presence of the orthosteric ligand (Schwartz and Holst, 2007). This is nicely illustrated by two compounds acting on orphan 7TM proteins. The first compound, CGP7930, allosterically enhances GABA binding to the $GABA_B$ receptor heterodimer. Importantly, CGP7930 can activate the 7TM domain of the orphan $GABA_{B2}$ subunit alone (Binet *et al.*, 2004). Similar observations have been made for taste T1R receptors. Lactisole and cyclamate have been proposed to bind to the 7TM domain of the orphan $T1R_3$ subunit and thus allosterically regulate ligand binding to the bilobate extracellular orthosteric sites of $T1R_1$ and $T1R_2$ in their respective heterodimers. Collectively, this indicates that orphan 7TM proteins can be targeted by allosteric agonists that may be interesting compounds even in the absence of orthosteric ligands for these proteins.

In addition, the signalling profile of synthetic ligands does not necessarily overlap with that of the natural agonist raising the question of how closely the effects of a receptor activated by synthetic ligand receptor correspond to those of the receptor activated by its natural agonist. Indeed, synthetic ligands, by binding to orthosteric or allosteric sites, might have biased properties favouring receptor conformations that promote a different repertoire of downstream signalling responses. Synthetic surrogate ligands therefore complement, rather than substitute, for the natural ligand.

When searching for synthetic ligands activating orphan 7TM proteins, a recurrent question concerns the use of an appropriate functional readout. The recent work from the Kostenis group may lead to a new way to solve this question as the authors employed a label-free technology by monitoring dynamic mass redistribution within the cell that occurs as a consequence of cell stimulation. This non-invasive technique provides a global, integrative and signalling pathway-unbiased measure of cellular activation. Using this method, the compound MDL29,951 was identified as a selective agonist for the orphan GPR17, involved in orchestration of oligodendrocyte differentiation and myelination in the CNS (Hennen *et al.*, 2013). Subsequent pathway analysis confirmed that this compound triggers a range of downstream signalling molecules including G_i, G_s, G_q and β-arrestin. The benefit of having identified a surrogate ligand is further illustrated by this study as MDL29,951 provided a reliable positive control to monitor GPR17 activation in many assays. Using this positive control, some previously proposed GPR17 ligands, such as the uracil nucleotides and LTCs, failed to show activity, confirming previous reports and putting GPR17 back to orphan status. Other previously proposed surrogate ligands of GPR17, based on high throughput virtual screening of the GPR17 binding site (Eberini *et al.*, 2011), can now be validated in parallel with MDL29,951 in the dynamic mass redistribution assay.

The deorphanization of GPR3, a potential new target for Alzheimer's disease treatment that also modulates early phases of cocaine reinforcement, is at a similar stage. Although sphingosine 1-phosphate (S1P) has been reported as a putative ligand for GPR3 (Uhlenbrock *et al.*, 2002), these results remain controversial as several groups were unable to confirm these results. In addition, S1P activates many other GPCRs including the $S1P_{1\text{-}5}$ receptors, making S1P unsuitable for further investigation of GPR3-specific signalling and subsequent functions. Low MW surrogate ligands such as diphenyleneiodonium chloride represent an interesting alternative in this context as it specifically promotes GPR3-mediated cAMP accumulation (Ye *et al.*, 2014).

Even for those 7TM proteins, for which a surrogate ligand has already been identified, further screening campaigns might be justified. In some cases, positive hits have limited use because of off-target effects by targeting other receptors in a complex biological environment such as tissues. In other cases, such ligands may exhibit variable potency across different species. Confronted by such a situation, several studies have been undertaken to identify surrogate GPR35 agonists with improved profiles for this receptor that modulates the

immune response and is involved in pain perception (Funke *et al.*, 2013; Neetoo-Isseljee *et al.*, 2013; Thimm *et al.*, 2013).

Computer-aided pharmacophore modelling based on identified surrogate ligands represents another strategy to optimize known surrogate ligands or to make predictions about the structure of putative endogenous ligands. This approach was successfully applied to GPR139, which led to the proposal that dipeptides containing aromatic aminoacids, are putative endogenous agonists for GPR139 (Shi *et al.*, 2011; Isberg *et al.*, 2014). GPR139 is expressed in the brain and controls locomotor activity.

Furthermore, based on the patent literature, the first GPR88 surrogate agonist, (1R, 2R)-2-pyridin-2-yl-cyclopropane carboxylic acid ((2S,3S)-2-amino-3-methyl-pentyl)-(4′-propylbiphenyl-4-yl)-amide), has been recently designed. In functional assays, inhibition of cAMP production was observed upon activation of GPR88, an orphan 7TM protein highly expressed in the striatum and associated with psychiatric disorders (Jin *et al.*, 2014).

General perspectives

The repertoire of ligand-independent functions of orphan 7TM proteins is steadily increasing. Proteomic approaches produce an increasing list of proteins interacting with orphan 7TM proteins. In addition, formation of heterodimeric GPCR complexes is shown in an increasing number of cases *in vivo*. The existence of 'conditional' orphan 7TM proteins, typically consisting of deorphanized GPCRs with identified ligand, which however behave as orphans in the absence of ligand, largely expands the number of potential orphan 7TM proteins particularly in the context of receptor heterodimers. Another future focus would be the identification of further unexplored functions such as the nuclear translocation of orphan 7TM proteins, either of the entire proteins or part of it, as recently shown for GPR50 (Li *et al.*, 2011) and GPR158 (Patel *et al.*, 2013) respectively. These examples demonstrate that the function of orphan 7TM proteins may go far beyond its potential ligand-dependent function and open new conceptual and therapeutic avenues that may even apply to already deorphanized GPCRs.

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Conflict of interest

None.

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