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Review

Structural features of the G-protein/GPCR interactions

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ABSTRACT

Background: The details of the functional interaction between G proteins and the G protein coupled receptors (GPCRs) have long been subjected to extensive investigations with structural and functional assays and a large number of computational studies.
Scope of review: The nature and sites of interaction in the G-protein/GPCR complexes, and the specificities of these interactions selecting coupling partners among the large number of families of GPCRs and G protein forms, are still poorly defined.
Major conclusions: Many of the contact sites between the two proteins in specific complexes have been identified, but the three dimensional molecular architecture of a receptor-Gα interface is only known for one pair. Consequently, many fundamental questions regarding this macromolecular assembly and its mechanism remain unanswered.
General significance: In the context of current structural data we review the structural details of the interfaces and recognition sites in complexes of sub-family A GPCRs with cognate G-proteins, with special emphasis on the consequences of activation on GPCR structure, the prevalence of preassembled GPCR/G-protein complexes, the key structural determinants for selective coupling and the possible involvement of GPCR oligomerization in this process.

1. Introduction

G-protein-coupled receptors (GPCRs) comprise a large superfamily of membrane proteins that carry out cell signaling processes and are therefore important targets of drugs and medications [1]. The signaling function of GPCRs involves the coupling of the activated receptor to a heterotrimeric G-protein (GTP-binding protein) [2]. These heterotrimeric proteins consist of two functional units, the guanine nucleotide-binding Gα subunit and the Gβγ dimer. Upon activation, the heterotrimer dissociates into the functional units, which modulate the activity of a large number of downstream effectors including enzymes and ion channels [3]. Although, a detailed structural characterization of these supermolecular signal transduction machines is of the utmost importance, the first structure of a GPCR/G-protein complex became available only very recently [4]. Consequently, a comprehensive structural context for the details of the functional mechanism of such complexes remains elusive, including the nature of the structural changes in GPCRs that allow for G-protein binding, the ligand-dependent change in the interface involved in the binding, and the oligomeric state of the physiologically relevant functional unit. Answers to these key questions are essential, as understanding the pharmacologically relevant interactions will help understand key mechanisms such as functional selectivity and receptor trafficking, which are not directly explained by the formation of a fully specific ligand–receptor–G-protein complex. To help attain some insight into the structural context required for such an understanding, we review here the information available about the GPCR/G-protein interactions in the A sub-family, connecting data about (i) the G-protein structure, (ii) the structure and activation of GPCRs; (iii) the key elements of the GPCR/G-protein interfaces and their specificity; and (iv) the roles of GPCR oligomerization and GPCR/G-protein.

2. Structural elements of heterotrimeric G-proteins

In humans, there are 21 Gα encoded by 16 genes, 6 Gβ subunits (35 kDa) encoded by 5 genes, and 12 Gγ subunits (8–10 kDa) [5]. Heterotrimers are typically grouped into four main classes according to the primary sequence similarity of Gα: Gαs (Gαs, Gαolf), Gαi (Gαt, Gαi1, Gαi2, Gαi3, Gαo1, Gαo2, Gαζ), Gαq/11 (Gαq, Gα11, Gα14, Gα15, Gα16), and Gα12, (Gα12, Gα13), which signal through distinct pathways involving second messenger molecules such as cAMP, inositol triphosphate (IO_3), diacylglicerol, intracellular Ca^{2+} and RHOA GTPases. Structures of many of these proteins have been resolved and determined crystallographically in various conformations, with at least one representative of each class bound to GTP or/and GDP. Some were obtained bounded to a ligand that mimics the transition state intermediate of the catalytic reaction (GDP, with tetrafluroaluminate (AlF^-), and magnesium ion), in which the phosphate is pentacoordinate [6]. Other structures were determined with a nonhydrolyzable GTP analog, GTPgS, that mimics the GTP-activated form of Gα resulting in which the affinity for Gβγ has decreased and affinity for Gα binding to effectors

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has increased [7]. Table 1 lists the available 3D structures of G-proteins, specifying the type of ligand, the PDBid, activation state and corresponding references.

The structures of the Gα subunit revealed a conserved Ras-like protein fold, composed of a GTPase domain and an α-helical domain that is unique to Gαβγ, which is composed of one long helix surrounded by five structurally distinct helices (dubbed "the helical domain"). The α-helical domain in the catalytic domain, three flexible loops are found to be essential for function: these switches I, II and III change conformation during the enzymatic cycle and switch I (11 amino acids) connects the two domains of Gα subunit. Switch II (21 amino acids) assumes a helical conformation in the active state and leads the interactions of Gα with Gβγ, effectors, RGS (regulator of G-protein signaling) proteins, GoLoco (Gαi/o-LOCO interaction) motifs and others [50]. Switch III (10 amino acids) is a coiled loop in the active conformation [51]. The N- and C-termini of the Gα subunit are key sites for coupling to the GPCRs, but in most of the Gα-crystal structures the C-terminus was either removed or appears disordered. In the heterotrimeric structures the N-terminus forms an α-helix that is ordered by its interaction with Gβ. The Gβ subunit is a toroidal structure composed of seven four-stranded antiparallel sheets — the WD40 sequence repeat [41]. The first 40 residues of Gβ are folded into an α-helical conformation that forms a coiled-coil with the N terminus of Gγ, and the C-terminus of Gγ binds to blades five and six. Gβγ acts as a modulator of G-protein signaling. Both Gα and Gβγ carry lipid modifications that target them to membranes. The Gγ is composed of two α-helices connected by a loop [41]. Gγ is prenylated at a C-terminal cysteine, which is required for GPCR/G-protein interaction and for membrane targeting. The G-protein

Table 1
Crystal structure of G-proteins retrieved from the RCSB Protein Data Bank on May of 2013. (GDP: Guanosine 5′-diphosphate; PI: Inositol monophosphate; $AlF4^-$: Tetrafluroaluminate; RGS14: Regulator of G-protein signaling 14; RGS9: Regulator of G-protein signaling 9; PDEγ: Cyclic GMP phosphodiesterase γ subunit; RGS4: Regulator of G-protein signaling 4; p64RhoGEF: RHOA/RAC/CDC42 exchange factor; RGS16: Regulator of G-protein signaling 16; p115RhoGEF: RHOA/RAC/CDC42 exchange factor; GRK-2: G-protein Receptor Kinase-2; GppNHp: Guanosine-5′-(βγ-imido)triphosphate; GTPγS: Guanosine 5′-γ-thio]triphosphate; AC: a complex between the C1 domain of adenylyl cyclase type V and the C2 domain of adenylyl cyclase type II; GTP: Guanosine 5′-triphosphate; KB-752: Guanine nucleotide-dependent Gα binding peptide.

