G Protein-Coupled Receptors

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Abstract

G protein-coupled receptors (GPCRs) include one of the largest gene families in the mammalian genome. The diversity of receptor binding sites and coupling mechanisms provides the signaling specificity necessary to maintain homeostasis. Various G protein-coupled receptors are critical for the functioning of every endocrine system in health and disease, and these proteins are the predominant targets of therapeutic drugs. GPCRs are grouped by primary sequence into different families that all have a canonical seven alpha helical transmembrane domain structure. In recent years, solving the crystal structure for an increasing number of these receptors has helped to resolve the molecular mechanisms of ligand interaction and activation. Despite their name, they couple to cellular signaling via both heterotrimeric G proteins and G protein-independent mechanisms. Receptor and signaling regulatory mechanisms contribute to controlling the level of the cellular responses elicited. A variety of endocrine and systemic diseases are caused by specific receptor mutations.

Keywords

Crystallography • Pharmacology • Signal transduction • Genetics • Regulation

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Introduction

Homeostasis is maintained via a plethora of extracellular factors that coordinate activity among organs and cell types. These mediators include hormones, peptides, neurotransmitters, proteins, ions, and lipids that act via specific receptors to elicit cellular responses. The functional classification of receptors includes at least three general types of cell surface receptors: G protein-coupled receptors

(GPCRs), ion channel receptors, and enzyme-associated receptors. The GPCRs form the largest and most diverse mammalian receptor group. This extreme diversity of binding sites serves the role of GPCRs throughout the endocrine system to maintain signaling specificity with hormones transmitted through the bloodstream and portal circulations. GPCRs are also promising therapeutic targets. In fact, 40–50% of drugs currently available on the market target GPCRs (Stewart and Fisher 2015).

GPCRs share a topology of seven α-helical transmembrane domains (Fig. 1). This structural template shows wide evolutionary conservation. Members of the largest rhodopsin-like GPCR family can be found in yeast, slime mold, plants, and protozoa. The rhodopsin-like GPCR family comprises one of the largest gene families known. GPCRs account for more than 1% of total cellular protein.

The term GPCR refers to the association with and signaling through heterotrimeric (α-, β-, and γ-subunit) G proteins. Although ligand-bound GPCRs were originally thought to activate downstream effectors only via G protein dissociation into G_α and $G_{\beta\gamma}$ subunits, many other heterotrimeric G protein-independent transduction mechanisms have been characterized. Hence, GPCRs interact with various GPCR regulatory proteins, multidomain scaffolding proteins, and chaperone molecules. Additional factors that affect signal transduction and specificity are GPCR homo- and heterodimerization. The diversity of GPCRs, of their signaling cascades, and of their regulatory factors underlies the specificity of the cellular response required for endocrine processes.

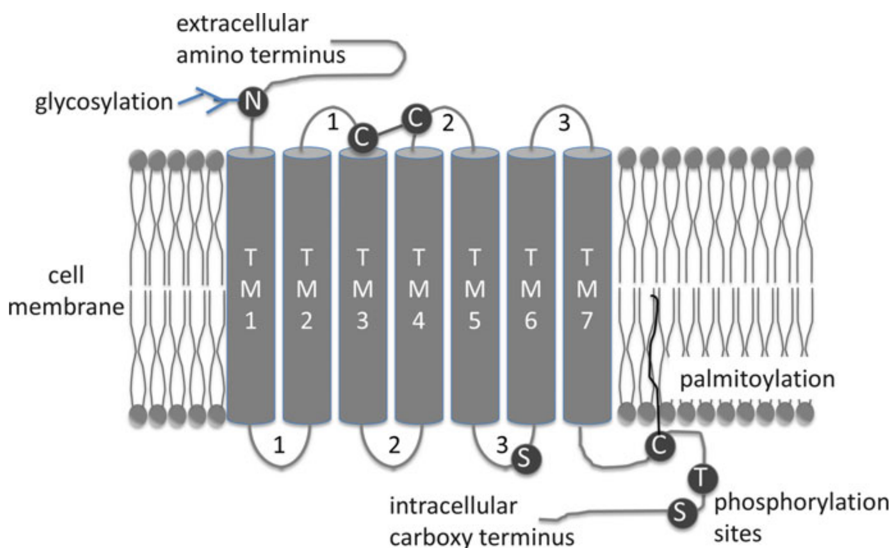

Fig. 1 Schematic of GPCR structure. Sites for extracellular glycosylation and disulfide bond formation as well as intracellular palmitoylation and phosphorylation are indicated

Classification of G Protein-Coupled Receptors

The visual pigment opsin and the β-adrenergic receptor were the first GPCRs resolved at the primary amino acid sequence level by molecular cloning in the mid-1980s. Approximately 800 GPCRs have been identified in the human genome (Davenport et al. 2013). Based on both physiological and structural features, GPCRs have been grouped into either five families (rhodopsin, adhesion, secretin, glutamate, frizzled) (Lee et al. 2015) or four classes (A, B, C, F) (Kolakowski 1994). Class A represents by far the largest group (for review, see Venkatakrishnan et al. 2014). Class A receptors have various functions, such as vision, olfaction, and regulation of immune response, and include most of the receptors for hormones. Class B comprises 47 receptors that are notably involved in glucose homeostasis and includes receptors for the hormones secretin, glucagon, and corticotropin-releasing factor. Class C consists of 15 receptors that are notably involved in synaptic transmission and includes the glutamate receptors. Class F receptors (11 members) participate in the Wnt and hedgehog signal transduction pathways. While the different GPCR classes lack significant sequence homology across families, the heptahelical transmembrane domain structure is preserved among all GPCR classes.

The structure of class A/rhodopsin family receptors has been the most studied, with the prototype rhodopsin structure being the first determined by X-ray crystallography, followed by the β_2-adrenergic receptor (β_2AR), and other class A receptors including the ternary structure of the agonist-bound (β_2AR)–Gs complex (Figs. 2 and 3). The crystal structure of the 7-transmembrane (7TM) domain has been obtained for two class B/secretin family GPCRs, the glucagon receptor (GCGR) and the corticotropin-releasing factor receptor type 1, and for two class C/glutamate family GPCRs, metabotropic glutamate receptors 1 and 5 (for review, see Lee et al. 2015).

Structural Features of G Protein-Coupled Receptors

All GPCRs share the same general structural organization, with seven hydrophobic transmembrane (7TM) α-helices interconnected by three extracellular loops (ECL) and three intracellular loops (ICL), an extracellular N-terminus, and a C-terminus located intracellularly (Fig. 1). While the size of each TM segment is conserved, varying from 20 to 27 residues, the N-terminus, loops, and C-terminus show considerable variability in length, typically ranging from tens to several hundreds of residues.

In order to compare similar amino acid sequences among different receptors, the most accepted consensus is the Ballesteros and Weinstein method. In this approach, the most conserved single residue in each transmembrane helical domain is assigned the arbitrary number 50, and each residue is numbered according to its position relative to this conserved residue. For example, 4.57 indicates an amino acid located in transmembrane segment 4, seven residues further along the sequence than the most conserved amino acid in helix 4, Trp(4.50). The most conserved amino acids of

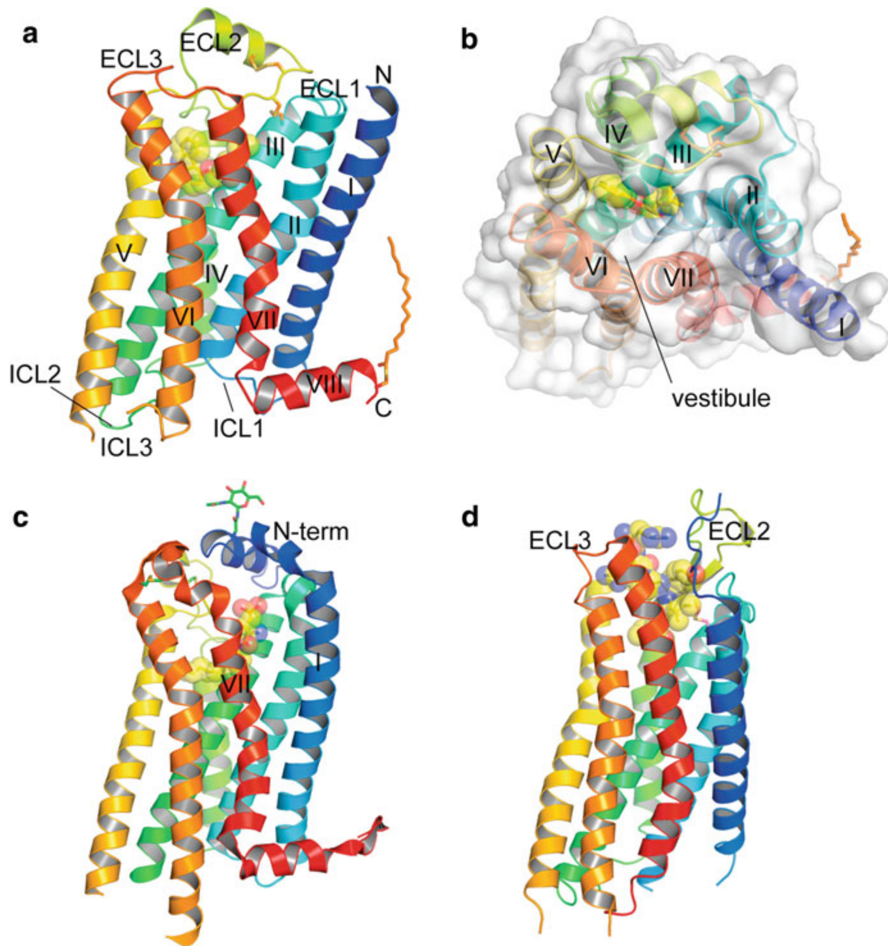

Fig. 2 Diversity of class A GPCR structures and binding sites. (**a**, **b**) Structure of the β_2-adrenergic receptor bound to the agonist carazolol from transmembrane and extracellular views. (**c**) Sphingosine-1-phosphate receptor structure bound to the antagonist ML056. (**d**) Neurotensin-bound neurotensin receptor structure. Disulfide bonds, palmitoylation, and N-terminus glycans are included (Reprinted from Lee et al. (2015))

each transmembrane segment in rhodopsin and rhodopsin-like GPCRs are $Asn^{1.50}$, $Asp^{2.50}$, $Arg^{3.50}$, $Trp^{4.50}$, $Pro^{5.50}$, $Pro^{6.50}$, and $Pro^{7.50}$. Implicit in this numbering scheme is the hypothesis that many relatively conserved amino acids at corresponding positions serve analogous structural and functional roles.

