Fine-tuning of GPCR activity by receptor-interacting proteins

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Abstract | G protein-coupled receptors (GPCRs) mediate physiological responses to various ligands, such as hormones, neurotransmitters and sensory stimuli. The signalling and trafficking properties of GPCRs are often highly malleable depending on the cellular context. Such fine-tuning of GPCR function can be attributed in many cases to receptor-interacting proteins that are differentially expressed in distinct cell types. In some cases these GPCR-interacting partners directly mediate receptor signalling, whereas in other cases they act mainly as scaffolds to modulate G protein-mediated signalling. Furthermore, GPCR-interacting proteins can have a big impact on the regulation of GPCR trafficking, localization and/or pharmacological properties.

Agonist
A molecule that binds to and stimulates a receptor to trigger a cellular response.

Clathrin-coated pit
An invaginated membrane structure involved in receptor endocytosis. It consists of a cluster of transmembrane receptors that are attached by adaptor proteins to the protein clathrin, on the cytosolic side of the membrane.

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G protein-coupled receptors (GPCRs) are the largest family of transmembrane proteins in vertebrates and they are the molecular targets for nearly half of the therapeutic drugs that are prescribed worldwide[1]. The approximately 1,000 members of the GPCR family exhibit a conserved 7-transmembrane domain topology and can be divided into 3 main subfamilies, termed A, B and C, based on sequence similarity. The canonical view of how GPCRs regulate cellular physiology is that the binding of ligands (such as hormones, neurotransmitters or sensory stimuli) induces conformational changes in the transmembrane and intracellular domains of the receptor, thereby allowing interactions with heterotrimeric G proteins. Activated GPCRs act as guanine nucleotide exchange factors (GEFs) for the α subunits of heterotrimeric G proteins, catalysing the release of GDP and the binding of GTP for G protein activation. The activated G protein subunits (α•GTP and βγ) can then associate with downstream effectors to modulate various aspects of cellular physiology.

In addition to interacting with G proteins, agonist-bound GPCRs associate with GPCR kinases (GRKs), leading to receptor phosphorylation. GRKs are a family of seven related kinases (GRK1–GRK7) that have differential patterns of distribution across tissues and distinct preferences for binding to certain receptors[2–4]. However, a common outcome of GPCR phosphorylation by GRKs is a decrease in GPCR interactions with G proteins and an increase in GPCR interactions with arrestins (members of a family of four closely related scaffold proteins). The interaction of GPCRs with arrestins further inhibits GPCR signalling through G proteins and simultaneously turns on other signalling pathways that are initiated by the arrestin-mediated recruitment of signalling proteins to activated GPCRs[2]. Furthermore, arrestins can directly link active receptors to clathrin-coated pits to facilitate receptor endocytosis, which is an important process controlling the desensitization and resensitization of GPCR activity[5].

Interactions with G proteins, GRKs and arrestins have been intensively studied for numerous GPCRs and have been exhaustively reviewed elsewhere[2–5]. For this reason, these broadly important interactions, which now represent a canonical model of GPCR regulation (BOX 1), are not further reviewed here. Similarly, the importance of homomeric and heteromeric interactions between GPCRs has also been thoroughly reviewed elsewhere[6–8] and is not addressed here. The focus of this Review is on recent advances in the characterization of receptor-selective GPCR associations with various proteins outside the four previously mentioned families of general GPCR-interacting proteins (G proteins, GRKs, arrestins and other receptors). These GPCR-selective partners can mediate GPCR signalling, organize GPCR signalling through G proteins, direct GPCR trafficking, anchor GPCRs in particular subcellular areas and/or influence GPCR pharmacology. As many of these partners exhibit limited patterns of tissue expression, these interactions can also help to explain cell type-specific fine-tuning of GPCR functional activity.

Mediation of GPCR signalling

For a GPCR-interacting protein to be considered a mediator of GPCR signalling, it would seem that the protein's interaction with the receptor needs to be regulated by agonist stimulation, as agonist-induced

changes are the essence of receptor-initiated signalling. For example, GPCR interactions with G proteins, GRKs and arrestins are strongly enhanced by agonist stimulation[1–5]. In addition, other GPCR-interacting proteins interact with specific GPCRs in an agonist-promoted manner to mediate particular aspects of receptor signalling, and these examples are considered in this section. By contrast, some GPCR-interacting proteins associate with receptors in an agonist-independent manner. Such proteins can potentially modulate G protein-mediated signalling, as described in the next section, but they should not be considered mediators of GPCR signalling as their interactions with GPCRs are not influenced by agonist stimulation. If a GPCR can be considered analogous in some ways to a gun, then ligand-dependent interactors that mediate signalling are analogous to bullets and ligand-independent interactors that modulate signalling are analogous to silencers and scopes, which influence gun function but do not directly mediate the effects of guns on targets.

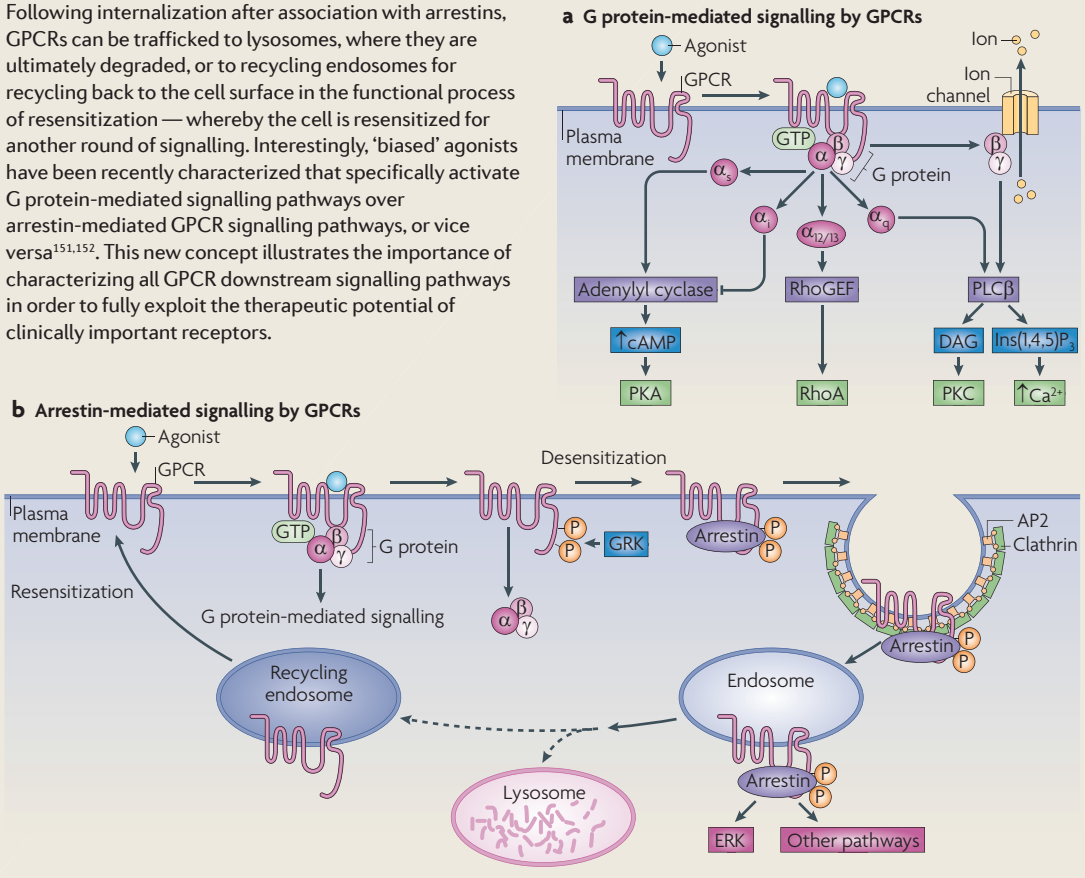
Agonist-promoted interactors that mediate signalling. Several GPCRs have been shown to initiate cellular signalling through agonist-promoted interactions with members of the janus kinase (Jak) family of non-receptor protein tyrosine kinases. For example, the