PDBid	Protein	Co-factors	State	Resolution/[Å]	Release date	Reference
1TAG	Gαt	Ca^{2+} and GDP	Inactive	1.80	1995	[8]
1GOT	$G\alpha tb_1y_1$	GDP	Inactive	2.00	1997	[9]
1GDD	$G\alpha i_1$	GDP	Inactive	2.20	1995	[10]
1GIT	$G\alpha i_1$	GDP and PI	Inactive	2.60	1997	[11]
1BOF	$G\alpha i_1$	Mg^{2+} and GDP and SO_4^{2-}	Inactive	2.20	1999	[12]
3FFA	Gαi	Mg^{2+} and GSP and SO_4^{2}	Inactive	2.30	1999	[13]
1Y3A	Gαi1/KB-752	GDP	Inactive	2.50	2005	[14]
2OM2	Gαi/RGS14	GDP and Mg^{2+}	Inactive	2.20	2007	[15]
3FFB	Gαi	GDP and SO_4^{2}	Inactive	2.57	2009	[13]
3ONW	Gαi/RGS14	GDP and SO_4^{2-}	Inactive	2.38	2010	[16]
2XTZ	Gαi	Mg^{2+} and GSP and SO_4^{2}	Inactive	2.34	2011	[17]
3QI2	Gαi/RGS14	GDP and SO_4^{2-}	Inactive	2.80	2012	[18]
3QE0	Gαi/KB752	GDP and SO_4^{2-}	Inactive	3.00	2012	[18]
3UMS	Gαi	GDP and SO_4^{2-}	Inactive	2.34	2012	[19]
1ZCB	Gα13	GDP	Inactive	2.00	2005	[20]
3AH8	$G\alpha i_1\beta_1\gamma_2$	GDP	Inactive	2.90	2010	1[21]
1KJY	Gαi/RGS14	GDP and Mg^{2+}	Inactive	2.70	2002	[22]
1TAD	Gαt	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	1.70	1995	[23]
1FQJ	Gαt/RGS9/PDEy	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.02	2001	[24]
1FQK	Gαt/RGS9	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.30	2001	[24]
1AGR	Gαi1/RGS4	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.80	1997	[25]
1SVK	Gαi	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.00	2004	[26]
3D7M	Gαi	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.90	2009	[27]
1ZCA	Gα12	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.90	2005	[20]
2RGN	Gαqi/p63RhoGEF	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	3.50	2008	[28]
4EKC	Gαq/RGS2	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	7.40	2013	[29]
4EKD	Gαq/RGS2	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.70	2013	[29]
3C7K	Gαo/RGS16	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.90	2008	[30]
1SHZ	$G\alpha 13i_1$/p115RhoGEF	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	2.85	2005	[31]
2BCJ	$G\alpha q\beta_1\gamma_2$/GRK-2	Mg^{2+} and GDP and $AlF4^-$	Transition-state mimetic conformation	3.06	2005	[32]
1TND	$G\alpha t/i_1$	Mg^{2+} and GTPγS	Active	2.20	1994	[33]
1CIP	$G\alpha i_1$	Mg^{2+} and GppNHp	Active mimetic conformation	1.50	1999	[34]
1GIA	$G\alpha i_1$	Mg^{2+} and GTPγS	Active	2.00	1994	[35]
1AZT	Gαs	Mg^{2+} and GTPγS	Active	2.30	1998	[36]
1AZS	Gαs/AC	Mg^{2+} and GTPγS	Active	2.30	1998	[37]
1CJU	Gαs/AC	Mg^{2+} and GTPγS	Active	2.80	1999	[37]
1AQG	Gαt C-terminus		Active	NMR	1998	[38]
1LVZ	Gαt C-terminus		Active	NMR	2002	[39]
1MF6	Gγ C-terminus		Active	NMR	2003	[40]
1TBG	$Gt\beta_1\gamma_1$			2.10	1997	[41]
3CIK	$G\beta_1\gamma_2$/GRK-2	Mg^{2+}		2.75	2009	[42]
1OMW	$G\beta_1\gamma_2$/GRK-2			2.50	2003	[43]
3KRX	$G\beta_1\gamma_2$/GRK-2	Mg^{2+}		3.10	2010	[44]
3PSC	$G\beta_1\gamma_2$/GRK-2			2.67	2011	[45]
3PVU	$G\beta_1\gamma_2$/GRK-2			2.48	2011	[45]
3PVW	$G\beta_1\gamma_2$/GRK-2			2.49	2011	[45]
1TBG	$G\beta_1\gamma_1$/Phosducin			2.10	1997	[46]
1AOR	$G\beta_1\psi_1$Phosducin			2.30	1995	[47]
1B9X	$G\beta_1\psi_1$Phosducin			3.00	1999	[48]
2TRC	Gβ1γ1/Phosducin			2.40	1997	[41]
2PBI	$G\beta_5$/RGS9			1.95	2008	[49]
1OMW	Gβ1γ2/GRK-2			2.50	2003	[43]

β- and γ subunits form a combined functional unit held together by a coiled-coil interaction between the N-terminus of Gγ and the N-terminus of Gβ [52]. Although most Gβ subunits can interact with most Gγ subunits, not all of the 60 possible dimer combinations are allowed [53]. Gβγ dimers can also interact with the same Gα isoform [54]. Fig. 1 depicts a typical structure of a G-protein.

3. GPCR structure and activation

The structures of sub-family A GPCRs contain seven transmembrane-spanning α-helices (TM1–7), which are connected by three inter-helical loops in each side of the membrane: three extracellular (ECL) and three intracellular (ICL) loops and a cytoplasmic C-terminus containing an α-helix (HX8) parallel to the cell membrane; the N-terminus is extracellular. Based on phylogenetic analysis, human GPCRs can be classified into five main families of receptors: rhodopsin (672 members), secretin (15 members), glutamate (22 members), adhesion (33 members) and frizzled–taste 2 (36 members). They mediate signaling in sensory perception, chemotaxis, neurotransmission, cell communication, the senses of sight, smell and taste and many other vital physiological events by responding to a vast variety of structurally diverse ligands such as small molecules (biogenic amines, nucleotides and ions), lipids, peptides, proteins and light [1,55]. Their members share >20% sequence identity in their TM domains [56]. GPCR proteins prove difficult to express and purify as they constitute large allosteric proteins embedded in membranes. After decades of great effort, a high number of GPCR structures have become available (Table 2), leading to great advances in the organization of the accumulated biochemical, mutational, and biophysical data in a reliable structural context [57]. Until recently, rhodopsin structures were the main basis for molecular modeling and computational studies seeking a structural context for the results of functional studies and the properties of active and inactive states of members of the rhodopsin-like family (family A) of GPCRs [58].