Bovine rhodopsin was the first GPCR whose crystal structure was determined, confirming the existence of seven transmembrane helices. The seven transmembrane domains form a structural core, which is involved in ligand binding and in signal transduction through structural rearrangements. The N-terminus and extracellular

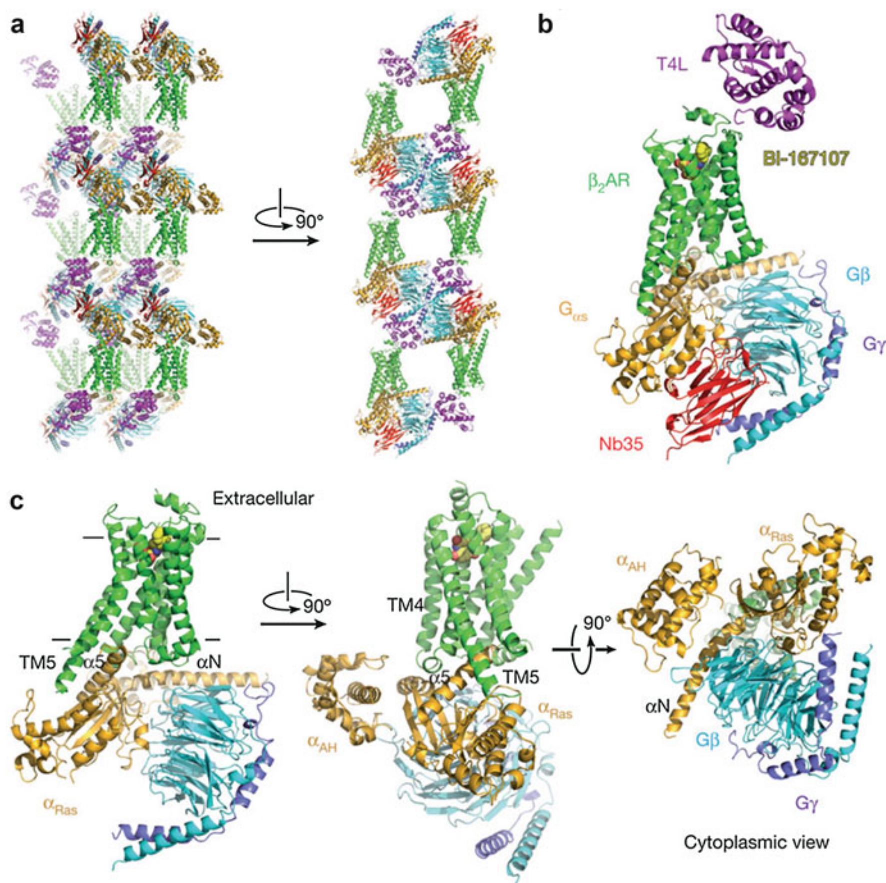

Fig. 3 Structure of the β_2AR–Gs complex. (**a**) Alternating layers of receptor and G protein within the crystal are shown. (**b**) The overall structure showing the β_2AR in *green* bound to an agonist (*yellow* spheres) and interacting with Gαs (*orange*). Gβ is *cyan* and Gγ is *purple*. A G_S binding nanobody (*red*) and T4 lysozyme (*magenta*) fused to the amino terminus of the receptor were included to facilitate crystallization. (**c**) The biological complex omitting nanobody and T4 lysozyme (Reprinted from Rasmussen et al. (2011))

loops play fundamental roles in processes related to ligand recognition and ligand access. The intracellular loops interact physically with heterotrimeric G proteins, G protein-coupled receptor kinases (GRKs), and other downstream signaling components (for review, see Zhang et al. 2015).

Crystallographic studies over the past decade and a half have confirmed the hypothesis that, although transmembrane regions display high sequence variability among GPCRs, they share conserved residues at key topological positions (for review, see Venkatakrishnan et al. 2014). One of the most conserved motifs among class A GPCRs is the D[E]$R^{3.50}$Y motif, which frequently forms an

"ionic lock" via a salt bridge with $D/E^{6.30}$. This ionic lock was identified in the rhodopsin structure (Palczewski et al. 2000). It was proposed as a domain involved in the inactive conformation of GPCRs, hindering G protein coupling at the cytoplasmic region. $W^{6.48}$xP is also described as one of the components that switch conformations between the active and inactive state of the receptor. A third conserved motif involved in GPCR activation is the $NP^{7.50}$xxY motif. Besides the transmembrane domains, extracellular loops also have some conserved motifs. Hence, most GPCRs harbor a highly conserved $Cys^{3.25}$ disulfide bond between the extracellular tip of the third transmembrane domain and a cysteine residue in the second extracellular loop. This disulfide bond stabilizes the conformation of extracellular domains and constrains the structural arrangement forming the entrance to the ligand-binding pocket. Similarly, the conformation of the intracellular loops is relatively conserved, which may be related to the limited range of GPCR binding partners.

The secondary structures in the extracellular loop region vary considerably between different receptors. For instance, the second extracellular loop has an α-helical structure in adrenergic GPCRs and a hairpin structure in all peptide GPCRs. In contrast, the first and third extracellular loops are relatively shorter and do not show distinct secondary structures.

Posttranslational Modifications

Glycosylation

Most GPCRs have at least one glycosylation site in their N-terminal domain (Wheatley and Hawtin 1999). A few GPCRs, such as the α_{2B}-adrenoceptor, lack identifiable glycosylation sites. In GPCRs that are glycosylated, complex or hybrid high-mannose oligosaccharides are linked to the Asn side chain (N-linked glycosylation).

The effects of glycosylation differ in specific GPCRs. Glycosylation is important for the stability of the GnRH and vasopressin V_{1a} receptors, but does not affect ligand binding. Glycan chains are essential for folding and trafficking of the TRH receptor, the FSH receptor, and the vasoactive intestinal peptide (VIP) 1 receptor. For the TRH, somatostatin, β_2-adrenergic, and gastrin-releasing peptide receptors, glycosylation contributes to high-affinity ligand binding and may also influence receptor–G protein coupling. For many GPCRs however, no function for glycosylation has been identified.

Palmitoylation

Covalent lipid modifications that interact with the cytoplasmic face of the cell membrane serve to anchor numerous signaling proteins (Qanbar and Bouvier 2003).

Protein fatty acylation may occur either through thioester linkages (S-acylation) or amide linkages (N-acylation). N-Acylation occurs on the amino-terminal glycines and S-acylation occurs on cysteine residues. Palmitate is the most commonly used S-linked fatty acid. Protein palmitoylation is reversible and can be regulated.

Many GPCRs are palmitoylated at cysteine residues in the intracellular C-terminal tail. Palmitoylation of GPCRs anchors the C-terminal tail to the plasma membrane, creating in effect a fourth intracellular loop. The elimination of palmitoylation sites attenuates G protein coupling of endothelin ET_B, β_2-adrenoceptors, and somatostatin SST_5 receptors (Qanbar and Bouvier 2003). The palmitoylation state governs receptor internalization by regulating accessibility to the arrestin-mediated internalization pathway (Charest and Bouvier 2003; Ponimaskin et al. 2005).

GPCR phosphorylation, which is crucial for regulation of receptor activity, is described in a later section.

Diversity of Receptor–Ligand Interaction

The cumulative resolution of structures of class A, B, C, and F receptors (for review, see Cooke et al. 2015) has not only allowed to better grasp the mechanistic details of ligand recognition, including diverse ligand-binding modes, but also improved strategies for structure-based drug design. Variations in the location and size of the ligand-binding sites are found among class A receptors (Venkatakrishnan et al. 2013) (Fig. 2). Furthermore, the antagonist CP-376395 of the class B corticotropin-releasing factor receptor 1 (CRF1) binds to a much deeper pocket than any class A receptor ligand (Hollenstein et al. 2014). The ligand-binding pocket of the negative allosteric modulator (NAM) in class C metabotropic glutamate GluR5 is narrow and located in the transmembrane region, halfway between those of class A and class B receptors (Dore et al. 2014). In contrast, the ligand-binding pocket of the class F receptor smoothened (SMO) is closer to the extracellular space than those of class A receptors, interacting with the second and third extracellular loops (Wang et al. 2013).

The physicochemical properties of the binding sites help to make ligand-binding predictions and thus have implications in drug discovery. The two main attributes of binding sites are the presence of hydrogen bonds or ionic interactions and the presence of lipophilic hotspots. Computational methods can evaluate the relative energies of water molecules and determine which ones favor or reduce ligand binding. To illustrate this, the GPCR CXCR4 has a small-molecule binding site with a single lipophilic hotspot high in the ligand-binding pocket and an unfavorable ionic interaction due to more solvent exposure. By contrast, the dopamine D3 ligand eticlopride binds at lipophilic hotspots deep in the pocket, thus dislodging several water molecules (for review, see Cooke et al. 2015).

Mechanism of Receptor Activation

In the classical model of GPCR activation, receptors are in equilibrium between an inactive (R) and an active (R*) state. Thus, a small fraction of receptors in the active state account for GPCR basal or constitutive activity (i.e., activity in the absence of agonist). In accordance with the two-state model, agonists shift the equilibrium toward the active state, whereas inverse agonists displace it toward the inactive state. Partial agonists shift the equilibrium toward the active state less strongly. Pure antagonists inhibit agonists in a competitive manner, without altering the equilibrium. However, a multi-state model has emerged, where the receptor can assume multiple distinct active and inactive states and a ligand is proposed to stabilize specific conformational states of a given GPCR (for review, see Sato et al. 2016). This multi-state model explains the existence of the phenomenon of biased agonism, described below.