Box 1 | **Canonical mechanisms of GPCR signalling**

In the classical view of G protein-coupled receptor (GPCR) signalling, an agonist binds to extracellular and/or transmembrane regions of the receptor, leading to its interaction with heterotrimeric G proteins. The GPCR acts as a guanine nucleotide exchange factor, catalysing the exchange of GDP for GTP on the Gα subunit and inducing dissociation of the Gα and Gβγ subunits from each other and from the GPCR (see the figure, part **a**). Activated α•GTP subunits, of which there are multiple subtypes, including $G\alpha_s$, $G\alpha_i$, $G\alpha_{12/13}$ and $G\alpha_q$, subsequently bind to and regulate the activity of effectors such as adenylyl cyclase, RhoGEF and phospholipase Cβ (PLCβ). These modulate downstream effectors directly or by generating second messengers (such as cyclic AMP, diacylglycerol (DAG) and inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) that modulate further downstream effectors, such as protein kinase A (PKA) and protein kinase C (PKC). Following their liberation from the heterotrimeric G protein complex, the βγ subunits can also bind to and regulate certain downstream effectors, such as ion channels and PLCβ.

G protein-mediated signalling by agonist-activated GPCRs can be terminated through GPCR phosphorylation by GPCR kinases (GRKs) and concomitant GPCR association with arrestins, which interact with clathrin and the clathrin adaptor AP2 to drive GPCR internalization into endosomes (see the figure, part **b**)[2–5]. GPCR internalization regulates the functional process of receptor desensitization. Recruitment of arrestins to activated GPCRs can also lead to the initiation of distinct arrestin-mediated signalling pathways, including activation of the mitogen-activated protein kinase extracellular signal-regulated kinase (ERK) pathway.

Following internalization after association with arrestins, GPCRs can be trafficked to lysosomes, where they are ultimately degraded, or to recycling endosomes for recycling back to the cell surface in the functional process of resensitization — whereby the cell is resensitized for another round of signalling. Interestingly, 'biased' agonists have been recently characterized that specifically activate G protein-mediated signalling pathways over arrestin-mediated GPCR signalling pathways, or vice versa[151,152]. This new concept illustrates the importance of characterizing all GPCR downstream signalling pathways in order to fully exploit the therapeutic potential of clinically important receptors.



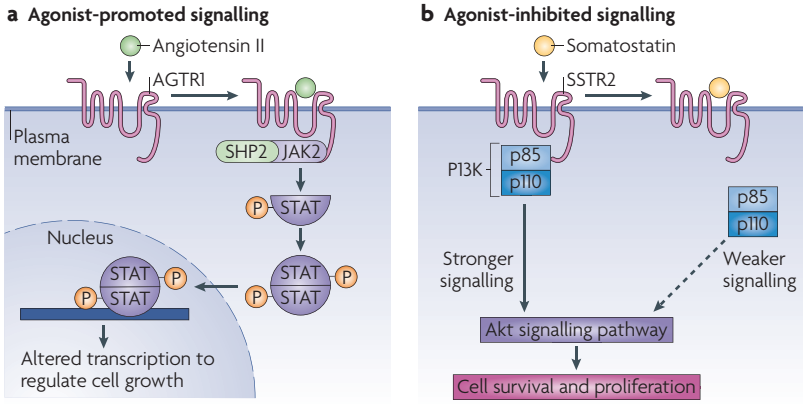


Figure 1 | **GPCR signalling can be mediated by receptor-interacting proteins.** **a** | Certain G protein-coupled receptor (GPCR)-interacting proteins can act as mediators of agonist-induced GPCR signalling, independently of G protein- or arrestin-mediated signalling pathways. An example of this phenomenon is the interaction of the non-receptor tyrosine kinase janus kinase 2 (JAK2) with type 1 angiotensin II receptor (AGTR1). Association of JAK2 with tyrosine protein phosphatase non-receptor type 11 (PTPN11; also known as SHP2) and stimulation of AGTR1 with angiotensin II together promote JAK2 association with AGTR1 and the initiation of JAK2-dependent signalling. Activated JAK2 can phosphorylate members of the signal transducers and activators of transcription (STAT) family of transcription factors, which leads to STAT dimerization, STAT translocation into the nucleus and the regulation of genes controlling cell growth. **b** | In other cases, the agonist-dependent dissociation of an interacting protein from a GPCR can alter the activity of an intracellular signalling pathway. For example, the interaction between the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K) and somatostatin receptor type 2 (SSTR2) is disrupted by agonist stimulation, leading to reduced PI3K-mediated Akt signalling and the suppression of cell survival pathways.

agonist-dependent activation of type 1 angiotensin II receptor (AGTR1) can recruit a complex of JAK2 and tyrosine phosphatase non-receptor type 11 (PTPN11; also known as SHP2) to associate with the AGTR1 carboxyl terminus, which facilitates JAK2 phosphorylation and activation[9,10]. Activated JAK2 can then recruit and phosphorylate members of the signal transducers and activators of transcription (STAT) family of transcription factors. Phosphorylated STAT dissociates from JAK2 and translocates to the nucleus to regulate the transcription of target genes (FIG. 1a). The agonist-promoted interaction between JAK2 and AGTR1 shows an additional signalling avenue for AGTR1, beyond the receptor's well-established coupling to the G protein subunit $G\alpha_q$. It can also help to explain certain effects of AGTR1 stimulation on cellular physiology that are not explained by G protein-mediated signalling[11]. Interestingly, AGTR1 coupling to $G\alpha_q$ can induce a rise in intracellular Ca^{2+} that further potentiates Jak–STAT signalling by this GPCR[12,13], providing an example of how G protein-dependent and G protein-independent signalling mechanisms can in some cases work synergistically. Another GPCR that can interact with JAK2 is platelet-activating factor receptor (PTAFR), which associates with a tyrosine kinase 2 (TYK2)–JAK2 complex in an agonist-regulated manner[14,15]. A mutant version of PTAFR that does not couple to G proteins but still interacts with the TYK2–JAK2 complex is fully capable of activating downstream STATs, showing the physiological importance of PTAFR's recruitment of JAK2 (REF. 15).