The structural commonalities of the GPCRs, and the results of structure–function studies interpreted in an increasingly clearer structural context have validated the properties and functionalities of specific structural elements shared by the activation mechanisms of this family of receptors, the SM/FM (conserved Structural Motifs that have roles as Functional Microdomains [58]. These SM/FM include groups of residues, which are numbered here according to the Ballesteros & Weinstein nomenclature, where a first digit identifies the TM helix number (from 1 to 7) and the second identifies the position of the residue in the TM with respect to the most conserved residue in that TM identified from a comprehensive sequence alignment and assigned arbitrarily the index number 50; the numbers decrease towards the helix N-terminus and increase towards its C-terminus [101].) The most important SM/FM are: (i) the "ionic lock" — designating the interaction between $Arg^{3.50}$ of the consensus (D/E)R(Y/M) in TM3 with $D/E^{3.49}$ and $D/E^{6.30}$ [102]; (ii) the hydrophobic arginine cage — conserved hydrophobic amino acids at positions 3.46 (L:15%, V:8%, I:58%, M:15%) and 6.37 (L:37%, V:24%, I:20%, M:5%) that form a cage restraining the conformation of the absolutely conserved $Arg^{3.50}$ [103]; (iii) the NPxxYxF motif in TM7 that is also responsible for the direct interaction of $Tyr^{7.53}$ in TM7 with $Phe^{7.60}$ in HX8 and with the side chain and backbone (via water molecule) of $Arg^{2.40}$ in TM2; and [103–105] (iv) the Rotamer Toggle Switch — an interaction among juxtaposed aromatic residues in TM6 that senses the binding of the ligand and through a coordinated change in rotameric angles triggers the regulation of the ionic lock through a series of specific rearrangements in the extracellular part of the GPCRs. This cluster of aromatic residues in TM6 surrounding $Trp^{6.48}$ of the CWxP motif undergoes a conformational transition from pointing towards TM7 in the inactive state to pointing towards TM5 in the active state [106]. The SM/FM motifs were proposed first from computational models incorporating results from various experimental assays, and were validated by the published crystal structures of GPCRs [106–108]. Unlike the transmembrane domains that maintain great similarity in overall architecture and secondary structure among family A GPCRs, there is much greater diversity among loop regions, including the sizes of the N-terminus (62 ± 98 amino-acids), the C-terminus (53 ± 36 amino-acids) and intracellular loop 3, ICL3, (41 ± 43 amino-acids). The ICL2 is the most conserved, with 20 ± 2 amino-acids [109], but like other ICLs, it presents little sequence conservation [110].

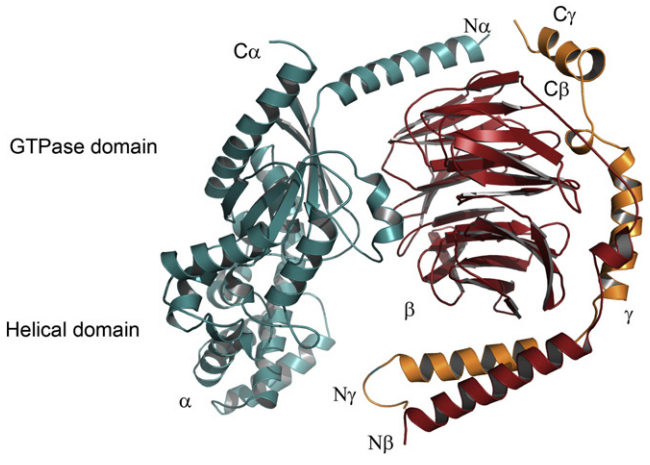

Fig. 1. Structural representation of a typical G-protein (transducin). Gα, Gβ and Gγ are colored in cyan, red and orange respectively. The Gtα subunit was built by homology modeling using the MODELLER software [342] and the crystal structure of the complex Gtα/Giα chimera of the Gtβψ subunits (PDBid: 1GOT [9]). The Gα C-terminus was grafted using the activated peptide of Gt (PDBid: 1LVZ [39]). The 1GOT structure of Gtαβγ was used to build Gtβγ subunits and 1MF6 [343] was used to build the last residues of Gγ.

Structural information about the ICL2 and ICL3 loops in the currently available structural data is most relevant to the coupling between GPCRs and G-proteins. It was found that closely related receptors show clear differences in the secondary structure of the ICL2. For example, ICL2 was found to adopt helical structure in M2R (PDBid: 3UON [96]), in β1AR (PDBid: 2VT4 [84], 2Y00 [85], 2Y01 [85], 2Y02 [85], 2Y03 [85], 2Y04 [85], 2YCY [86], 2YCW [86], 2YCX [86], 2YCZ [86]) and in α2AR (PDBid: 3RFM [82], 3PWH [82], 3UZA [146], 3UZC [146], 4EIY [147], 3EML [96]). In contrast, ICL2 has a two-turn α-helix in some β2AR structures (PDBid: 3P0G [81] and 3SN6 [4]), but not in other β2AR structures (PDBid: 2R4R [79], 2R4S [79], 2RH1 [78], 3D4S [80], 3KJ6 [82], 3NY8 [77], 3NY9 [77], 3NYA [77], 3PDS [83]). The helix in ICL2 is also absent in α2AR (PDBid: 3REY [82], 2YDO [145], 2YDV [145], 3QAK [144]), H1R (PDBid: 3RZE), CXCR4 (PDBid: 3ODU [75], 3OE0 [75], 3OE6 [75], 3OE8 [75], 3OE9 [75]) and in all structures of rhodopsin. Shan et al. demonstrated that the ICL2 structure can interconvert between a non-helical L-shaped strand and a helix, and that this may represent a transition to an active state of the receptor [111]. The α-helix structure may accommodate a hydrogen bond between a conserved tyrosine on ICL2 and an aspartate of the ionic lock. In the two slightly different structures identified for κ-OR (PDBid:4DJH [98]), ICL2 has a two-turn α-helix in monomer B and only a one-turn α-helix in monomer A. A similar difference was observed for the D3R structure, in which in monomer A there is a one turn a-helix and in monomer B ICL2 is disordered [76]. Fig. 2 shows the difference between these two structural forms of ICL2.