The high-resolution crystal structures of the GPCR–G protein (Fig. 3) and several GPCR-agonist complexes have provided insights into the molecular mechanisms of ligand binding and the conformational changes induced by the ligand (for review, see Zhang et al. 2015). Receptor activation involves conserved motifs, called molecular microswitches, that are involved in the transitions between inactive and active states. For instance, the ionic lock involved in the inactive conformation of rhodopsin is broken during receptor activation. Transmembrane helices 3 and 6 form an ionic lock via interaction of R135 and E134 of the conserved E(D)$R^{3.50}$Y motif (TM3) and E247 and T251 of TM6. Similarly, ligand interaction induces a conformational change within the side chain of $W^{6.48}$ in the $CW^{6.48}$xP motif, resulting in the sixth transmembrane segment moving outward with subsequent GPCR activation (Park et al. 2008; Scheerer et al. 2008).

Another important feature of GPCR activation is the rearrangement of the transmembrane helices around proline bends. Class A GPCRs have highly conserved prolines in TM5, TM6, and TM7. The activation of class A GPCRs involves helical rearrangement, such as a proline-induced deformation of TM5, rotation and translation of TM6, and inward repositioning of TM7 (for review, see Venkatakrishnan et al. 2014). Classes B and F GPCRs have prolines at similar positions in TM4 and TM5, and class C receptors have two conserved prolines in TM6 and TM7 (see Venkatakrishnan et al. 2014). It is hypothesized that these prolines also contribute to conformational changes occurring during receptor activation.

Biased Agonism

Many GPCRs activate multiple downstream signaling pathways. Different agonists acting at the same GPCR may induce very different relative activation of these multiple signaling pathways coupled to that receptor. The signaling selectivity represented by this biased agonism (originally called “stimulus trafficking”) can

contribute to the effects of therapeutic agonists and is important in drug discovery (for review, see Violin et al. 2014).

Agonist bias is believed to result from GPCRs having distinct active conformations that differ in their activation of different signaling pathways and from the capacity of certain agonists to stabilize a particular pattern of active GPCR conformations. An example of agonist bias is the signaling effects of psychedelic or non-psychedelic serotonin 5-HT_{2A} receptor agonists (Gonzalez-Maeso et al. 2007; Schmid and Bohn 2010). Additional findings that support the fundamental role of biased agonism in whole animal models include the modulation of circadian glucocorticoid oscillation via CXCR7 receptors recruiting β-arrestin-dependent signaling by intermediate peptides (Ikeda et al. 2013), as well as the recent discovery of an opioid analgesic (PZM21) that activates Gi proteins with high selectivity for the μ-opioid receptor and minimal β-arrestin-2 recruitment (Manglik et al. 2016). Considering that morphine and other opioids induce respiratory depression via μ-opioid through the β-arrestin interaction, whereas their analgesic effects are G protein-dependent, these findings may provide the basis for the development of new opioid ligands with improved analgesic and less unwanted respiratory effects.

Receptor–G Protein Coupling and Selectivity

The binding of a ligand to a GPCR induces a conformational change that promotes the formation of active Gα-GTP and the release of Gβγ dimer (Fig. 4). The G proteins in turn stimulate downstream effectors including enzymes (adenylate cyclases, phospholipases), ion channels, and protein kinases (for review, see Stewart and Fisher 2015) (Fig. 5).

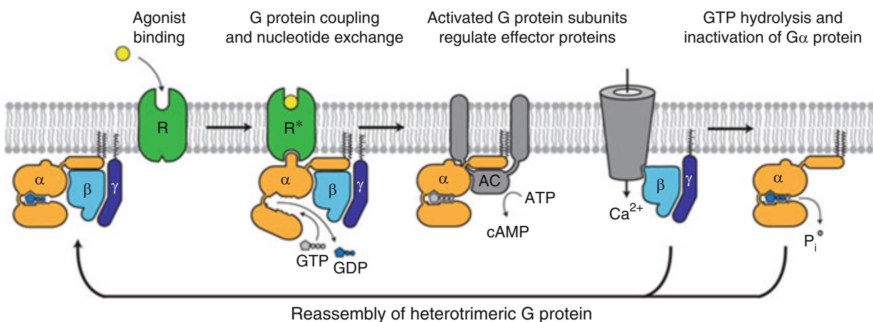

Fig. 4 G protein cycle. Agonist binding to the receptor leads to conformational rearrangements of the cytoplasmic ends of transmembrane segments that enable the G_s heterotrimer (α, β, and γ) to bind the receptor. GDP is released from the α-subunit upon formation of GPCR–G protein complex. GTP binds to α-subunit resulting in dissociation of the α- and βγ-subunits from the receptor. The subunits regulate their respective effector proteins. The G protein heterotrimer reassembles from α- and βγ-subunits following hydrolysis of GTP to GDP by the intrinsic GTPase activity of the α-subunit (Reprinted from Rasmussen et al. (2011))

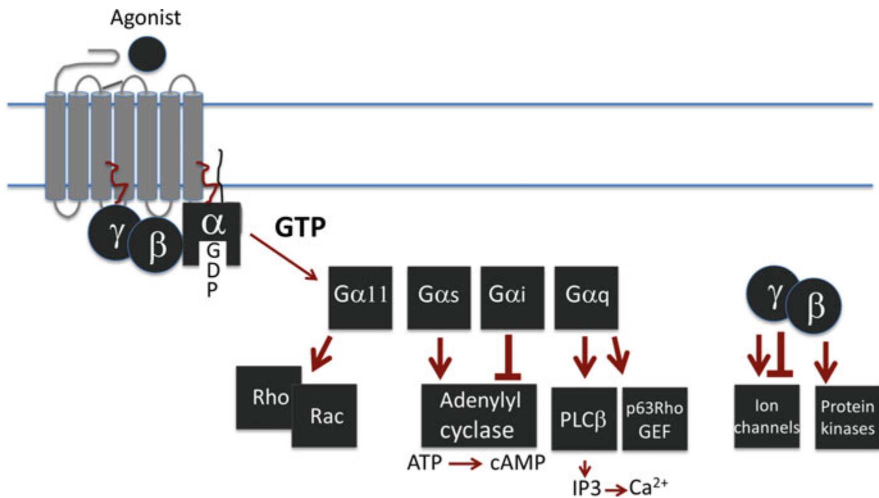

Fig. 5 Diversity of G protein signaling mechanisms. Heterotrimeric G proteins stimulate second messengers such as Ca^{2+}, cAMP, protein kinase activity, and ion channel activity. In conjunction with additional non-G protein mechanisms, these signals generate an integrated cellular response

Heterotrimeric G Proteins

Gilman and Rodbell received the Nobel Prize in Physiology in 1994 for the discovery of G proteins and their role in signal transduction. In the 1990s, scientists characterized the crystal structures of G proteins such as Gαs, Gαt, Gαi, Gβγ dimer, and Gαβγ heterotrimer (for review, see Duc et al. 2015).

G proteins bind and cause the hydrolysis of guanine nucleotides. Heterotrimeric G proteins are composed of three subunits (α, β, and γ) (Milligan and Kostenis 2006; Oldham and Hamm 2008). In its inactive state, the Gα subunit binds guanosine diphosphate (GDP), and in its active state, it binds guanosine triphosphate (GTP). The β- and γ-subunits are tightly bound to each other to form a dimer. The exchange of GDP for GTP is facilitated by the conformational change induced by the agonist binding to the GPCR. The GTP-bound Gα subunit and the βγ-dimer each activate downstream effectors (Fig. 4).

Twenty-one Gα, 6 Gβ, and 12 Gγ subunits are found in humans. Most Gα subunits are expressed ubiquitously. The four major classes (Gs, Gi/o, Gq/11, and G12/13) of G proteins are based on Gα subunit sequence similarities (Baltoumas et al. 2013). The various heterotrimeric complexes generated by combining these different Gα, Gβ, and Gγ subunits influence the specificity of both GPCRs and their downstream signal transduction (Oldham and Hamm 2008).

Crystallography revealed that the Gα subunit consists of two domains, a domain similar to Ras-like small GTPases with binding sites for $G_{\beta\gamma}$ and an α-helical domain, which is thought to segregate the guanine nucleotide in the GTP-binding

domain; the guanine nucleotide-binding pocket is positioned between the Ras-like and the helical domains (for review, see Duc et al. 2015; Stewart and Fisher 2015). The Gβ subunit includes an N-terminal α-helix and a seven-bladed β-propeller motif. The Gγ subunit is composed of two α-helices connected by a linker loop. Gβ dimerizes with Gγ via a coiled-coil interaction between the N-terminal helix of Gβ and the N-terminal helix of Gγ (Sondek et al. 1996).

How a G protein gets activated and transmits the signal from a GPCR to its effectors can be recapitulated as follows: ligand binding to the GPCR induces a conformational change in the receptor, as described earlier, that promotes G protein binding and the release of GDP from the Gα G protein subunit. Next, GTP binds to Gα, which induces the dissociation of Gα from the Gβγ dimer. Both GTP-bound Gα and the released Gβγ dimer can then activate downstream effector molecules. Deactivation of the G protein occurs via the GTPase activity of Gα, which hydrolyzes GTP into GDP and leads to heterotrimer reassociation (Fig. 4). The rate of hydrolysis varies among the different classes of G proteins (for review, see Duc et al. 2015; Stewart and Fisher 2015).

Molecular Basis of Receptor–G Protein Coupling

Although GPCRs are numerous and diverse, and G proteins exhibit some degree of variety, GPCRs interact only with a few G proteins, as defined by their Gα subunit (Milligan and Kostenis 2006; Oldham and Hamm 2008). Accordingly, GPCRs are typically distinguished by their $G_{i/o}$, G_s, or $G_{q/11}$ coupling (Table 1).