GPCR interactions with proteins that possess PDZ domains can also, in some cases, mediate agonist-promoted GPCR signalling. PDZ domains, named after the first three proteins in which they were discovered (Postsynaptic density protein 95, Discs large and Zonula occludens protein 1), can mediate high-affinity interactions with specific motifs at the distal C termini of target proteins[16]. For example, the PDZ protein Na^+–H^+ exchange regulatory factor 1 (NHERF1; also known as EBP50 and SLC9A3R1) has been shown to associate in an agonist-promoted manner with the C terminus of the β_2-adrenergic receptor (β_2AR)[17,18]. The recruitment of NHERF1 to β_2AR disrupts the ability of NHERF1 to inhibit Na^+–H^+ exchanger type 3 (NHE3; also known as SLC9A3), providing a G protein-independent mechanism by which β_2AR can activate Na^+–H^+ exchange in kidney cells[17]. The κ-type opioid receptor (κOPR) is another GPCR that can regulate Na^+–H^+ exchange through agonist-induced interactions with NHERF1 (REFS 19,20). Interestingly, the studies on κOPR provide an example of how interactions between a GPCR and a protein such as NHERF1 can confer cell type-specific signalling to a given GPCR: κOPR stimulation robustly activates NHE3 activity in cell lines expressing high levels of NHERF1 but not in other cell lines that lack significant NHERF1 expression[20].

Agonist-disrupted interactors that mediate signalling. In addition to the above examples of how agonist-promoted associations of GPCR-interacting proteins with GPCRs mediate aspects of GPCR signalling, the disruption of interactions between a GPCR and a cytoplasmic binding partner can also initiate cellular signalling. For example, it has been shown that agonist stimulation of somatostatin receptor type 2 (SSTR2) disrupts its constitutive association with the phosphoinositide 3-kinase (PI3K) subunit p85 to negatively regulate PI3K signalling[21] (FIG. 1b). In the absence of agonist, association of p85 with the first intracellular loop of SSTR2 constitutively enhances PI3K activity to promote cell survival through the Akt pathway. However, following agonist stimulation of SSTR2, association of the receptor with p85 is disrupted, leading to decreased PI3K activity and sensitization of cells to stimuli that induce apoptosis[21]. Thus, even though the classical examples of proteins that mediate GPCR signalling (G proteins and arrestins) exhibit enhanced associations with receptors following agonist stimulation, it is evident that as long as a GPCR-interacting partner exhibits some type of change in its location and/or activity in response to agonist stimulation, this can be sufficient to initiate cellular signalling.

Modulation of GPCR signalling

Some GPCR-interacting partners increase the speed and efficiency of GPCR signalling by acting as scaffolds to tether downstream effectors in close proximity to the receptor. Other GPCR-interacting partners can decrease the intensity and/or time course of GPCR signalling by disrupting the association of GPCRs with G proteins or, in some cases, by recruiting negative regulators of GPCR signalling. By finely tuning the spatial and

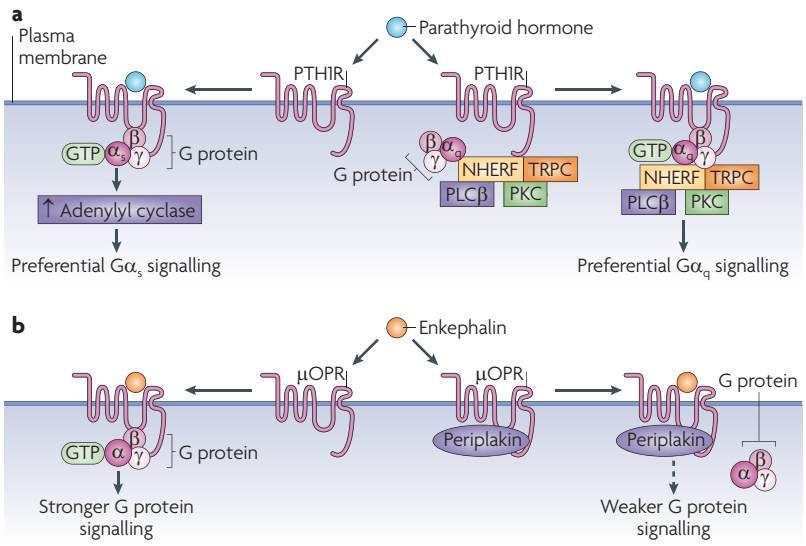


Figure 2 | **GPCR-interacting proteins can modulate G protein-mediated signalling.** **a** | Interaction between the Na^+–H^+ exchange regulatory factor (NHERF) scaffold proteins and parathyroid hormone 1 receptor (PTH1R) leads to a preferential enhancement of downstream $G\alpha_q$-mediated signalling by PTH1R. Through its various protein–protein interaction domains, NHERF not only binds to PTH1R but also tethers multiple downstream signalling effectors, such as phospholipase Cβ (PLCβ), protein kinase C (PKC) and transient receptor potential channel (TRPC), in close proximity to the PTH1R. This creates an efficient complex for preferential $G\alpha_q$-mediated signalling. However, when PTH1R is in a cell type or cellular compartment in which NHERF proteins are absent, PTH1R preferentially signals through $G\alpha_s$ to activate adenylyl cyclase. **b** | By contrast, periplakin can associate with μ-type opioid receptor (μOPR) to impair G protein-mediated signalling by an unknown mechanism. However, in cell types or cellular compartments where periplakin is not found, μOPR ligands can more robustly activate the receptor to stimulate signalling by G proteins. GPCR, G protein-coupled receptor.

temporal resolution of signalling, certain GPCR interactors can dramatically affect the ability of GPCRs to transduce extracellular stimuli into changes in cellular physiology.

Interactors that enhance G protein-mediated signalling. The visual system of *Drosophila melanogaster* has long been an important model system for studying fast and efficient G protein-mediated signalling. Visual signalling in *D. melanogaster* is mediated by light stimulation of the GPCR rhodopsin, which couples to $G\alpha_q$ to activate phospholipase Cβ (PLCβ). Active PLCβ leads to the generation of the second messengers inositol-1,4,5-trisphosphate ($Ins(1,4,5)P_3$) and diacylglycerol and to the opening of the Ca^{2+} transient receptor potential channels (TRPCs). The influx of Ca^{2+} and the production of diacylglycerol lead to activation of protein kinase C (PKC), which plays a key part in the termination of visual signalling. Interestingly, almost all of the components of the *D. melanogaster* visual signalling pathway are tethered together by a large PDZ domain-containing scaffold protein known as Inactivation-no-after-potential D protein (INAD), which associates with rhodopsin, TRPC, PLCβ and PKC[22–28]. By tethering these downstream effectors in close proximity to rhodopsin, INAD creates an efficient signalling complex that dramatically increases the speed and amplitude of physiological responses to light stimulation[29].

Second messenger
An intracellular signal, such as cAMP, diacylglycerol or inositol triphosphate, that is rapidly and transiently synthesized following receptor activation in order to further amplify the signal transduction cascade.