The differences in lengths of ICL3 have been proposed to relate to the selectivity for different G-proteins. The crystallized forms of the GPCR complexes with a variety of stabilizing protein fragments present mostly disordered ICL3 regions, but a variety of experimental approaches have produced convincing evidence that the C-terminal part of TM5 and the N-terminal section of TM6 form α-helices elongating these segments by at least 2 or 3 turns beyond the membrane [71]. The structure of opsin exhibits such an elongation of TM5 (by 1 to 2.5

Table 2
Crystal structure of GPCRs retrieved from the RCSB Protein Data Bank on May of 2013. RHO: Rhodopsin receptor; β-ionone: (3E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one; CXCR4: C-X-C chemokine receptor type 4; IT1T: (6,6-dimethyl-5,6-dihydroimidazo [2,1-b] [1,3]thiazol-3-yl)methyl N,N′-dicyclohexylimidothiocarbamate; CVX15: a 16-residue cyclic peptide analog of the horseshoe crab peptide polyphemusin; D3R: Dopamine receptor type 3; eticlopride: 3-chloro-5-ethyl-N-{[(2S)-1-ethylpyrrolidin-2-yl]methyl}-6-hydroxy-2-methoxybenzamide; β2AR: β2 adrenergic receptor; ICI118,551: (2S,3S)-1-[(7-methyl-2,3-dihydro-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]butan-2-ol; alprenolol: (2S)-1-[(1-methylethyl)amino]-3-(2-prop-2-en-1-ylphenoxy)propan-2-ol; carazolol: (2S)-1-(9H-Carbazol-4-yloxy)-3-(isopropylamino)propan-2-ol; timolol: (2S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol; FAUC50: 8-hydroxy-5-[(1R)-1-hydroxy-2-({2-[3-methoxy-4-(3-sulfanylpropoxy)phenyl]ethyl}amino)ethyl]quinolin-2(1H)-one; β1AR: β1 adrenergic receptor; Cyanopindolol: 4-{[(2S)-3-(tert-butylamino)-2-hydroxypropyl]oxy}-3H-indole-2-carbonitrile; dobutamine: 4-[2-[[(2R)-4-(4-hydroxyphenyl)butan-2-yl]amino]ethyl]benzene-1,2-diol; carmoterol: 8-hydroxy-5-[(1R)-1-hydroxy-2-[[(2R)-1-(4-methoxyphenyl)propan-2-yl]amino]ethyl]-1H-quinolin-2-one; isoprenaline: 4-[(1R)-1-hydroxy-2-(propan-2-ylamino)ethyl]benzene-1,2-diol; salbutamol: 4-[(1R)-2-(tert-butylamino)-1-hydroxy-ethyl]-2-(hydroxymethyl)phenol; iodocyanopindolol: 4-{[(2S)-3-(tert-butylamino)-2-hydroxypropyl]oxy}-3-iodo-1H-indole-2-carbonitrile; α2AR: α2 adenosine receptor; ZM241385: 4-[2-[(7-amino-2-furan-2-yl [1,2,4]triazolo[1,5-a] [1,3,5]triazin-5-yl) amino]ethyl}phenol; UKA: 6-(2,2-diphenylethylamino)-9-[(2R,3R,4S,5S)-5-(ethylcarbamoyl)-3,4-dihydroxy-oxolan-2-yl]-N-[2-[(1-pyridin-2-ylpiperidin-4-yl)carbamoylamino]ethyl] purine-2-carboxamide; NECA: 5′(N-ethylcarboxaido)-adenosine; T4E: 4-(3-amino-5-phenyl-1,2,4-triazin-6-yl)-2-chlorophenol; T4G: 6-(2,6-dimethylpyridin-4-yl)-5-phenyl-1,2,4-triazin-3-amine; XAC: 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-l,3-dipropylxanthine; CLR: calcitonin receptor-like receptor; olcegepant: N-{(1S)-5-amino-1-[(4-pyridin-4-ylpiperazin-1-yl)carbonyl]pentyl}-3,5-dibromo-Nalpha-{[4-(2-oxo-1,4-dihydroquinazolin-3(2H)-yl)piperidin-1-yl]carbonyl}-D-tyrosinamide; telcagepant: N-[(3R,6S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl]-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxamide; H1R: histamine H1 receptor; doxepin: (3Z)-3-(dibenzo[b,e]oxepin-11(6H)-ylidene)-N,N-dimethylpropan-1-amine; M2R: M2 muscarinic acetylcholine receptor; M3R: M3 muscarinic acetylcholine receptor; QNB: (3R)-1-azabicyclo[2.2.2]oct-3-ylhydroxy(diphenyl)acetate; Tiotropium; (1R,2R,4S,5S,7S)-7-{[hydroxy(dithiophen-2-yl)acetyl]oxy}-9,9-dimethyl-3-oxa-9-azoniatricyclo[3.3.1.0 ~ 2,4~]nonane; κ-OR: kappa opioid receptor; NOP: nociception/orphanin FQ peptide receptor; JDTic: (3R)-7-hydroxy-N-{(2S)-1-[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]-3-methylbutan-2-yl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide; C-24: 1-benzyl-N-{3-[spiroisobenzofuran-1(3H),4′-piperidin-1-yl]propyl} pyrrolidine-2-carboxamide; S1p(1): sphingosine 1-phosphate receptor.