In 2011, the Kobilka group elucidated how a GPCR activates a G protein when they determined the X-ray crystal structure of the β_2-adrenoceptor-G protein complex (Fig. 3). They observed a major displacement of the α-helical domain of Gα relative to the Ras-like domain upon receptor binding, causing the opening of the nucleotide-binding pocket (Rasmussen et al. 2011). The main interactions between the receptor and Gαs involve the rotation and movement of the C-terminal α5-helix of the Gαs Ras-like domain toward the β_2-adrenergic receptor, which propagates the conformational changes from the agonist-bound receptor to the nucleotide-binding pocket. With regard to the GPCR regions involved in the GPCR–G protein interface, the X-ray crystal structure of receptor–Gs complex revealed that the binding regions of the receptor comprise transmembrane domains 3, 5, and 6 and intracellular loops 2 and 3. The C-terminus of Gαs, which contacts TM3, TM5, TM6, and parts of ICL2 (Rasmussen et al. 2011), may provide the selectivity of the GPCR–G protein coupling. Other G protein regions may interact with GPCRs (Mnpotra et al. 2014; Rasmussen et al. 2011).

Regulation of Receptor–G Protein Coupling by RNA Editing

RNA editing is a molecular process that creates diversity both at the RNA and at the protein level. Deamination of adenosine into inosine (A to I) is a typical RNA editing

Table 1 Classification of selected GPCRs relevant to endocrinology, according to the current IUPHAR database

GPCR class	Family name	Ligand	Principal transduction
A (rhodopsin-like)	5-Hydroxytryptamine receptors	5-Hydroxytryptamine	$G_{i/o}$ (subtypes 1A, 1B, 1D, 1E, 1F), $G_{q/11}$ (subtypes 2A, 2B, 2C), G_s (subtypes 4, 6, 7), G_i/G_o (subtype 5A)
	Acetylcholine receptors (muscarinic)	Acetylcholine	$G_{q/11}$ (subtypes M1, M3, M5), $G_{i/o}$ (subtypes M2, M4)
	Angiotensin receptors	Angiotensin	$G_{q/11}$ (subtype 1), G_i/G_o, Tyr and Ser/Thr phosphatases (subtype 2)
	Apelin receptor	Apelin-36, apelin-13, apelin-17; apelin receptor early endogenous ligand	$G_{i/o}$
	Bradykinin receptors	Bradykinin, kallidin, T-kinin	$G_{q/11}$ (B1 and B2 receptors)
	Galanin receptors	Galanin, galanin-like peptide	$G_{i/o}$ (subtypes 1, 3), $G_{i/o}$, $G_{q/11}$ (subtype 2)
	Ghrelin receptor	Ghrelin	$G_{q/11}$
	Glycoprotein hormone receptors	FSH, hCG, LH, TSH	G_s (FSH receptor), G_s, $G_{q/11}$ (LH receptor), all four families of G proteins (TSH receptor)
	Gonadotropin-releasing hormone receptors	GnRH I, GnRH II	$G_{q/11}$ (subtypes 1, 2)
	G protein-coupled estrogen receptor	17β-estradiol	G_s, $G_{i/o}$
	Kisspeptin receptor	Kisspeptin-10, kisspeptin-13, kisspeptin-14, kisspeptin-54	$G_{q/11}$
	Neurotensin receptors	Large neuromedin N, large neurotensin, neuromedin N, neurotensin	$G_{q/11}$
	Orexin receptors	Orexin-A, orexin-B	$G_{q/11}$
	Prolactin-releasing peptide receptor	PrRP-20, PrRP-31	$G_{q/11}$
	Somatostatin receptors	CST-17, SRIF-14, SRIF-28	G_i
	Thyrotropin-releasing hormone receptors	TRH	G_q
	Vasopressin and oxytocin receptors	Oxytocin, vasopressin	$G_{q/11}$ (subtypes V_{1A}, V_{1B}), G_s (subtype V_2), $G_{q/11}$, $G_{i/o}$ (subtype OT)

(*continued*)

Table 1 (continued)

GPCR class	Family name	Ligand	Principal transduction
B (secretin receptor family)	Calcitonin receptors	Amylin, calcitonin, α-CGRP, β-CGRP, etc.	G_s
	Corticotropin-releasing factor receptors	Corticotropin-releasing hormone, urocortin 1, 2, 3	G_s
	Glucagon receptor family	GHRH, gastric inhibitory polypeptide, glucagon, secretin, etc.	G_s
	Parathyroid hormone receptors	PTH	G_s, $G_{q/11}$
	VIP and PACAP receptors	PACAP-38, PACAP-27, PHM, PHV, VIP	G_s
C (metabotropic glutamate)	Calcium-sensing receptors	Ca^{2+}, Mg^{2+}	$G_{q/11}$, $G_{i/o}$, $G_{12/13}$ (CaS receptor), unknown ($GPRC_6$ receptor)
	Class C orphans	Unknown	Unknown
	$GABA_B$ receptors	GABA	$G_{i/o}$
	Metabotropic glutamate receptors	L-Glutamic acid	$G_{q/11}$ (subtypes 1, 5), $G_{i/o}$ (subtypes 2, 3, 4, 6, 7, 8)
F (frizzled/ smoothened)	Class frizzled GPCRs	WNTs	Unknown

event that affects precursor and mature mRNAs and results in an alteration of amino acid sequences (as inosine is recognized as guanosine during translation). Transcripts of the human serotonin 2C receptor, for example, are subject to A-to-I RNA editing, thereby generating multiple receptor isoforms that vary in constitutive activity and G protein coupling efficacy (for review, see O'Neil and Emeson 2012). RNA editing also can increase receptor diversity. 5-HT_{2C} transcripts have five A-to-I editing sites, with predicted amino acid sequence alterations affecting the second intracellular loop; up to 24 receptor isoforms can be produced.

Effect of Posttranslational Modifications on Receptor–G Protein Coupling Selectivity

The best defined GPCR regulatory mechanisms are mediated by G protein-coupled receptor kinases (GRKs), arrestins, and regulator of G protein signaling (RGS) proteins.

The standard allosteric two-state (off–on) model of GPCR activation has evolved into a complex paradigm of functional selectivity based on multisite phosphorylation. G protein-coupled receptor kinases (GRKs) are recruited to the receptor and

phosphorylate cytosolic segments, thereby recruiting β-arrestins, which besides sterically hindering the G protein interaction also can serve as signal transducers (Lefkowitz and Shenoy 2005). Based on mass spectrometry analyses, β_2AR has 13 serine/threonine phosphorylation sites in the third intracellular loop and the C-terminal tail, which are phosphorylated by multiple kinases. GRK2 and GRK6, which have different phosphorylation sites on the receptor, induce distinct conformations of β-arrestin upon its recruitment to the receptor and subsequently distinct patterns of downstream signaling. Overall, evidence suggests that the different phosphorylation patterns of GRKs establish a "barcode" that ultimately determines different β-arrestin functional capabilities (for review, see Prabakaran et al. 2012).

Regulators of G Protein Signaling Proteins (RGS Proteins)

RGS proteins are negative regulators of G protein signaling. They accelerate the GTPase activity of Gα, thereby promoting the reassociation of the heterotrimeric complex with the GPCR and the termination of signaling to downstream effectors (Fig. 6). Thus, RGS proteins determine the extent of the cellular response to GPCR stimulation (for review see Stewart and Fisher 2015).

There are 20 mammalian RGS proteins that function as GTPases, accelerating proteins or GAPs for $G\alpha_{i/o}$, $G\alpha_{q/11}$, or both. Another 20 RGS proteins contain nonfunctional RGS homology domains that frequently serve as an interface with GPCRs or Gα subunits. Resolution of the crystal structure of the RGS protein–Gα complex has revealed the mechanism by which RGS catalyzes GTP hydrolysis by Gα by stabilizing the transition state of Gα for nucleotide hydrolysis (Berman et al. 1996; Tesmer et al. 1997). As they can compete for effector binding, RGS proteins also have the ability to modulate adenylate cyclase, MAPK, IP_3/Ca^{2+} signaling, K^+ conductance, and visual signaling (Neubig and Siderovski 2002; Yan et al. 1997).

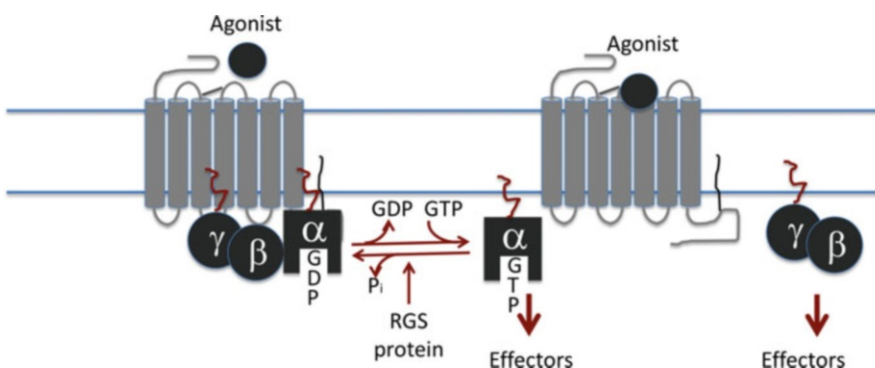

Fig. 6 G protein regulation by RGS proteins. RGS proteins accelerate the intrinsic GTPase activity of Gα and promote reassociation of the heterotrimeric complex with the receptor at the cell membrane, thereby terminating signaling

Activators of G Protein Signaling (AGS)

AGS proteins, in contrast with RGS proteins, use diverse mechanisms to activate G proteins. They are organized in four groups: group I, guanine nucleotide exchange factors (GEFs); group II, guanine nucleotide dissociation inhibitors (GDIs); group III, *G*βγ binding proteins; and group IV, Gα16 binding proteins (for review, see Park 2015).