Analogous to *D. melanogaster* INAD, some mammalian PDZ scaffold proteins have been found to interact with GPCRs to enhance the efficiency of GPCR-stimulated G protein signalling. For example, the association of NHERF1 and/or the closely related protein NHERF2 (also known as SLC9A3R2) with various GPCRs, including parathyroid hormone 1 receptor (PTH1R)[30–34], lysophosphatidic acid receptor 2 (LPAR2)[35], purinergic receptor (P2RY1)[36] and metabotropic glutamate receptor 5 (mGluR5)[37], can enhance their PLCβ-mediated signalling. Unlike the aforementioned interactions of NHERF1 with β_2AR and κOPR, which are regulated by agonists, the associations of the NHERF proteins with PTH1R, LPAR2, P2RY1 and mGluR5 are not altered by agonist stimulation and they mainly seem to enhance G protein-mediated signalling. Interestingly, many known NHERF-binding partners (in addition to GPCRs and NHE3) are components of $G\alpha_q$–PLCβ signalling pathways; for example, $G\alpha_q$[38], several TRPCs[39,40], various isoforms of PLCβ[36,41], PKC[42] and protein kinase D (PKD)[43].

The interaction of NHERF2 with PTH1R provides a particularly compelling example as to how receptor-interacting scaffolds can help to explain cell type-specific fine-tuning of GPCR signalling. In osteoblast-like ROS 17/2.8 cells (which do not express detectable levels of NHERF proteins) PTH1R signals mainly by regulating adenylyl cyclase, but in human umbilical vein endothelial ECV304 cells (which contain high levels of both NHERF1 and NHERF2) PTH1R signals mainly through PLCβ regulation[30] (FIG. 2a). Thus, the enigmatic ability of PTH1R to signal by regulating adenylyl cyclase in some cell types but PLCβ in other cell types might be accounted for in many cases by differential cellular expression of the NHERF proteins[44].

In addition to NHERF proteins and INAD, various other PDZ scaffold proteins have been shown to associate with specific GPCRs to enhance certain signalling pathways (TABLE 1). For example, association of multiple PDZ domain protein (MUPP1; also known as MPDZ) with γ-aminobutyric acid B ($GABA_B$) receptors[45] and melatonin type 1 receptors[46] results in markedly enhanced $G\alpha_i$-mediated signalling following receptor stimulation. Similarly, interactions of the PDZ scaffold membrane-associated guanylate kinase, WW and PDZ domain-containing protein 3 (MAGI3) with the GPCRs Frizzled 4 (REF. 47) and LPAR2 (REF. 48) enhance receptor-mediated activation of the mitogen-activated protein kinase (MAPK) extracellular signal-regulated kinase (ERK) pathway. Conversely, associations of LPAR2 with two related PDZ scaffold proteins, PDZ-RhoGEF and leukaemia-associated RhoGEF (LARG; also known as ARHGEF12), do not result in enhanced downstream MAPK activation but rather potentiate LPAR2-induced stimulation of Rho signalling to modify cytoskeleton dynamics[49]. Thus, LPAR2 can preferentially couple to downstream PLCβ activation[35], MAPK activation[48] or Rho activation[49], in a cell type-specific manner, depending on which LPAR2-interacting PDZ scaffold protein is expressed. Other prominent examples of GPCR-associated proteins that enhance the efficiency of G protein-mediated signalling are the Homer proteins,

Table 1 | **GPCR interactors that mediate or modulate GPCR signalling**

Interactor*	Associated GPCR	Site of interaction	Impact on GPCR	Refs
AKAP79 (AKAP5) and AKAP250 (AKAP12, gravin)	β_2AR and β_1AR	CT	Tethers PKA near to the receptor	54–58
Calmodulin	5-HT1A	i3L	Competes with PKC for GPCR phosphorylation	78
	5-HT2A	i2L and CT	Impairs G protein coupling	79
	5-HT2C	CT	Promotes arrestin-dependent ERK activation	80
	D2R	i3L	Modulates G protein signalling	81,82
	mGluR7	CT	Regulates GPCR phosphorylation	76,77
	PTH1R	CT	Inhibits GPCR activity	85
	V2R	CT	Enhances GPCR-induced Ca^{2+} signalling	84
	μOPR	i3L	Inhibits G protein coupling	83
Homer	mGluR1 and mGluR5	CT	Regulates GPCR signalling and localization	51,52, 126–128, 153,154
INAD	Rhodopsin	CT	Enhances speed and efficiency of GPCR signalling	22–29
JAK2	AGTR1 and PTAFR	CT	Promotes Jak–STAT signalling	9,10,12–15
LARG (ARHGEF12)	LPAR2	CT	Facilitates GPCR-mediated activation of Rho	49
MAGI3	Frizzled 4 and LPAR2	CT	Potentiates GPCR-mediated activation of the MAPK ERK	47,48
MUPP1 (MPDZ)	$GABA_B$ and MT_1	CT	Enhances GPCR-mediated $G\alpha_i$ signalling	45,46
Neurochondrin	MCHR1	CT	Disrupts G protein-mediated signalling	88
NHERF1 (EBP50, SLC9A3R1)	PTH1R	CT	Enhances $G\alpha_q$-mediated receptor signalling	31–34
	β_2AR and κOPR	CT	Mediates activation of Na^+–H^+ exchange	17–20,122
NHERF2 (SLC9A3R2)	LPAR2	CT	Enhances $G\alpha_q$-mediated receptor signalling	35
	mGluR5 and P2RY1	CT	Prolongs GPCR-mediated Ca^{2+} signalling	36,37
	PTH1R	CT	Enhances $G\alpha_q$-mediated receptor signalling	30
p85	SSTR2	i1L and CT	Mediates survival signalling by receptor	21
PDZ-RhoGEF (ARHGEF11)	LPAR2	CT	Facilitates GPCR-mediated activation of Rho	49
Periplakin	MCHR1 and μOPR	CT	Impairs G protein-mediated signalling	86,87
Spinophilin	D2R	i3L	Reduces G protein and arrestin-mediated signalling	61
	α_2AR and mAChR	i3L	Reduces GPCR-mediated Ca^{2+} signalling	62–65

*Not included in this list are GPCR interactions with G proteins, GPCR kinases, arrestins, regulators of G protein signalling proteins or other receptors. Alternative protein names are provided in brackets. AGTR1, type 1 angiotensin II receptor; AKAP, A-kinase anchor protein; AR, adrenergic receptor; CT, carboxyl terminus, D2R, D2 dopamine receptor; GABA, γ-aminobutyric acid; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; i1L, first intracellular loop; i2L, second intracellular loop; i3L, third intracellular loop; INAD, Inactivation-no-after-potential D protein; JAK2, janus kinase 2; LARG, leukaemia-associated RhoGEF; LPAR2, lysophosphatidic acid receptor 2; mAChR, muscarinic acetylcholine receptor; MAGI3, membrane-associated guanylate kinase, WW and PDZ domain-containing protein 3; MAPK, mitogen-activated protein kinase; MCHR1, melanin-concentrating hormone receptor 1; mGluR, metabotropic glutamate receptor; MT1, melatonin type 1 receptor; MUPP1, multiple PDZ domain protein; NHERF, Na^+–H^+ exchange regulatory factor; OPR, opioid receptor; P2RY1, purinergic receptor; PKA, protein kinase A; PKC, protein kinase C; PTAFR, platelet-activating factor receptor ; PTH1R, parathyroid hormone 1 receptor; SSTR2, somatostatin receptor type 2; STAT, signal transducers and activators of transcription; V2R, vasopressin V2 receptor.

which associate with mGluR1 and mGluR5 (REFS 50–53), and members of the A-kinase anchoring protein (AKAP) family, which interact with β-adrenergic receptors[54–58]. In addition, Homer proteins also interact with intracellular $Ins(1,4,5)P_3$ receptors, thereby linking $Ins(1,4,5)P_3$ receptors, mGluRs and other components to increase the efficiency of mGluR-stimulated Ca^{2+} signalling[52,59,60].