PDBid	Protein	Ligand	Resolution/[Å]	Release date	Reference
1F88	RHO	Inverse agonist 11-*cis* retinal.	2.80	2000	[59]
1HZX	RHO	Inverse agonist 11-*cis* retinal.	2.80	2001	[60]
1L9H	RHO	Inverse agonist 11-*cis* retinal.	2.60	2002	[58]
1GZM	RHO	Inverse agonist 11-*cis* retinal.	2.65	2003	[61]
1U19	RHO	Inverse agonist 11-*cis* retinal.	2.20	2004	[62]
2HPY	RHO	All-trans-retinal	2.80	2006	[63]
2G87	RHO	All-trans-retinal	2.60	2006	[64]
2I35	RHO	Inverse agonist 11-*cis* retinal.	3.80	2006	[65]
2I36	RHO	Inverse agonist 11-*cis* retinal.	4.10	2006	[65]
2I37	RHO	All-trans-retinal	4.15	2006	[65]
2J4Y	RHO	Inverse agonist 11-*cis* retinal.	3.40	2007	[66]
2PED	RHO	Inverse agonist 9-*cis* retinal.	2.95	2007	[67]
2Z73	Squid RHO	Inverse agonist 11-*cis* retinal.	2.50	2008	[68]
2ZIY	Squid RHO	Inverse agonist 11-*cis* retinal.	3.70	2008	[69]
3C9M	RHO	Inverse agonist 11-*cis* retinal.	3.40	2008	[70]
3C9L	RHO	Inverse agonist 11-*cis* retinal.	2.65	2008	[70]
3CAP	Opsin	Ligand-free	2.90	2008	[71]
3PXO	RHO-MetaII	All-trans-retinal	3.00	2011	[72]
3OAX	RHO	β-ionone	2.60	2011	[73]
4BEZ	RHO	G90D mutant	3.30	2013	[74]
3ODU	CXCR4	Antagonist IT1T	2.50	2010	[75]
3OE0	CXCR4	Antagonist CVX15	3.20	2010	[75]
3OE6	CXCR4	Antagonist IT1T	3.20	2010	[75]
3OE8	CXCR4	Antagonist IT1T	3.10	2010	[75]
3OE9	CXCR4	Antagonist IT1T	3.10	2010	[75]
3PBL	D3R	Antagonist eticlopride	2.89	2010	[76]
3NY8	β2AR	Inverse agonist ICI 118,551	2.84	2010	[77]
3NY9	β2AR	Inverse agonist	2.84	2010	[77]
3NYA	β2AR	Neutral antagonist alprenolol	3.16	2010	[77]
2RH1	β2AR	Inverse agonists carazolol	2.40	2007	[78]
2R4R	β2AR	Inverse agonists carazolol	3.40	2007	[79]
2R4S	β2AR	Inverse agonists carazolol	3.40	2007	[79]
3D4S	β2AR	Inverse agonists timolol	2.80	2008	[80]
3P0G	β2AR	Nanobody-stabilized active state	3.50	2011	[81]
3KJ6	β2AR/FAB	Antibody	3.40	2010	[82]
3PDS	β2AR	Inverse agonist FAUC50	3.50	2011	[83]
2VT4	β1AR	Antagonist cyanopindolol	2.70	2008	[84]
2Y00	β1AR	Partial agonist dobutamine	2.50	2011	[85]
2Y01	β1AR	Partial agonist dobutamine	2.60	2011	[85]
12Y02	β1AR	Agonist carmoterol	2.60	2011	[85]
2Y03	β1AR	Agonist isoprenaline	2.85	2011	[85]
2Y04	β1AR	Partial agonist salbutamol	3.05	2011	[85]
2YCX	β1AR	Antagonist cyanopindolol	3.25	2011	[86]
2YCW	β1AR	Antagonist carazolol	3.00	2011	[86]
2YCZ	β1AR	Antagonist iodocyanopindolol	3.65	2011	[86]
2YCY	β1AR	Antagonist cyanopindolol	3.15	2011	[86]
3ZPQ	β1AR	Indole	2.80	2013	[87]
3ZPR	β1AR	Quinoline	2.70	2013	[87]
3EML	α2AR	Inverse-agonist ZM241385	2.60	2008	[88]
3QAK	α2AR	Agonist UKA	2.71	2011	[89]
2YDV	α2AR	Agonist NECA	2.60	2011	[90]
2YDO	α2AR	Agonist adenosine	3.00	2011	[90]
3UZA	α2AR	Antagonist T4G	3.27	2012	[91]
3UZC	α2AR	Antagonist T4E	3.34	2012	[91]
3RFM	α2AR	Antagonist caffeine	3.60	2012	[92]
3REY	α2AR	Antagonist XAC	3.31	2012	[92]

(continued on next page)

Table 2 (*continued*)

PDBid	Protein	Ligand	Resolution/[Å]	Release date	Reference
3PWH	α2AR	Inverse-agonist ZM241385	3.30	2012	[92]
4EIY	α2AR	Inverse-agonist ZM241385	1.80	2012	[93]
3N7S	CLR	Antagonist olcegepant	2.10	2010	[94]
3N7R	CLR	Antagonist telcagepant	2.90	2010	[94]
3RZE	H1R	Inverse agonist doxepin	3.10	2011	[95]
3UON	M2R	Antagonist QNB	3.00	2012	[96]
4DAJ	M3R	Inverse agonist tiotropium (Spiriva)	3.40	2012	[97]
4DJH	κ-OR	Antagonist JDTic	2.90	2012	[98]
4EA3	NOP	Antagonist C-24	3.01	2012	[99]
3V2Y	S1P(1)	Antagonist ML056	2.80	2012	[100]
3V2W	S1P(1)	Antagonist ML056	3.35	2012	[100]

helix turns, depending in the reference structure and corresponding crystal form) and a tilt of TM5 towards TM6 of about 2 to 3 Å; this opens a pocket on the cytoplasmic face of the receptor that accommodates the C-terminus of Gα [112]. Similarly, in the 3PWH [92] structure, and in a few other structures of α2AR, TM5 and TM6 are projected approximately 15 Å into the cytoplasm just as seen for the squid rhodopsin [68] (Fig. 3). Crystallization of β1AR (PDBid: 2YCY, 2YCX, 2YCW) with three different ligands led to different conformations of the cytoplasmic end of TM6, which can be bent or straight [86]. The bent conformation is very similar to the one seen in rhodopsin, and the straight one is very similar to a form of β2AR that was obtained in an MD simulation with closed ionic lock [113].