Group I AGS proteins, which facilitate the exchange of GDP for GTP on Gα, do so in the absence of GPCRs (for review, see Blumer and Lanier 2014). They also demonstrate selectivity in their interaction with G proteins. Group II AGS proteins carry one to four GPR motifs that stabilize GDP-bound Gα. Group III are *G*βγ-interacting proteins that show nonselectivity for Gα. The fourth group comprises AGS11–13 that are selective for Gα16, but their mechanism of action remains to be elucidated. Groups I–III are thought to act together in a core signaling triad (GEF/GαGPR/*G*βγ-interacting proteins) that is akin to the one formed by GPCR/Gαβγ/effector.

G Protein-Dependent Signaling Effectors

GPCRs generate a variety of cellular responses, ranging from intracellular production of cAMP to induction of gene transcription. GPCRs can stimulate different families of G proteins ($G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12/13}$ in mammals). Receptors that interact predominantly with $G\alpha_s$ stimulate adenylate cyclase as the downstream effector, while those coupled to $G\alpha_{q/11}$ activate phospholipase C, which increases intracellular Ca^{2+} levels (Fig. 5). Some GPCRs transduce extracellular signals in the absence or nearly absence of G protein activation (Brzostowski and Kimmel 2001; DeWire et al. 2007).

Adenylyl Cyclase Signaling

The discovery of adenylate cyclases (Manning and Gilman 1983) preceded that of G proteins. There are nine membrane adenylate cyclase isoforms (AC1–AC9) in mammals. They consist of two transmembrane hydrophobic domains and two cytosolic domains, C1 and C2, which represent the enzyme catalytic core and are significantly homologous (for review, see Seifert et al. 2012). Although all ACs are activated by stimulatory G proteins, AC5 and AC6 are also negatively regulated by inhibitory G proteins (for review, see Bodmann et al. 2015). Hence, Gilman's group and others established the direct interaction of adenylate cyclases with Gαs and Gαi by protein biochemistry (Dessauer et al. 1998; Sunahara et al. 1997). In particular, six subtypes of inhibitory G proteins ($G\alpha i_{1,2,3}$, $G\alpha o_{A,B}$, and Gαz) were shown to bind AC5 and AC6 (Dessauer et al. 1998).

Phospholipase C Signaling

Phospholipase C-β (PLC-β) is the major effector of $G\alpha_q$, and it also displays a GTPase-activating protein (GAP) function that is selective for $G\alpha_q$ (for review, see Litosch 2016). Rapid hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2) by PLC-β results in the accumulation of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP3 induces the release of cytosolic Ca^{2+} from intracellular stores, while DAG activates protein kinase C (PKC). There are four PLC-β isoforms (β1–β4), which are activated by $G\alpha_q$ with variable efficiencies (Smrcka and Sternweis 1993). The amplitude of the signal transmitted from the agonist-bound receptor to the effector is determined by the relative rates of the receptor-promoted activation of $G\alpha_q$ and the GTPase-activating protein (GAP) activity of PLC-β (Biddlecome et al. 1996; Mukhopadhyay and Ross 1999).

Thirteen PLC family members have been cloned. They belong to six classes, β , γ , δ , ε , η, and ζ (for review, see Vines 2012). Although the residues are poorly conserved between members, there is 40–50% homology for the N-terminal pleckstrin homology domain (PH), the EF hand, the X and Y domains, and the C2 domain. Except for PLC-ζ, all PLC family members have a PH domain, which is implicated in signal transduction. The EF hand, X, Y, and C2 domains form the catalytic core.

Other $G\alpha_q$ effectors include p63RhoGEF, G protein-regulated kinase 2 (GRK2), as well as type 1A phosphatidylinositol-3-kinase (PI3K) and tetratricopeptide repeat 1 (TPR1). The guanine nucleotide exchange factor (GEF) activity of p63RhoGEF catalyzes the exchange of bound GDP for GTP on Rho GTPases, which in turn control key cellular processes such as regulation of actin cytoskeleton (for review, see Sanchez-Fernandez et al. 2014). Interestingly, p63RhoGEF and PLC-β_2 compete with each other following $G\alpha_q$ activation. GRK2 acts as a suppressor of $G\alpha_q$ signaling via binding and most likely sequestration of activated $G\alpha_q$ (Carman et al. 1999). PI3K, which is involved in the regulation of the Akt pathway, is inhibited by activated $G\alpha_q$ via a direct interaction (Ballou et al. 2003). TPR1 is a scaffold protein that functions as an adaptor between $G\alpha_{16}$ and Ras (Marty et al. 2003). Two other atypical effectors, PKCζ and MEK5, associate with $G\alpha_q$ upon GPCR activation to activate the ERK5 MAPK in a PLC-β-independent manner (Garcia-Hoz et al. 2010).

Ion Channel Signaling

Ion channel activation is mediated by G proteins following GPCR ligand binding, thereby inducing specific downstream signaling cascades. The activation of ion channels by G proteins can either be direct or indirect via a second messenger. Examples of direct interactions with G proteins include high-voltage N-type calcium channels/Gαo (inhibition) and L-type calcium channels/Gαs (stimulation; for review, see Luttrell 2006). Moreover, the Gβγ heterodimer can also activate ion channels, as in the case of the inward-rectifying muscarinic-gated potassium channel.

Voltage-gated calcium channels (VGCC) are modulated by a variety of GPCRs following agonist activation. Hence, presynaptic N, P/Q, and R-type calcium channels are negatively regulated by GPCRs, while the sodium leak channels non-selective (NALCN) are activated by the acetylcholine M3 muscarinic receptor (M3R) (for review, see Altier 2012).

G Protein-Coupled Receptor Signaling Networks

In the early model of GPCR signaling, receptor activation leads to dissociation of heterotrimeric G proteins into α- and βγ-subunits that activate effector molecules, including second messenger systems. Activation of these pathways modulates cellular responses in the target cells. However, the number of effectors is much smaller than the number of GPCRs. Cells express multiple different GPCRs, leading to integration and cross regulation among the different signaling pathways. The presence of G protein-independent signaling pathways further increases the complexity of GPCR regulation of signaling and cell responses.

Multiple G Protein Coupling

Although GPCRs usually preferentially stimulate one G protein type, many receptors can activate several different G protein classes (Hermans 2003). The α_2-adrenoceptor can suppress or activate adenylate cyclase activity via $G_{i/o}$ or G_s, with the signaling altered according to agonist concentration. Promiscuous coupling (the capacity for a receptor to couple to more than one G protein type) has also been demonstrated using receptor–G protein fusion proteins, in which a G_α subunit is fused to the receptor (Milligan et al. 2004).

Membrane Microdomains and GPCR Signaling

Different GPCRs that signal through the same G protein in a single cell type have been found to sometimes activate different cellular responses (Ostrom 2002). The concept of random mixing of receptors and signaling components cannot easily account for these observations because it does not include compartmentalization of molecules in cells (Gonzalez-Maeso et al. 2002; Remmers et al. 2000). The compartmentalization of receptors and effectors in membrane microdomains is an important determinant of receptor signaling (Ostrom 2002; Ostrom et al. 2000).

Caveolae are plasma membrane microdomains enriched in caveolins, cholesterol, and sphingolipids (Insel et al. 2005). Several GPCRs, G proteins, and other signaling proteins are located in caveolae (Ostrom 2002). This compartmentalization may cause receptor coupling to multiple effect systems, increase signaling, or influence which pathway is activated.

Cross Talk Between GPCRs

One mechanism of integration of signaling of different GPCRs occurs through modulation of signaling pathways of one GPCR by activation of a different GPCR on the same cell (Hur and Kim 2002; Jordan et al. 2000; Neves et al. 2002). For example, activation of phospholipase C by purinergic $P2Y_2$ receptors via G_q proteins inhibits cAMP synthesis stimulated by β-adrenoceptors via G_s proteins (Suh et al. 2001).

In addition to modulating other GPCR-signaling pathways, signaling pathways activated by GPCRs also influence the signaling of other structural classes of receptors. Epidermal growth factor receptors, for example, can be transactivated by stimulation of a number of GPCRs (Hur and Kim 2002). GPCRs may also cause cross talk regulation of downstream signaling pathways. For example, vasopressin and bombesin (acting at G_q-coupled receptors) act synergistically with several growth factors to stimulate growth. Morphine desensitization, internalization, and downregulation of the $G_{i/o}$-coupled μ-opioid receptor are facilitated by activation of the $G_{q/11}$-coupled 5-HT_{2A} receptor (Lopez-Gimenez et al. 2008). Another important area of cross talk is via heterodimerization of different GPCRs, discussed in a subsequent section.

G Protein-Coupled Receptor Interacting Proteins

Besides GRKs, arrestins, RGS and AGS, additional GPCR-interacting proteins (GIPs) including other GPCRs (via homo- or heterodimerization), scaffolding, and accessory proteins have been identified. Dimerization plays an important role in ligand recognition, signaling, and receptor trafficking. GIPs assist nascent receptors in correct folding, target them to the appropriate subcellular compartments, and accomplish their signaling tasks. GIPs include receptor activity-modifying proteins (RAMPs), PDZ domain-containing proteins, various ions, lipids, and peptides that act as allosteric modulators (for review, see Brady and Limbird 2002; Maurice et al. 2011; van der Westhuizen et al. 2015). Thus, GIPs are involved in the regulation of GPCR function, may play a role in pathophysiology, and represent potential targets for drug development.

Receptor Activity-Modifying Proteins (RAMPs)

RAMPs are a family of accessory proteins that alter the ligand pharmacology or signaling of several GPCRs (Gingell et al. 2016). Structurally, they contain an extracellular N-terminal domain, a single transmembrane-spanning domain, and a short intracellular C-terminal domain. RAMPs were initially characterized as coupling partners for the class B calcitonin receptor-like receptor (CRLR). Hence, the induction of cAMP production by the calcitonin gene-related peptide (CGRP) receptor is dependent on RAMP1 expression. Additionally, RAMPs act as molecular chaperones in receptor trafficking, as it is the case with CRLR and the class C extracellular calcium-sensing receptor (CaSR). Based on in vivo studies, RAMPs

play major roles in the cardiovascular, renal, and respiratory systems, as well as in inflammation. In terms of drug development, RAMPs and the RAMP–GPCR interface both represent promising targets.