The AKAPs were originally named because of their associations with protein kinase A (PKA). Indeed, the interactions of A-kinase anchor protein 79 kDa (AKAP79; also known as AKAP5)[55] and AKAP250 (also known as AKAP12 and gravin)[54,56,57] with β_2AR tether PKA in the vicinity of the receptor and increase the efficiency of PKA-mediated phosphorylation of various substrates that

are downstream of receptor activation, including β_2AR itself. The consequences of the increased functional relationship between PKA and β_2AR include the enhanced efficiency of receptor resensitization[54,57] and more robust β_2AR-mediated ERK signalling[55].

Interactors that reduce G protein-mediated signalling. In contrast to the above examples of GPCR-interacting proteins, which increase the efficiency of certain GPCR-stimulated signalling pathways, some GPCR-interacting proteins associate with GPCRs to decrease the efficiency of G protein-mediated signalling. The arrestins are perhaps the most general example of this phenomenon. A more receptor-specific example is spinophilin, which interacts with the third intracellular loop of a few GPCRs, including members of the dopamine, adrenergic and muscarinic acetylcholine receptor families[61–65]. Spinophilin also binds to several members of the regulators of G protein signalling (RGS) family of proteins. RGS proteins tightly regulate the intensity and time course of GPCR signalling by accelerating the inherent GTPase activity of activated Gα subunits. Thus, spinophilin tethers RGS proteins in close proximity to GPCRs to attenuate receptor-stimulated G protein signalling[63,64]. Interestingly, RGS proteins can also associate directly with the intracellular regions of some GPCRs to inhibit their signalling and exert cell type-specific regulation of their activity[66–71]. RGS proteins have been reviewed in detail elsewhere[72,73] and are therefore not discussed further here.

Calmodulin is another protein that can interact with a variety of GPCRs to modulate their functional properties. This widely expressed Ca^{2+}-binding protein can associate in a Ca^{2+}-sensitive manner with mGluRs[74–77] and serotonin (also known as 5-hydroxytryptamine (5-HT))[78–80], dopamine[81,82] and other[83–85] receptors. The functional effects of calmodulin interaction vary depending on the GPCR, but perhaps the most commonly reported effect of calmodulin–GPCR interaction is an attenuation of G protein coupling[79,81,83,85]. As stimulation of many GPCRs can result in a downstream increase in cellular Ca^{2+} levels, the Ca^{2+}-dependent interaction of calmodulin with GPCRs can, in some cases, represent a form of feedback inhibition that restrains GPCR-initiated G protein signalling. However, G protein-independent signalling pathways can actually be potentiated by GPCR interactions with calmodulin in some situations, as calmodulin association with the 5-HT2C receptor strongly promotes arrestin-mediated signalling (but not G protein-mediated signalling) by the receptor in both transfected cells and cultured neurons[80].

Two other proteins that can associate with a few GPCRs to tone down G protein-mediated signalling are periplakin and neurochondrin. Periplakin was first reported to associate with the C-terminus of μ-type opioid receptor (μOPR)[86] and melanin-concentrating hormone receptor 1 (MCHR1)[87], and neurochondrin was found to interact with the same region of the MCHR1 C terminus as periplakin[88]. Both periplakin and neurochondrin have recently been shown to also interact with a few other GPCRs[89]. For all of the GPCRs that associate with periplakin and neurochondrin, the primary functional consequence is an attenuation of G protein-mediated signalling[86–89] (FIG. 2b). As periplakin and neurochondrin exhibit discrete patterns of distribution in the brain and other tissues[89], it seems likely that they contribute to the cell-context-dependent sculpting of receptor signalling for the various GPCRs with which they interact. Considered together with the other GPCR partners discussed in this section, the emerging theme is that GPCR-interacting proteins can exert bidirectional effects on the efficiency of G protein-mediated signalling to impart cell type-specific fine-tuning of GPCR activity.

Regulation of GPCR trafficking

GPCRs are typically trafficked to the plasma membrane to achieve functional activity. Following agonist stimulation, most receptors are internalized into endosomes and then either targeted for lysosomal degradation or recycled back to the plasma membrane. A number of GPCR-interacting proteins have been shown to exert dramatic effects on both the biosynthetic trafficking and the post-endocytic sorting of particular GPCRs.

Interactors that regulate biosynthetic trafficking. GPCRs must be properly folded after translation and, in most cases, transported to the plasma membrane to achieve functional activity. A number of GPCR-interacting proteins can regulate the folding, biosynthetic trafficking and surface expression of receptors in a cell type- and receptor-specific manner. A classic example is the *D. melanogaster* neither inactivation nor afterpotential protein A (NINAA), which associates with the GPCR rhodopsin to enhance the receptor's folding and forward trafficking[90,91]. Similarly, Ran-binding protein 2 is a vertebrate homologue of NINAA that associates with vertebrate opsins to enhance the biosynthetic trafficking of these GPCRs[92]. Another protein that regulates rhodopsin trafficking is the dynein light chain component T-complex testis-specific protein 1 homologue (TCTEX1; also known as DYNLT1), which directly associates with vertebrate rhodopsin to promote its trafficking to the cell surface[93,94]. Other vertebrate proteins that have been found to act as chaperones to enhance GPCR-specific surface expression include glandular epithelial cell protein 1 (GEC1; also known as GABARAPL1)[95,96], receptor of activated protein kinase C1 (RACK1; also known as GNB2L1)[97], dopamine receptor-interacting protein of 78 kDa (DRIP78; also known as DNAJC14)[98,99], AT2 receptor binding protein of 50 kDa (ATBP50)[100] and ubiquitin-specific-processing protease 4 (USP4)[101], which are listed in TABLE 2. For each of these GPCR-interacting proteins, their expression level in a given cell type can strongly influence the level of functional receptor expression for the particular GPCRs with which they interact.

Olfactory receptors, the largest subfamily of GPCRs, have proved notoriously difficult to study in heterologous cells (that is, in cells other than olfactory sensory neurons, which naturally express olfactory GPCRs) because of their poor trafficking to the plasma membrane[102]. This trafficking deficiency suggests a key role for chaperone proteins in the cell type-specific control of anterograde olfactory receptor trafficking in olfactory sensory neurons. Indeed, *Caenorhabditis elegans* odorant response

Heterologous cell
A cell that lacks endogenous expression of a gene of interest but is manipulated, for example by transfection or viral infection, to express the gene.