Responding to the type of ligand and the mode of ligand binding, GPCRs are expected to attain various conformations, which constitute the basis of functional selectivity [55,107,114]. Thus, it has been established that activation of GPCRs involves an outward movement of the intracellular end of TM6, which opens a crevice within the intracellular surface of the receptors into which a G-protein can bind [54,115,116]. The rotation of TM6 around its longitudinal axis has as a pivot point the helix kink, with the rotameric change of Trp265$^{6.48}$ ("rotamer toggle switch") causing key sequences to be exposed to cytoplasmic binding partners [117,118]. In early crosslinking studies, Sheik et al. showed that by preventing the movement of TM3 and TM6 it was possible to inhibit G-protein activation in members of the rhodopsin and secretin families [119]. It appears that an activation-dependent rotation or reorientation of the cytoplasmic end of TM6 is a common feature in the rhodopsin family of GPCRs, [54] which also entrains the SM/FM referred above [120]. Nevertheless, there are some differences between closely related members of sub-family A. For example, in the β2AR structure Trp$^{6.48}$ lacks direct ligand interactions. In H1R, like in dark-side rhodopsin, Trp$^{6.48}$ participates in direct interactions with the antagonist, which is unique among the known non-rhodopsin structures [95]. The outward movement is a consequence of the ionic lock breaking and protonation of D/E$^{6.30}$. Predicted more than a decade ago by Ballesteros et al. for the GnRH receptor [102] and speculated for the β2-adrenergic receptor [121], the thyrotropin-releasing hormone receptor [122] and the M3-muscarinic receptor, [123] this conformational rearrangement was finally observed in the recent X-ray crystallographic structures of the complexes Opsin/Gt C-terminus [124], RHO MetaII/Gt C-terminus [72] as well as β2AR/Gs [125]. In the κ-OR structure, although lacking Asp/Glu at position 6.30, Arg156$^{3.50}$ forms a hydrogen bond with Thr273$^{6.34}$ inactivating its structure [78]. For the α2AR structure (PDBid: 3PWH) the ionic lock can also be perceived [92]. Instead of participating in the ionic lock, the arginine in D/ERY motif may also play a role in stabilizing the deprotonated state of the adjacent aspartate or glutamate residue. For example, (i) in the H1R the ionic lock is broken and Arg125$^{3.50}$ forms a hydrogen bond with Gln416$^{6.36}$; [95] (ii) in M2R the ionic lock is also broken and Arg121$^{3.50}$ forms a salt bridge only with Asp120$^{3.49}$; [96] (iii) in the α2AR structure the ionic lock is also broken and instead Asp101$^{3.49}$ forms a hydrogen bond with Tyr112$^{3.60}$ in ICL2 and Thr412$^{2.39}$ at TM2 [88]. It is hypothesized that different structures of the ionic lock regions could be caused by modification in ICL3 or are related with different levels of activity of the various

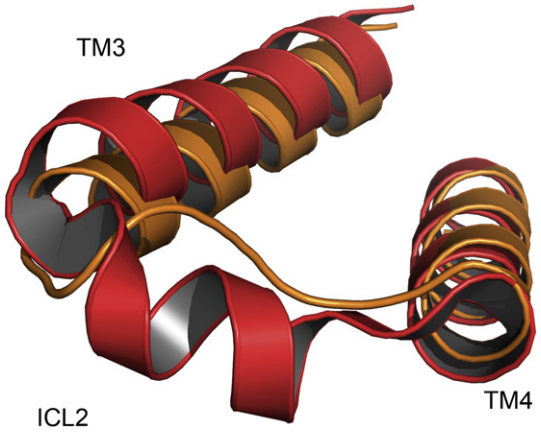

Fig. 2. Structural representation of the ICL2 of β2AR. The activated form (PDBid: 3P0G [81]) and inactivated form (PDBid: 2RH1 [78]) are in red and orange, respectively.

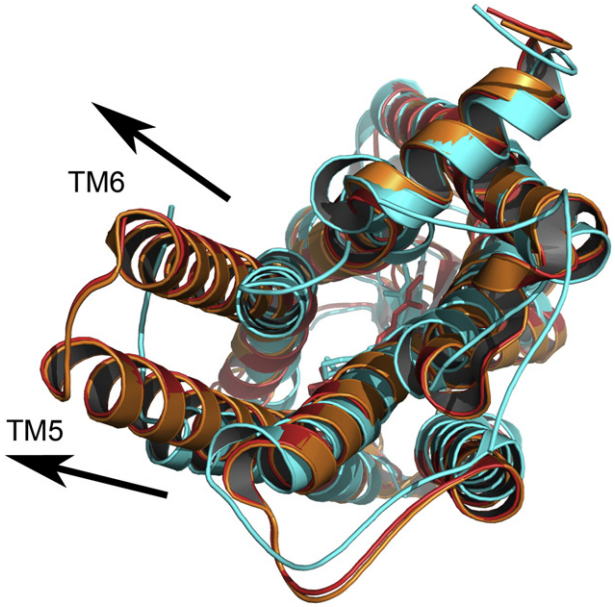

Fig. 3. Structural representation of the elongation of TM5 and TM6 (represented by arrows) that creates a crevice for G-protein coupling. Colored in cyan, orange and red are the structures of inactive rhodopsin (PDBid: 1F88 [59]), opsin (PDBid: 3CAP [71]) and rhodopsin MetaII (PDBid: 3PXO [72]), respectively.

Table 3
Key G protein/GPCR interactions — Rho: rhodopsin, TSHR: thyrotropin receptor; FSHR: follicle-stimulating hormone receptor receptor; LHR: lutropin receptor; AT1: angiotensin type 1 receptor; C5aR: C5a receptor; CB1: cannabinoid receptor; CXCR4: C-X-C chemokine receptor type 4; IL-8: interleukin 8 or NAP-1 neutrophil-activating peptide-1; CCK-AR: cholecystokinin A receptor; CCK-BR: cholecystokinin B receptor; GnRG: gonadotropin-releasing hormone receptor; MC3R: melanocortin-3-receptor; MOR: μ-opioid receptor; V2R: vasopressin receptor; M1R: acetylcholine (muscarinic) receptor type 1; M2R: acetylcholine (muscarinic) receptor type 2; M3R: acetylcholine (muscarinic) receptor type 3; M5R: acetylcholine (muscarinic) receptor type 5; α1bAR: epinephrine/norepinephrine adrenoreceptor type α1b; β2AR: epinephrine/norepinephrine adrenoreceptor type β2; H2R: histamine receptor type 2; 5-HT1A: histamine receptor type 1A; A2AR: adenosine receptor type 2A; PAFR: platelet-activating factor receptor; PAR1: protease-activated receptor type 1; IPR: prostacyclin receptor.