Interestingly, the receptor component protein (RCP) is a small intracellular peripheral membrane protein that is critical for CGRP signaling, as loss of RCP expression results in a decrease in CGRP-induced cAMP production. A functional CGRP receptor thus consists of the receptor itself, the chaperone RAMP, and RCP that couples the receptor to downstream effectors (for review, see Brady and Limbird 2002).

Melanocortin Receptor Accessory Proteins (MRAPs)

Like RAMPs, MRAPs are also single transmembrane-spanning proteins, which modulate the expression, trafficking, and signaling of members of the melanocortin receptor (MCR) family. Without MRAP, the melanocortin 2 receptor MC2R stays in the ER (for review, see Maurice et al. 2011).

Homer Family Proteins

The Homer proteins, which contain a PDZ-like domain in their N-terminal region, bind the C-terminal tail of the metabotropic glutamate receptors $mGlu_1$ and $mGlu_5$ at huge postsynaptic membrane-associated protein complexes termed postsynaptic densities (PSD) and thus contribute to postsynaptic signaling and plasticity (for review, see Maurice et al. 2011). In neurons, the ratio between Homer1a and Homer1b regulates the cell surface expression of $mGlu_5$ and thus the calcium signaling response of the receptor.

Cytoskeleton-Associated Proteins

Numerous proteins modulate GPCR intracellular trafficking by playing the role of adaptors between the receptors and cytoskeleton-associated proteins. Examples include the dynein light chain Tctex-1 (t-complex testis expressed 1), which is critical for the apical surface targeting of rhodopsin (for review, see Maurice et al. 2011). Filamin A is an actin-binding protein that controls GPCR trafficking. The interaction of D2 and D3 dopamine receptors with protein 4.1N is crucial for their localization at the neuronal plasma membrane. Conversely, binding of protein 4.1G to the metabotropic glutamate receptor subtype 1 diminishes its anchoring to the cell surface membrane.

PDZ Domain-Containing Proteins

GPCRs interact with a number of PDZ domain-containing proteins that act as adaptors of multimeric complexes and modulate signaling (Maurice et al. 2011).

Hence, the sodium–hydrogen exchanger regulatory factors, NHERF-1 (also known as EBP50) and NHERF-2, bind to several GPCRs; β_2AR binding of NHERF-1 is involved in the receptor-mediated regulation of Na+/H+ exchange. NHERF-2 regulates P2Y1 purinergic receptor-induced calcium signaling. MUPP1 is a multi-PDZ domain protein that interacts with the melatonin MT_1 receptor and stimulates its coupling to the G_i/adenylate cyclase pathway.

Other GPCR-Interacting Proteins and Allosteric Modulators

Various other GIPs as well as ion, lipid, and peptide allosteric modulators have been reported. Details can be found in recent reviews (Brady and Limbird 2002; Maurice et al. 2011; van der Westhuizen et al. 2015).

G Protein-Independent Signaling by G Protein-Coupled Receptors

The traditional GPCR-mediated signaling transduction involves activation of downstream effectors via the catalysis of heterotrimeric G protein dissociation into G_α and $G_{\beta\gamma}$ subunits. However, the heptahelical receptors have the ability to stimulate G protein-independent signaling pathways, such as mitogen-activated protein kinase (MAPK) cascades.

Although β-arrestins are classically known for desensitizing GPCRs, they also have the ability to mediate the activation of MAPK signaling via the recruitment of signaling molecules distinct from G protein-mediated signaling, thus setting off a second wave of signaling (for review, see Smith and Rajagopal 2016). Hence, β-arrestins function as adaptors that bind both the nonreceptor tyrosine kinase c-Src and ligand-bound β_2AR, causing c-Src recruitment to the membrane and the GPCR sequestration, which results in Ras-dependent activation of the MAPKs ERK1 and ERK2 (Luttrell et al. 1999). In the case of angiotensin II type 1a receptors (AT1aR), the receptor, β-arrestin-2, and components of the ERK cascade form a multiprotein complex. β-arrestins act as scaffolds that enhance c-Raf-1 and MEK-dependent activation of ERK2 (Luttrell et al. 2001). β-arrestins were also shown to scaffold JNK1/2 and thus promote their activation (Kook et al. 2013). β-arrestins mediate p38 MAPK activation, yet the underlying molecular mechanism remains to be elucidated (Sun et al. 2002).

G Protein-Coupled Receptor Dimerization

In addition to the plethora of GIPs that include scaffolding and accessory proteins modulating GPCRs, GPCRs can form homo-, heterodimers, or larger oligomers. GPCR homo-/heterodimerization and hetero-oligomerization have been implicated in the regulation of GPCR function, trafficking, and ligand pharmacology (for review, see Milligan 2009). This phenomenon has important biological implications,

i.e., the modulation of GPCR signaling and the mediation of cross talk between GPCR pathways. Clinically, GPCR heterodimers may be exploited as potential drug targets. While most studies have relied on expressing recombinant receptors in heterologous cells, very few have demonstrated the existence of GPCR heteromers in native tissues or in vivo. Probing close physical relationships between two GPCRs is technically challenging. Recent progress, notably in the development of proximity-based assays, may help to better evaluate the presence and function of GPCR heteromers in native tissues (for review, see Gomes et al. 2016). On the whole, there has been accumulating evidence supporting heteromerization between GPCRs, yet the underlying molecular mechanisms and functional effects of heteromerization remain to be elucidated.

Homodimerization

Although GPCRs were originally thought to function as monomers, co-expression and co-immunoprecipitation studies, reported nearly two decades ago, provided evidence for the existence of multiple copies of each of the β_2AR, the dopamine D2 receptor, and the δ-opioid peptide (DOP) receptor within a complex (for review, see Milligan 2009). Although DOP receptor dimerization was inhibited by specific agonists (Cvejic and Devi 1997), agonist-induced dissociation of the homomultimer was not corroborated by resonance energy transfer techniques (McVey et al. 2001), highlighting the need for independent confirmation. The dopamine D_2 receptor was shown to homodimerize as well as to heterodimerize with the 5-hydroxytryptamine 5-HT_{1B} receptor; furthermore, ligand selectivity was demonstrated for the receptor monomer vs. the dimer (Ng et al. 1996). More recent research on the β_2AR contradicted earlier work suggesting that the dimer, but not the monomer, was involved in G protein activation and receptor function (Whorton et al. 2007). Similarly, when rhodopsin is incorporated into a reconstituted phospholipid bilayer particle, it is monomeric and activates transducin (Whorton et al. 2008). Accordingly, it seems unlikely that GPCR dimerization is necessary for G protein activation and signal transduction.

Some GPCR dimers were previously described in crystal structure studies. For instance, the β_2AR dimer is formed via lipids composed of two cholesterol and two palmitic acid molecules in the carboxy-terminal region (Rasmussen et al. 2007). The CXCR4 homodimer involves interactions via helices V and VI (Wu et al. 2010). Overall, a number of studies have reported GPCR dimerization or oligomerization in heterologous systems, yet there is still a lack of physiological or pathological evidence.

Heterodimerization

The occurrence and significance of GPCR heterodimerization have remained elusive. Previous heterologous expression studies and/or yeast two-hybrid studies revealed that the $GABA_B$ receptors $GABA_B$R1 and $GABA_B$R2 form heterodimers

via their intracellular C-terminal tails and that these heterodimers are fully functional (White et al. 1998). Similarly, the amino acid taste receptor, T1R1+3, was characterized as a heterodimer of taste-specific T1R1 and T1R3 GPCRs (Nelson et al. 2002). GPCR heterodimerization is thought to happen early during protein synthesis and to be involved in the receptor delivery to the cell surface. Hence, the C-terminal endoplasmic reticulum (ER) retention motif of $GABA_{B1}$ is key in the cell surface delivery of a functional $GABA_B$ receptor heterodimer (Margeta-Mitrovic et al. 2000). Co-expression of the β_2AR with an altered β_2AR harboring the C-terminal tail of $GABA_{B1}$ results in intracellular ER retention of both the mutant and the wild-type receptor (Salahpour et al. 2004). In cells co-expressing the cannabinoid CB1 receptor and the orexin OX1 receptor, the CB1 receptor antagonist causes a redistribution of both receptors to the cell surface; likewise, the selective OX1 receptor antagonist causes a redistribution of both receptors to the cell surface when they are co-expressed, indicating that the two receptors most likely form stable heterodimers that are regulated by both CB1 and OX1 receptor ligands (Ellis et al. 2006). Thus, selective ligands may trigger co-trafficking of heterodimerized GPCRs.

With regard to pharmacology of a GPCR heterodimer, the effect of a selective ligand on the conformation of a GPCR can be relayed to G protein activation via the other GPCR, implying the induction of a conformational change of the other GPCR in the absence of direct ligand binding. This is illustrated with the neurotransmitter GABA, which binds to the N-terminal region of $GABA_{B1}$ within the $GABA_B$ receptor heterodimer $GABA_{B1}$–$GABA_{B2}$. Additionally, a ligand with no affinity for a given GPCR can regulate the function of that GPCR if it forms a heterodimer with a receptor for which the ligand has affinity. As an example, in cells co-expressing the human DOP opioid and chemokine CXCR2 receptors, a CXCR2 antagonist enhanced the function of DOP receptor agonists, although it had no affinity for the DOP receptor alone (Parenty et al. 2008). Thus, the CXCR2 antagonist functions as an allosteric modulator of a GPCR heterodimer.