Table 2 | **GPCR interactors that regulate GPCR trafficking, targeting and/or ligand binding**

Interactor*	Associated GPCR	Site of interaction	Impact on GPCR	Refs
ATBP50	AGTR2	CT	Enhances GPCR surface expression	100
DRIP78 (DNAJC14)	AGTR1 and D1R	CT	Promotes GPCR surface expression	98,99
GASP1	CNR1, D2R and δOPR	CT	Targets GPCR to lysosomes for degradation	114–117
GEC1 (GABARAPL1)	EP3R and κOPR	CT	Enhances GPCR surface expression	95,96
Homer	mGluR1 and mGluR5	CT	Regulates GPCR signalling and localization	51,52,126–128,153,154
M10 MHC	V2R	Unknown	Enhances GPCR surface expression	108
MAGI2	β_1AR	CT	Promotes agonist-induced β_1-AR internalization	138
MPP3	5-HT2C	CT	Inhibits agonist-induced 5-HT2C internalization	139
MRAP and MRAP2	MC2R	NT and TM	Promotes forward trafficking of GPCR	109–113
MUPP1	5-HT2C	CT	Induces GPCR clustering	155
	$GABA_B$	CT	Increases GPCR stability	45
	SSTR3	CT	Targets GPCR to epithelial tight junctions	144
NHERF1 (EBP50, SLC9A3R1)	β_2AR and κOPR	CT	Promotes GPCR recycling	17–20,122
NINAA	Rhodopsin	Unknown	Promotes GPCR biogenesis and trafficking	90,91
ODR4	ODR10	Unknown	Facilitates GPCR folding and surface expression	103
PICK1	mGluR7a	CT	Facilitates GPCR clustering in presynaptic active zones	140,141,156
PSD95	5-HT2A	CT	Impairs GPCR internalization and directs localization	134,135
	β_1AR	CT	Attenuates agonist-promoted GPCR internalization	136,138
RACK1 (GNB2L1)	TXA2R	i1L and CT	Promotes forward trafficking of GPCR	97
RAMP1	CRLR	NT and TM	Forms functional CGRP receptors	146
	CALCR	NT and TM	Forms functional amylin receptors	149,150
RAMP2	CRLR	NT and TM	Forms functional adrenomedullin receptors	146
RAMP3	CRLR	NT and TM	Forms functional adrenomedullin receptors	147,148
	CALCR	NT and TM	Forms functional amylin receptors	149,150
RanBP2	Opsin	Unknown	Promotes forward trafficking of GPCR	92
REEPs	OR	Unknown	Promotes forward trafficking of GPCR	104
RTPs	OR	Unknown	Promotes forward trafficking of GPCR	104,105
RTPs and REEPs	T2R	Unknown	Promotes GPCR surface expression	106
RTP4	μOPR and δOPR	CT	Promotes heterodimer surface expression	107
Shank	LPHN1 (CL1)	CT	Promotes GPCR clustering	132
	mGluR1 and mGluR5	CT	Anchors GPCR in mature dendritic spines	129
SNX1	PAR1 (F2R)	CT	Facilitates agonist-promoted GPCR degradation	119,120
Syntrophin	α_{1D}AR	CT	Enhances GPCR stability	142,143
TCTEX1 (DYNLT1)	Rhodopsin	CT	Promotes apical delivery of GPCR in polarized cells	93,94
USP4	α_{2A}AR	CT	Enhances GPCR surface expression	101

*Not included in this list are GPCR interactions with G proteins, GPCR kinases, arrestins, regulators of G protein signalling proteins or other receptors. Alternative protein names are provided in brackets. AR, adrenergic receptor; AGTR1, type-1 angiotensin II receptor; AGTR2, type-2 angiotensin II receptor; ATBP50, AT2 receptor binding protein of 50 kDa; CALCR, calcitonin receptor; CGRP, calcitonin gene-related peptide; CNR1, cannabinoid receptor 1; CRLR, calcitonin receptor-like receptor; CT, carboxyl terminus; D1R, D1 dopamine receptor; D2R, D2 dopamine receptor; DRIP78, dopamine receptor-interacting protein of 78 kDa; EP3R, prostaglandin E receptor 3; $GABA_B$, γ-aminobutyric acid B; GASP1, GPCR-associated sorting protein 1; GEC1, glandular epithelial cell protein 1; GPCR, G protein-coupled receptor; i1L, first intracellular loop; LPHN1, latrophilin 1; MAGI2, membrane-associated guanylate kinase, WW and PDZ domain-containing protein 2; mGluR, metabotropic glutamate receptor; MHC, major histocompatibility complex; MPP3, MAGUK p55 subfamily member 3; MC2R, melanocortin receptor 2; MRAP, MC2R-accessory protein; MUPP1, multiple PDZ domain protein; NHERF1, $Na^+ - H^+$ exchange regulatory factor 1; NINAA, neither inactivation nor afterpotential protein A; NT, amino terminus; ODR, odorant response abnormal protein; OPR, opioid receptor; OR, olfactory receptor; PAR1, proteinase-activated receptor 1; PICK1, protein interacting with C kinase; PSD95, postsynaptic density protein 95; RACK1, receptor of activated protein kinase C1; RanBP2, Ran-binding protein 2; RAMP, receptor activity-modifying protein; REEP, receptor expression-enhancing protein; RTP, receptor transporting protein; SNX1, sorting nexin 1; SSTR3, somatostatin receptor type 3; TCTEXI, T-complex testis-specific protein 1; T2R, taste receptor type 2; TM, transmembrane; TXA2R, thromboxane A2 receptor; USP4, ubiquitin-specific-processing protease 4; V2R, vasopressin V2 receptor.