Type of receptor	GPCR/ G-protein	Domain									References
		TM1	ICL1	TM2	TM3	ICL2	TM5	ICL3	TM6	HX8	
Sensory stimuli	Rho/Gt			Leu72$^{2.39}$	Glu134$^{3.49}$ Arg135$^{3.50}$ Tyr136$^{3.51}$ 1le137$^{3.52}$ 1le138$^{3.53}$ Val139$^{3.54}$	Cys140 Lys141	Tyr223$^{5.57}$	Leu226 Val227 Thr229 Val230 Ala233 Ala234 Ser240 Ala241 Thr242 Thr243 Thr244 Gln245	Ala246$^{6.29}$ Glu249$^{6.32}$ Val250$^{6.33}$ Thr251$^{6.34}$ Met253$^{6.36}$ Val254$^{6.37}$ Ile255$^{6.38}$ Tyr257$^{6.40}$	Asn310$^{8.47}$ Lys311$^{8.48}$ Gln312$^{8.49}$ Phe313$^{8.50}$ Ser316$^{8.53}$ Met317$^{8.54}$ Lys325$^{8.62}$– Ser338$^{8.75}$	[72,112,124,174–181]
Glycoprotein hormones	TSHR/Gq	Leu440$^{1.58}$ Thr441$^{1.59}$			Ile523$^{3.54}$	His443 Phe525 Leu529		Tyr605 Val608	Lys618$^{6.29}$ Lys621$^{6.32}$ Ile622$^{6.33}$ Ala623$^{6.34}$	Arg687$^{8.52}$	[182–184]
	TSHR/Gs&Gq					Met527 Arg528 Asp530					[183]
	FSHR/Gs					Arg467$^{3.50}$ Thr470$^{3.53}$					[185]
	LHR/Gs				Arg464$^{3.50}$ Thr467$^{3.53}$ Ile468$^{3.54}$	Tyr470		Tyr550	Asp564$^{6.30}$		[186]
Peptides	AT1/Gq				Ile130$^{3.54}$	Met134					[187]
	C5aR/Gq				Asp133$^{3.49}$ Arg134$^{3.50}$			Trp230 Ala234 Thr235	Arg236$^{6.29}$ Ser237$^{6.30}$ Lys239$^{6.32}$		[188]
	CB1/Gs					Leu222					[189]
	CXCR4/Gi				Asn119$^{3.35}$ Arg134$^{3.50}$						[190]
	IL-8/Gi				Tyr136$^{3.51}$ Ley137$^{3.52}$ Ile139$^{3.54}$	Val140			Met241$^{6.34}$		[191]
	CCK-AR/Gs	Arg68$^{1.59}$	Asn69 Met72								[192]
	CCK-BR/Gq								Lys333$^{6.30}$ Lys334$^{6.31}$ Arg335$^{6.32}$		[193]
	GnRG/Gs		Leu73 Ser74								[194]
	GnRG/Gq11					Ser153		Leu238	Ala261$^{6.29}$ Arg279$^{6.34}$		[195–197]
	MOR/Gi										[198]
	V2R/Gs					Met145	Gln225$^{5.57}$	Glu231			[199,200]
Biogenic amines	M1R/Gq11								Ly361$^{6.31}$ Lys362$^{6.32}$ Lys365$^{6.35}$	Phe425$^{8.50}$ Arg426$^{8.51}$ Thr428$^{8.53}$ Leu432$^{8.57}$	[201,202]
	M2R/Gq11							Val385 Thr386 Ile389 Leu390			[152]
	M3R/Gq					Leu173			Ala488$^{6.33}$	Lys548 Thr549 Thr552	[148]
	M3R/Gq11								Ala489$^{6.33}$ Leu493$^{6.37}$ Ser494$^{6.38}$		[152,203,204]
	M2/M3R/ Gq11				Ser168$^{3.53}$	Arg171 Arg176					[203]
	M5R/Gq					Ala141		Ile216 Tyr217 Thr220 Arg223	Arg439$^{6.32}$ Ala440$^{6.33}$ Ala441$^{6.34}$		[204–206]
	α1bAR/Gq							Arg254 Lys258	Lys291$^{6.32}$		[207]
	β2AR/Gs		Glu63 Arg64	Val67$^{2.38}$ Thr68$^{2.39}$	Asp130$^{3.49}$ Arg131$^{3.50}$	Thr136 Pro138 Phe139	Arg221$^{5.60}$	Val222 Glu225 Al226	Ala271$^{6.33}$ Thr274$^{6.36}$ Leu275$^{6.37}$	Arg328$^{8.46}$ Ser329$^{8.47}$ Pro330$^{8.48}$	[125,208]

(continued on next page)

Table 3 (*continued*)

Type of receptor	GPCR/ G-protein	Domain								References
		TM1	ICL1	TM2	TM3	ICL2	TM5	ICL3	TM6	HX8	
					Ala134$^{3.53}$ Ile135$^{3.54}$	Lys140 Tyr141Gln142 Ser143 Leu144 Thr146 Lys147		Arg228 Gln229 Leu230 Lys232 Ile233 Lys235 Ser236 Glu237 Arg239			
	H2R/Gs				Arg116$^{3.50}$						[209]
	5-HT1A/Gi					Thr149 Tyr144 Asn146	Arg218$^{5.60}$	Arg227			[210,211]
Miscellaneous receptors	PAFR/Gq11			Asp63$^{2.50}$							[212]
	PAR1/Gq11				Arg200$^{3.50}$ Val204$^{3.54}$ Pro207$^{3.57}$ Met208$^{3.58}$						[213]
	IPR/Gs		Arg42 Ala44 Arg45								[214]