GPCR heterodimers may be implicated in disease etiology and/or represent potential targets for disease treatment. Hallucinogenic drug models of psychosis have some similitudes with characteristics of schizophrenia, and the contribution of the 5-hydroxytryptamine 5-HT_{2A} receptor $G_{i/o}$-mediated signaling pathway is essential for distinguishing hallucinogenic from non-hallucinogenic agonists of this receptor (Gonzalez-Maeso et al. 2007). Gonzalez-Maeso and his collaborators carried out co-immunoprecipitation experiments in human brain tissues and RET-based assays in transfected cells to demonstrate that the 5-HT_{2A} receptor (2AR) interacts with the $mGlu_2$ but not the $mGlu_3$ receptor (Gonzalez-Maeso et al. 2008). The 2AR–mGluR2 complex may be involved in the altered cortical processes of schizophrenia.

Identifying small-molecule ligands that target specific GPCR heterodimers may enable researchers to study heterodimer expression and function in native tissues or cells and in vivo, as well as to ultimately treat diseases. The opioid agonist 6′-guanidinonaltrindole, which activates the DOP–KOP receptor heterodimer (but not homomers), is the best heterodimer-selective ligand described so far (Waldhoer et al. 2005). In most cases of reported heterodimers, however, either data validation

in native cells has been missing, or data observed in native tissues have not replicated those obtained in heterologous systems. Investigators have proposed the following criteria for claiming evidence of heterodimerization in endogenous systems: (i) heterodimer components should colocalize and physically interact; (ii) heterodimers should have biochemical properties that differ from those of their individual components; (iii) heterodimer disruption should result in a loss of heterodimer-specific properties (for review, see Gomes et al. 2016).

Mechanisms of G Protein-Coupled Receptor Desensitization

Receptor desensitization occurs when there is a rapid decline in the receptor response to repeated or sustained agonist stimulation. By contrast, receptor downregulation, which is a decrease in the number of receptors on the cell surface, is a slower process that extends over hours (for review, see Smith and Rajagopal 2016).

GPCR desensitization entails (i) receptor phosphorylation and subsequent uncoupling of the receptor from its cognate G protein and (ii) receptor internalization to intracellular compartments. Receptor desensitization can be separated into homologous mechanisms, in which the activated receptor is desensitized, and heterologous densensitization, in which downstream signaling such as adenylate cyclase activation leads to desensitization of any GPCRs with cAMP-activated protein kinase A phosphorylation sites. Homologous desensitization typically involves the sequential actions of G protein-coupled receptor kinases (GRKs) and β-arrestins (Ferguson 2001; Krupnick and Benovic 1998) (for review, see Walther and Ferguson 2013).

Uncoupling of Receptors from G Proteins

β-Arrestins are thought to suppress G protein signaling by preventing the G protein–GPCR interaction at the second (ICL2) and third (ICL3) intracellular loops of the receptor (DeGraff et al. 2002; Marion et al. 2006). This competition between arrestin and G protein for receptor binding results in desensitization of the downstream effector pathways (for review, see Smith and Rajagopal 2016). β-Arrestin binding requires (i) activation of the GPCR by its ligand and (ii) GRK-mediated receptor phosphorylation, as previously reported for β_2AR (Krasel et al. 2005).

GPCR phosphorylation can either target the ligand-bound receptor (homologous desensitization) or various GPCRs throughout the cell (heterologous desensitization). While phosphorylation of the GPCR intracellular residues is predominantly mediated by GRKs in homologous desensitization, heterologous desensitization is often mediated by PKA or PKC. In both cases, the residues that are phosphorylated are serine and threonine.

β-Arrestins were originally proposed to be involved in β_2AR desensitization and were found to share homology with the retinal visual arrestin (for review, see Smith and Rajagopal 2016). Visual arrestin (arrestin-1) blocks rhodopsin signaling in the

retina. The arrestin family consists of four members: two visual arrestins, arrestin-1 (visual arrestin) and arrestin-4 (cone arrestin), and two nonvisual arrestins, β-arrestin-1 and β-arrestin-2 (also referred to as arrestin-2 and arrestin-3). The structurally related α-arrestins have been implicated in receptor endocytosis (Patwari and Lee 2012). Based on high-resolution crystallography and mutagenesis experiments, visual arrestin comprises an N-terminal and a C-terminal domain, each of which is organized as a seven-strand β-sandwich. The N-terminal domain is responsible for recognition of the activated receptor, while the C-terminal domain contains a secondary receptor-binding region (Luttrell and Lefkowitz 2002).

There are seven human GRKs (GRK1–7). GRK1 and GRK7 are expressed in the eye; GRK2, GRK3, GRK5, and GRK6 are ubiquitous; and GRK4 is predominantly in the reproductive tract (Premont and Gainetdinov 2007). GRKs have similar structures, with an N-terminal RGS-like domain involved in receptor recognition, a central catalytic domain, and a C-terminal domain that facilitates the translocation of the kinase to the plasma membrane. Besides targeting the C-terminal tail of the GPCR for phosphorylation (e.g., for rhodopsin and β_2AR), GRK can target other intracellular GPCR sites such as ICL3 (e.g., for α2-adrenergic receptor and M2 muscarinic receptor (Liggett et al. 1992; Pals-Rylaarsdam and Hosey 1997)).

Unlike GRK phosphorylation, which depends on receptor activation by its ligand, PKA- or PKC-mediated phosphorylation of the receptor is dependent upon the increase in second messenger intracellular concentration (e.g., cAMP or DAG). Hence, besides their classical function in signal transduction via phosphorylation of downstream effectors, PKA and PKC are involved in a negative feedback mechanism, namely, heterologous receptor desensitization, through GPCR phosphorylation.

Endocytosis and Internalization of G Protein-Coupled Receptors

Receptor internalization can lead to either receptor resensitization or degradation (for review, see Walther and Ferguson 2013). Besides their role in receptor desensitization through the uncoupling of GPCRs from G proteins, arrestins target GPCRs for internalization via clathrin-coated pits (for review, see Lee et al. 2016). Additionally, they indirectly regulate GPCR trafficking by coordinating their ubiquitination and deubiquitination.

Arrestins represent an adaptor between the GPCR and key components of the internalization machinery. They bind to trafficking proteins (clathrin, clathrin adaptor protein 2 AP2), thus functioning as scaffolds in the receptor-mediated endocytosis pathway (Gurevich and Gurevich 2015). Mechanistically, binding of nonvisual arrestins to GPCR stimulates the release of the arrestin C-terminal tail, which contains binding sites for clathrin and AP2. Binding of clathrin and AP2 to those C-terminal sites induces receptor internalization via coated pits. This is exemplified by β_2AR, whose internalization is promoted by arrestin-2 and arrestin-3 (Goodman et al. 1996).

Arrestin-Independent Internalization

GPCRs use more than one internalization pathway. In the muscarinic M2 receptor, mutation of the two clusters of serine/threonine residues that are required for arrestin binding and receptor desensitization does not block receptor endocytosis, suggesting that the receptor internalizes in an arrestin-independent manner (Pals-Rylaarsdam et al. 1997). Similarly, while wild-type protease-activated receptor 2 (PAR2) internalizes in a β-arrestin-dependent manner, mutation of all the serine/threonine in the receptor C-tail results in β-arrestin-independent internalization (Ricks and Trejo 2009). The type II GnRH receptor can internalize both in an arrestin-dependent and arrestin-independent fashion (Ronacher et al. 2004). Alternatively, GPCRs can internalize in a β-arrestin-independent manner via caveolae (for review, see Walther and Ferguson 2013). Hence, β1AR mutants lacking GRK phosphorylation sites are internalized via caveolae following PKA phosphorylation, independently of β-arrestin, suggesting that PKA- and GRK-mediated phosphorylation influence β1AR internalization in an additive fashion (Rapacciuolo et al. 2003). Although diverse internalization mechanisms have been reported, β-arrestin and clathrin dependent, β-arrestin and clathrin independent, β-arrestin independent and clathrin dependent, and β-arrestin dependent and clathrin independent (Marchese et al. 2003), the majority of GPCRs are internalized via the β-arrestin-dependent, clathrin-mediated internalization pathway.

Stability of the GPCR/β-Arrestin Complex and GPCR Intracellular Fate

Distinct β-arrestins differentially regulate GPCR internalization. For instance, β-arrestin-2 promotes β_2AR internalization 100 times more efficiently than β-arrestin-1 (Kohout et al. 2001). Thus, GPCRs have been grouped into two categories based on the strength of the GPCR/arrestin interaction, which is thought to determine whether they are preferentially recycled or degraded. One class of receptors (e.g., β_2AR, μ-opioid receptors, dopamine D1A receptors) has a higher affinity for β-arrestin-2, and their association with arrestins is transient, resulting in their transfer to endosomes and their recycling to the plasma membrane and their resensitization. Conversely, other receptors (e.g., V2 vasopressin, neurotensin 1, angiotensin II type 1a receptors) bind β-arrestin-1 and β-arrestin-2 with comparable affinity, and their association with arrestins remains steady through endocytosis, predominantly resulting in lysosomal degradation (for review, see Walther and Ferguson 2013).

Alternate Role of Arrestins in GPCR Signaling

Although β-arrestins are known for regulating GPCR signaling, they can also mediate signaling via the recruitment of signaling molecules distinct from G protein-mediated signaling, thus setting off a second wave of signaling (Luttrell et al. 1999, 2001). In the case of angiotensin II type 1a receptors (AT1aR), β-arrestins act as scaffolds to enhance ERK2 activation. Interestingly, there is a correlation between the affinity of the GPCR/arrestin complex and the degree of β-arrestin–ERK binding, as the class of GPCRs forming stable receptor–β-arrestin complexes activates ERK more persistently than those forming transient receptor–β-arrestin complexes (Tohgo et al. 2003).