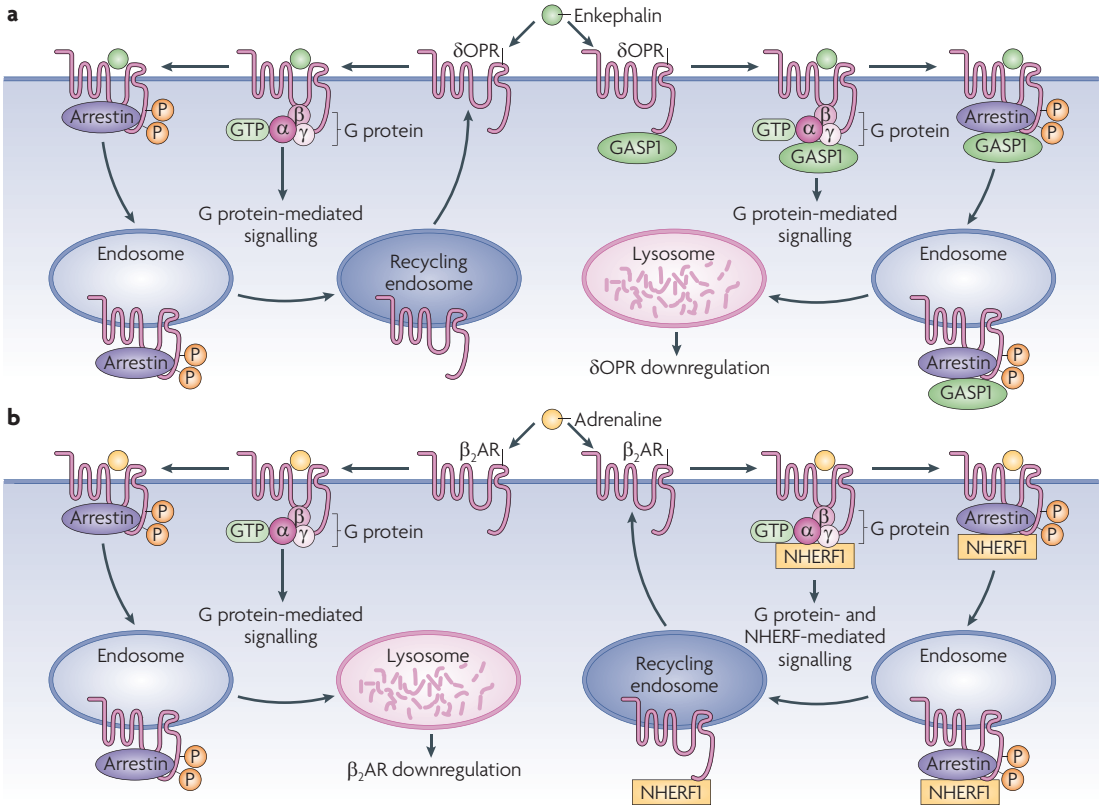


Figure 3 | **GPCR-interacting proteins can regulate the post-endocytic trafficking of GPCRs.** Following agonist-induced receptor endocytosis, some G protein-coupled receptors (GPCRs) are targeted for proteolytic and/or lysosomal degradation, whereas other GPCRs rapidly recycle back to the plasma membrane. **a** | The interaction between GPCR-associated sorting protein 1 (GASP1) and δ-type opioid receptor (δOPR) promotes the endocytic targeting of agonist-internalized δOPRs to lysosomes, where the receptors are degraded. However, in a distinct cellular compartment (or distinct cell type) that lacks GASP1, as shown on the left, δOPRs are rapidly recycled back to the plasma membrane. **b** | By contrast, the interaction between the GPCR-interacting protein Na^+–H^+ exchange regulatory factor 1 (NHERF1; also known as EBP50 and SLC9A3R1) and the β_2-adrenergic receptor (β_2AR) promotes the rapid recycling of receptors following agonist-promoted internalization. However, in a distinct cellular compartment (or distinct cell type) that lacks NHERF1, as shown on the left, β_2ARs are preferentially targeted to lysosomes for degradation.

abnormal protein 4 (ODR-4) is expressed exclusively in chemosensory neurons, where it regulates the forward trafficking of chemosensory receptors such as the GPCR ODR-10 (REF. 103). In vertebrates, two unrelated families of transmembrane proteins have been shown to associate with olfactory receptors to enhance receptor trafficking to the plasma membrane: the receptor transporting proteins (RTPs) and the receptor expression-enhancing proteins (REEPs)[104,105]. Certain RTP and REEP isoforms are expressed exclusively in the olfactory epithelium, where they exert a cell type-specific enhancement of olfactory receptor trafficking[104]. However, other RTP and REEP isoforms are more widely expressed and can interact with other GPCRs, including bitter taste receptor type 2 (REF. 106) and opioid receptors[107], to enhance the trafficking of these GPCRs to the plasma membrane.

Analogous to the RTPs and REEPs, two other types of single-transmembrane proteins have been shown to control the biosynthetic trafficking of particular GPCRs. Vomeronasal type 2 receptors (V2Rs) had proved difficult to study in heterologous cells until the observation that they interact with M10 major histocompatibility complex (MHC) molecules, which associate with the MHC component β2-microglobulin to promote the surface expression of V2Rs in heterologous cells[108]. In a similar manner, melanocortin receptor 2 (MC2R)-accessory protein (MRAP) and MRAP2 can directly interact with MC2R and dramatically enhance the receptor's surface expression[109–113]. The physiological importance of MC2R–MRAP interactions *in vivo* has been well established by studies revealing that naturally occurring MRAP mutations cause defects in MC2R trafficking and function, resulting in an inherited disease known as familial glucocorticoid deficiency type 2 (REF. 109).

Familial glucocorticoid deficiency type 2
A rare, autosomal recessive disorder in which affected individuals are unresponsive to adrenocorticotropin owing to mutations in the gene encoding MRAP.

Interactors that influence post-endocytic trafficking. Most GPCRs undergo significant endocytosis from the plasma membrane in response to agonist stimulation. In some cases the receptors are recycled back to the plasma membrane, but in other cases they are targeted to lysosomes for degradation[5]. GPCR internalization is heavily influenced by two of the canonical families of GPCR-interacting proteins, the GRKs and the arrestins[2–5]. However, certain other GPCR-interacting proteins can also regulate

the endocytic trafficking of GPCRs in a more receptor-selective manner. For example, the GPCR-associated sorting proteins (GASPs) comprise a family of ten proteins, and the founding member, GASP1, was originally identified in a yeast two-hybrid screen for proteins that interact with the C terminus of the δ-type opioid receptor (δOPR)[114]. Association of δOPR with GASP1 promotes receptor trafficking to lysosomes following agonist-stimulated endocytosis[114] (FIG. 3a), and GASP1 has a similar effect on a few other GPCRs, including D2 dopamine receptor[115] and cannabinoid receptor 1 (REFS 116,117). Most of the GASP family members are preferentially expressed in the central nervous system (CNS)[118], suggesting that they might act in a cell type-specific manner to control the post-endocytic fate (recycling versus degradation) of certain CNS-enriched GPCRs.

In addition to the members of the GASP family, a few other GPCR-interacting proteins influence the post-endocytic sorting of particular receptors. For example, sorting nexin 1 (SNX1) directly associates with proteinase-activated receptor 1 (PAR1; also known as F2R) to promote PAR1 post-endocytic trafficking to lysosomes[119,120]. SNX1 can also bind to the C termini of several other GPCRs[121], but how this influences the trafficking of these receptors is still unknown. In contrast to the effects of GASPs and SNX1, which associate with GPCRs to decrease receptor recycling to the plasma membrane, GPCR interactions with NHERF1 have been found to promote receptor recycling following endocytosis[19,122–124]. As mentioned earlier, NHERF1 interactions with β_2AR and κOPR mediate certain aspects of GPCR signalling, and NHERF interactions with other GPCRs can modulate signalling by G proteins. Interestingly, mutant versions of β_2AR that cannot associate with NHERF1 are targeted much more robustly than wild-type receptors to lysosomes after agonist stimulation[122], revealing that their interaction with NHERF1 promotes receptor recycling (FIG. 3b). The association of κOPR with NHERF1 also favours GPCR recycling over GPCR targeting to lysosomes[19], and interaction of PTH1R with NHERF1 increases the plasma membrane retention of this receptor[125]. Moreover, transplantation of the NHERF1 binding motif onto the C termini of GPCRs that do not normally interact with NHERF1, such as δOPR, dramatically enhances the efficiency of receptor recycling back to the plasma membrane following endocytosis[123,124]. These findings provide an example of how certain GPCR-interacting proteins, such as NHERF1, can act as signalling intermediates, regulators of G protein signalling and regulators of receptor trafficking, analogous to the multiple roles of arrestins in the signalling and trafficking of many GPCRs[2].

Control of GPCR targeting

In addition to the effects of many of the aforementioned GPCR-interacting proteins on GPCR signalling and trafficking, some of these interactions control GPCR anchoring to discrete regions of the plasma membrane. For example, Homer proteins not only enhance the efficiency of mGluR1- and mGluR5-mediated Ca^{2+} signalling (as described above), they also facilitate the clustering and anchoring of mGluR1- and mGluR5 in postsynaptic dendritic spines[126–128]. Consequently, mGluR1 and mGluR5 can be selectively localized near the postsynaptic density, positioning them to respond to the glutamate that is released into the synaptic cleft. Members of the Shank family of PDZ scaffold proteins interact with both Homer proteins and mGluRs to further strengthen the anchoring of mGluRs to postsynaptic regions[129–131]. The Shank family of scaffolding proteins also induce clustering of the latrotoxin-binding GPCR latrophilin 1 (LPHN1; also known as CL1 and CIRL1) in heterologous cells and colocalize with latrophilin 1 at synapses in native brain tissue[132,133].

A variety of PDZ scaffold proteins, in addition to the Shank family, have been shown to regulate GPCR-specific clustering and anchoring. For example, interaction of PSD95 (also known as DLG4) with 5-HT2A receptors induces the clustering of 5-HT2A receptors in heterologous cells[134] and facilitates the targeting of 5-HT2A receptors to postsynaptic dendritic compartments in cultured cortical pyramidal neurons[135]. PSD95 also associates with the C terminus of β_1AR to facilitate the clustering of this receptor with other components of the postsynaptic density, such as NMDA (*N*-methyl-D-aspartate) receptors[136]. The anchoring of both 5-HT2A receptor and β_1AR to the plasma membrane as a result of their association with PSD95, which is known to be palmitoylated and therefore tightly membrane associated[137], greatly reduces their agonist-induced internalization[134,136,138]. By contrast, PSD95 association with 5-HT2C receptors facilitates agonist-dependent internalization, and another PDZ scaffold protein, MAGUK p55 subfamily member 3 (MPP3), associates with 5-HT2C receptors to prevent agonist-dependent internalization and to stabilize the receptors at the cell surface in primary cortical neurons[139]. Furthermore, interaction of the mGluR7a C terminus with the PDZ scaffold protein PICK1 (protein interacting with C kinase) results in the specific clustering of mGluR7a at presynaptic sites[140,141], interaction of the α_{1D}-adrenergic receptor with syntrophins enables linkage to the dystrophin-associated glycoprotein complex in smooth muscle cells[142,143], and the interaction of SSTR3 with MUPP1 enables SSTR3 to be targeted to tight junctions in epithelial cells[144]. These examples show how certain GPCR-interacting partners can selectively target GPCRs to specialized cellular compartments to promote the receptors' physiological activities.

Regulation of ligand binding

The vast majority of GPCR-interacting proteins described above associate with intracellular regions of the GPCRs. Therefore, they have no direct effects on the pharmacological properties of the GPCRs, which are typically determined by their extracellular and/or transmembrane domains. However, a few GPCR-interacting proteins have been shown to exert striking effects on the agonist selectivity of the GPCRs with which they interact. The most intensively studied examples of GPCR-interacting partners that regulate receptor pharmacology are receptor activity-modifying protein 1 (RAMP1), RAMP2 and RAMP3 (REF. 145). The RAMP proteins were initially identified in experiments designed to search for the receptor that was activated by calcitonin gene-related peptide (CGRP).

Postsynaptic density
A specialized postsynaptic compartment, highly enriched in various scaffold proteins and receptors, that is found at all asymmetric (usually glutamatergic) synapses in vertebrate central nervous systems.

Cortical pyramidal neuron
The predominant type of neuron in the neocortex. It is named after its triangular cell body.

Palmitoylation
Post-translational modification of a protein by the covalent attachment of a palmitate (a 16-carbon saturated fatty acid) to a cysteine residue through a thioester bond.

Dystrophin-associated glycoprotein complex
A series of protein subcomplexes, including complexes of the adaptor protein dystrophin, cytoskeletal proteins, the sarcoglycan complex and the α,β-dystroglycan complex, which together are required to link the cytoskeleton to the extracelluar matrix in muscle cells.

Surprisingly, it was found that expression of a functional CGRP receptor required co-expression of an orphan GPCR, known as the calcitonin receptor-like receptor (CALCRL; also known as CRLR), with a receptor-interacting partner — the single-transmembrane protein RAMP1 (REF. 146). When CALCRL was co-expressed with RAMP2, this was shown to result in the formation of receptors activated not by CGRP, but rather by a related peptide known as adrenomedullin[146]. Subsequent work has revealed that CALCRL can also interact with RAMP3 to form a distinct subtype of adrenomedullin receptor[147,148]. Moreover, expression of a distinct receptor, calcitonin receptor (CALCR), with any of the RAMPs results in the formation of receptors with unique pharmacological properties, including the preferential activation of some RAMP–CALCR combinations by a distinct peptide known as amylin[149,150]. It is clear from work in this area that the pharmacological properties of CALCR and CALCRL are heavily regulated in a cell type-specific manner depending on which of the RAMP proteins is expressed, and furthermore the RAMP proteins can also dramatically affect the surface expression levels of CALCR and CALCRL[145]. Thus, a comprehensive understanding of RAMP–GPCR interactions is essential for developing therapeutics that might target the various RAMP-interacting receptors[145].

Conclusions and perspectives

The signalling and trafficking of most GPCRs involves receptor interactions with G proteins, GRKs, arrestins and other receptors. In addition to these widespread canonical GPCR associations, there are many other types of GPCR-interacting proteins that can interact with particular receptors to fine-tune receptor activity. GPCRs are important drug targets and, because it is often desirable to achieve cell type-specific drug action in order to minimize side effects, it can be clinically useful to consider the ways in which GPCRs can be differentially modulated by therapeutics depending on the cellular context. Along these lines, there has been increasing interest over the past few years in studies on 'biased agonists' that can preferentially activate G protein-mediated over arrestin-mediated GPCR signalling pathways[151,152]. It is worth keeping in mind that agonists are likely to be differentially biased depending on the cellular context, which is determined largely by the set of GPCR-interacting proteins that regulate receptor signalling, trafficking and/or localization in a particular cell type. Thus, uncovering the full set of GPCR-interacting proteins for receptors of clinical interest could provide novel therapeutic insights by shedding light on the fundamental mechanisms controlling the fine-tuning of GPCR activity.

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DATABASES

UniProtKB: http://www.uniprot.org
AGTR1 | AKAP79 | AKAP250 | β_2AR | β_1AR | CALCR | CALCRL | CGRP | δOPR | DRIP78 | GASP1 | GEC1 | 5-HT2A | 5-HT2C | INAD | JAK2 | κOPR | LARG | LPHN1 | LPAR2 | MAGI3 | MCHR1 | mGluR5 | μOPR | MPP3 | MRAP | MRAP2 | MUPP1 | NHE3 | NHERF1 | NHERF2 | ODR-4 | ODR-10 | PAR1 | PICK1 | P2RY1 | PSD95 | PTAFR | PTH1R | PTPN11 | RACK1 | RAMP1 | RAMP2 | RAMP3 | SNX1 | SSTR2 | SSTR3 | TCTEX1 | USP4

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