G Protein-Coupled Receptor Ubiquitination

To recapitulate the principal functions of arrestins, they are implicated in GPCR desensitization, GPCR internalization, and GPCR post-endocytic trafficking. Both arrestin ubiquitination and GPCR ubiquitination regulate GPCR trafficking (for review, see Gurevich and Gurevich 2015). Upon agonist stimulation, both GPCR and arrestin are ubiquitinated. While arrestin ubiquitination may affect the stability of the GPCR/arrestin complex (Shenoy and Lefkowitz 2003) and appears to be required for receptor internalization (Shenoy et al. 2001), GPCR ubiquitination is dependent upon arrestin and may mediate lysosomal sorting (Marchese and Benovic 2001; Shenoy et al. 2001). However, one study reports that agonist-stimulated ubiquitination of arrestin-2 by E3 ubiquitin ligase Mdm2 has no effect on β_2AR internalization (Ahmed et al. 2011). Notably, the pattern of β-arrestin ubiquitination correlates with the stability of the GPCR/β-arrestin complex, as receptors forming a stable complex are linked with persistent ubiquitination (Shenoy and Lefkowitz 2003).

Downregulation of G Protein-Coupled Receptors

Lysosomal targeting leads to a decline in the number of receptors on the cell surface. Gurevich's group has proposed two distinct models for the recycling versus degradation of GPCRs. According to the first model, the longer time the GPCR spends in the endosome, the higher the likelihood that it will be targeted to lysosomal degradation. This hypothesis is in agreement with the fate of internalized receptors being determined by the stability of GPCR/arrestin interaction (see above). The second model speculates that only phosphorylated forms of the receptor are targeted to lysosomes for degradation (Pan et al. 2003) (for review, see Gurevich and Gurevich 2015). It is based on studies indicating that GPCR dephosphorylation may be necessary for its recycling to the cell surface (Hsieh et al. 1999). Indeed, Gurevich's group previously showed that arrestin-2 mutants, which bind to activated GPCRs independently of receptor phosphorylation, prevent β_2AR downregulation (Pan et al. 2003).

G Protein-Coupled Receptor Signaling and Disease

The GPCR superfamily represents the most prevalent group of transmembrane receptors, thus mediating the majority of physiological responses to hormones, neurotransmitters, ions, light, and odors. Consequently, impairment of GPCR function can cause a wide range of diseases, including blindness, cancers, cardiovascular diseases, neuropsychiatric, and metabolic disorders. A large variety of endocrine diseases are due to GPCR mutations (Table 2).

Naturally occurring GPCR mutations may cause alterations in ligand binding, G protein coupling, receptor desensitization, and receptor recycling in a variety of

Table 2 GPCR mutations causing endocrine disease. Partial list of the large number of endocrine diseases caused by specific GPCR mutations

Receptor mechanism	Disease	Receptor
Adrenocorticotropin receptor (ACTHR/ MC2R)	Isolated glucocorticoid deficiency	Loss of function
Arginine vasopressin receptor 2 (AVPR2)	Nephrogenic diabetes insipidus	Loss of function
Calcium-sensing receptor (CASR)	Familial hypocalciuric hypercalcemia	Loss of function
	Neonatal severe hyperparathyroidism	Loss of function
Ghrelin receptor	Short stature	Loss of basal activity
Gonadotropin-releasing hormone	Idiopathic hypogonadotropic	Reduced/loss of function
Hormone receptor (GnRHR)	Hypogonadism	
Growth hormone-releasing hormone	Short stature	Loss of function
Follicle-stimulating hormone receptor (FSHR)	Ovarian dysgenesis	Loss of function
KISS1 receptor	Central hypogonadotropic hypogonadism	Loss of function
Luteinizing hormone/chorionic gonadotropin receptor (LHCGR)	Familial male precocious puberty	Constitutive activity
	Leydig cell hyperplasia	Constitutive activity
Melanocortin 4 receptor (MC4R)	Autosomal dominant obesity	Loss of function
Parathyroid hormone receptor	Jansen's chondrodysplasia	Constitutive activity
	Blomstrand's chondrodysplasia	Lack of adenylyl cyclase signaling
Thyroid-stimulating hormone	Non-autoimmune	Constitutive activity
Receptor (TSHR)	Thyroiditis	
	Toxic adenoma	Constitutive activity
	Congenital hypothyroidism	Loss of function
	Familial gestational hyperthyroidism	Activation by HCG

human genetic diseases (for review, see Thompson et al. 2008b). Loss-of-function mutations result in reduced ligand binding, while gain-of-function mutations lead to constitutive activation or enhanced binding. As an example, polycystic kidney disease is an inherited disorder that can result in progressive loss of renal function. Genetic variants in the *PKD1* gene, which encodes the GPCR polycystin-1 (PC-1), are the predominant factor associated with the disease in nearly two-thirds of patients (Hama and Park 2016).

A large number of genetic endocrine disorders affecting every endocrine system result from specific GPCR mutations. Hence, mutations in the LH receptor (LHCGR) result in a constitutively active receptor and are linked to familial male

precocious puberty. An FSH receptor (FSHR) mutation that causes decreased affinity for its ligand is associated with ovarian dysgenesis. GnRHR reduced or loss-of-function mutations are associated with idiopathic hypogonadotropic hypogonadism (IHH). TSH receptor (TSHR) mutations lead to constitutive activity causing non-autoimmune thyroiditis as well as adenomas. Loss-of-function mutations in the calcium-sensing receptor (CASR) are responsible for familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Loss-of-function mutations in the melanocortin 4 receptor (MC4R), which is involved in energy homeostasis, are associated with severe autosomal dominant obesity. A mutation in the high-affinity binding site of the thyrotropin-stimulating hormone receptor has been identified as a cause of familial gestational hyperthyroidism. The altered receptor can be activated by chorionic gonadotropin as well as its native agonist, thyrotropin-stimulating hormone. Elevated levels of chorionic gonadotropin during pregnancy lead to unregulated activation of the thyrotropin-stimulating hormone receptor, resulting in clinical hyperthyroidism occurring only during pregnancy.

Genes encoding accessory proteins for GPCRs (e.g., G protein, RGS, AGS, GRK) are also disrupted in various hereditary diseases (Thompson et al. 2008a). Termination of GPCR signaling relies on the hydrolysis of GTP by the intrinsic GTPase activity of Gα. If impaired, this process can bring about a number of diseases. Hence, cholera toxin, which is produced by *Vibrio cholerae* upon infection, prevents GTP hydrolysis by covalently modifying an arginine residue located in the nucleotide-binding pocket of Gαs. This causes prolonged activation of GPCR signaling and thus elevated cAMP levels in mucous intestinal cells, leading to secretory diarrhea. In some pituitary tumors, mutation of the same arginine residue in the gene encoding Gαs also results in prolonged GPCR signaling and enhanced secretion of growth hormone (Vallar et al. 1987). Inactivating Gαs variants are associated with a form of pseudohypoparathyroidism called Albright's hereditary osteodystrophy (Spiegel 1990), whereas activating mutations are observed in patients with McCune–Albright syndrome and in various tumors (Turan and Bastepe 2015). RGS2 SNPs are linked to hypertension in African Americans, and GRK4 mutations that increase GRK4 activity are associated with hypertension and sodium sensitivity. Aberrant upregulation of GRK2 and/or GRK5, which interferes with GPCR signaling, can lead to cardiovascular diseases, neurodegenerative disorders, and cancer (Penela 2016). Moreover, epigenetic modulation of GPCR signaling has been associated with CNS disorders as well as pain disorders (Dogra et al. 2016).

Summary

G protein-coupled receptors (GPCRs) include one of the largest gene families in the mammalian genome. About 800 human GPCRs have been identified. The specificity provided by the diversity of GPCR receptor binding sites leads to their playing crucial roles in every endocrine system, and GPCRs represent the predominant target of therapeutic drugs. The term GPCR refers to the classical coupling of these

receptors via heterotrimeric G proteins. In addition, they can also couple via many other non-heterotrimeric G protein interactions.

GPCRs are grouped by primary sequence similarity into different families that all have a canonical seven alpha helical transmembrane domain structure. Covalent modifications of these receptors include extracellular glycosylation and intracellular palmitoylation and phosphorylation. By far, the largest family, class A, comprises the rhodopsin-like GPCRs that have over 700 members including the majority of receptors for hormones and neurotransmitters. A distinct gene family, class B, also includes receptors for hormones including secretin, glucagon, and corticotropin-releasing factor.

In recent years, the crystal structure has been solved for an increasing number of GPCRs. The increasing number of solved crystal structures for GPCRs includes rhodopsin, the β_2-adrenergic receptor, the glucagon receptor, the corticotropin-releasing factor receptor, and two metabotropic receptors. The agonist-bound crystal structure of the β_2-adrenergic receptor has clarified the mechanism of activation of the receptor, which involves helical movement around proline bends in the transmembrane helices. The crystal structures from the different classes reveal distinct location of the ligand-binding pockets both across and within classes.

Despite their name, they couple to cellular signaling via both heterotrimeric G proteins and G protein-independent mechanisms. Specific heterotrimeric G proteins can signal by regulating different mediators, including adenylyl cyclase, phospholipase C, and ion channel activity. A specific GPCR may couple to more than one heterotrimeric G protein, and agonists can influence the relative coupling to different pathways activated by the same receptor, a phenomenon called biased agonism. The activity of GPCRs can be modulated by regulators of G protein signaling proteins (RGS) and activators of G protein signaling (AGS). Receptor activity may also be modified by interaction with receptor activity-modifying proteins (RAMP) as well as a variety of other signal-transducing or signal-modulating proteins. GPCR activity can be regulated by phosphorylation by protein kinases. Receptor dimerization and cross dimerization of different GPCR subtypes contribute to creating functional diverse receptor complexes.

Hundreds of endocrine and systemic diseases are caused by specific receptor mutations. Examples include cases of male precocious puberty due to LH receptor mutations, idiopathic hypogonadotropic hypogonadism due to GnRH receptor mutations, and autoimmune thyroid disease due to TSH receptor mutations.

Cross-References

► Cytokine Receptors
► Genetic Disorders of Adrenocortical Function
► Molecular Mechanisms of Thyroid Hormone Synthesis and Secretion
► Principles of Endocrine Diseases
► Synthesis, Secretion, and Transport of Peptide Hormones

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