GPCRs [95]. Dror et al., [113] and Romo et al. [126] have performed micro-scale MD simulations and shown that the ionic lock forms and breaks spontaneously, suggesting that it is a dynamic process. Noteworthy, these key SM/FM motifs and their functional properties were inferred from both computational modeling and molecular simulation studies and confirmed by new structural data [106,108]. In a synergetic manner experimental and computational approaches had reveal some of the most important structural–functional aspects of the GPCR machinery. These findings can potentially change the way we see GPCR activation. Small changes such as protonation/deprotonation of key residues or reactions catalyzed by internal water molecules, may influence GPCR activation [104,105,127]. GPCR activation may also depend on the interaction of structural bound waters to conserved motifs in the receptor. Membrane dynamics, composition and charge density can also play a role in activation [104,105,128]. Studies have shown that negatively charged phospholipids influence the rhodopsin structure and provide a platform for Gt anchoring to the membrane [129,130]. It was also shown that phosphatidylethanolamine (PS) is organized in small clusters that help to attract the G-protein to the membrane [131]. Upon activation it was also observed that the movement of phosphatidylethanolamine (PE) across the membrane [132], affects membrane fluidity, which probably allows the activation driven structural changes in GPCRs [129]. By electron crystallography it was determined that the G-protein binds to the membrane [133] by its suggested membrane-binding motif (the myristoyl group attached to the N-terminus of Gα and the farnesyl group attached to the C-terminus of Gγ, which are in close proximity). Kosloff et al., by free energy calculations, have shown that lipid electrostatics influence membrane affinity and orientation of transducin on the membrane surface. These authors differentiate the role of the negatively charged lipids on Gβγ and Gα suggesting that Gβγ is attracted to the membrane and Gα repelled, facilitating membrane dissociation of Gα [134]. Therefore, we can inferred that subtle structural changes are, at least, as important as massive rearrangements of TM in GPCRs for coupling with G-proteins. The different crystallographic structures and computational techniques have shown that activation results from the allosteric communication between the ligand-binding region and the G-protein. This mechanism is a dynamic process that generates a vast ensemble of receptor structures [135]. By NMR and MD studies, Nygaard et al. proposed that the dynamic properties of the GPCR activation are not universal and that agonist binding destabilizes the inactive state but does not stabilize the fully active conformation observed in the β2AR–Gs complex [125]. A new model arises in which agonist binding allows the movement of the GPCRs towards a conformation that can interact with different protein depending on the physiological context (G-proteins, kinase and arrestins) [136,137]. Conformational flexibility can be crucial in fine-tuning the pair interactions upon G-protein coupling to the receptor [138].

4. Preassembled complexes between GPCRs/G-proteins

Two different models have been suggested to explain G-protein/GPCR coupling: (i) 'collisional coupling' and (ii) 'physical scaffold'. The first model hypothesizes that these interactions occur through collisional coupling and free lateral diffusion within the plasma membrane, wherein G-proteins only interact with activated receptors [139]. The classical models have been challenged by studies that suggest GPCR/G-protein complexes, G-proteins complexes or both may persist during the activation process [140–142]. This observation led to the 'physical scaffold' hypothesis that suggests direct or indirect interactions of specific protein components. It was proposed also as an explanation for the rapid activation of many G-protein-mediated signaling pathways, with responses occurring within milliseconds to seconds [143] and it indicates that the receptor-promoted activation of G-proteins involves organized modules [144]. This alternative model suggests that G-protein is preassembled with GPCRs before activation forming a stable complex during the early steps of activation, [145] and is supported by several experiments [145]. Gt was showed to bind to Rho* (activated rhodopsin) with high affinity (<1 nM) and to dark-adapted Rho with an affinity of 60 nM to 1 μM [2]. For example, for the α2-adenosine receptor it is hypothesized that it is precoupled with Gαs because the receptor can be solubilized in a complex with Gs in the absence of agonist [146,147]. Another study demonstrated that: (i) basal BRET (Bioluminescence Resonance Energy Transfer) signals were observed between receptors and either Gαi1, Gβ1 or Gγ2 in the absence of agonist stimulation; (ii) the decrease in the basal BRET between receptors and Gαi was detected upon the conformational changes imposed by the PTX-catalyzed ADP-ribosylation of Gαi1; (iii) after agonist stimulation BRET50 values did not reveal any change in the affinity of receptors for G-protein [145]. It was demonstrated that the majority of M3R–Gαq contact sites are identified in the inactive and active states [148]. It was also shown that Gαs and Gβγ subunits are always associated with the β2A receptor regardless of its state of activation [149]. Therefore, at least a fraction of the receptor exists in pre-associated complexes with Gαβγ, even in the absence of receptor activation [145]. FRET (Fluorescence Resonance Energy Transfer) or once again BRET techniques used

to examine interactions between different forms of GPCRs with specific heterotrimeric G-proteins confirmed the possible existence of complexes formed before receptor activation [142,150]. It has been shown for M3R that they are able of forming preassembly complexes with Gq by their C-terminus and HX8. The author hypothesized that pre-coupling facilitates signaling by obviating or accelerating the collision step [151].

5. GPCR/G-protein interface

It is essential to understand the structural basis of the coupling between G-proteins and GPCRs. Although a subject of study for a long time [152–154], with many of contact sites that comprise this interface identified, the molecular architecture of the receptor-Gα interface for the various GPCRs remains poorly defined [148]. Various techniques such as Ala-scanning mutagenesis, [155] chimeric studies, [156] sequence analysis of conserved residues in Gα subclasses, [157] chemical crosslinking [158] and tryptic proteolysis [159] have demonstrated that at least six regions within G-proteins are important for GPCR binding: the N terminus, [160–162] the α3–β5 loop, [163] the α2 helix and the α2–β4 loop [164], the α4 helix and α4–β6 loop domain, the α5 helix, the α3–β5 loop [163] and αN–β1 loop [165] of the GTPase domain. Of these, the extreme N- and C-termini, the αN–β1 loop, the α4–β6 region and the C-terminus of α5 helix contribute to the specificity of Gα–GPCR interactions [54,124,158,166–169]. Even though the selectivity is not known, there are specific amino-acid residues recognized to be important (summarize in Table 3 and shown in Fig. 4). Most of the literature offer information about single residues known to be important for GPCR/G-protein coupling. However, for some receptors, cross-linking studies have provided information about interacting pairs. For example, for the M3R–Gq complex, the following interactions have been identified: (i) Asp321 in the α4–β6 loop of Gαq and Lys548$^{8.48}$, Thr549$^{8.49}$ and Thr552$^{8.52}$ in the N-terminal segment of HX8; (ii) A488$^{6.33}$ in the cytoplasmic end of TM6 of the M3R and the last three residues Asn357, Leu358 and Val359 of Gαq; (iii) residue Arg31 from αN helix of Gαq is connected to M3R Leu173 at the ICL2; and (iv) the Thr549$^{8.49}$ and Thr552$^{8.52}$ in the N-terminal portion of H8 of the M3R and the C-terminus of Gαq [148]. The author hypothesized that upon activation there is a structural change at the receptor-Gαq interface that increases the proximity between the N-terminal portion of HX8 of the M3R and the α4–β6 loop and C-terminus of Gαq [148]. Most contacts were identified in inactive and active states indicating that M3R is precoupled to Gq before activation [148]. In the rhodopsin/transducin case several pairs of residues were highlighted as important for the coupling [3]: (i) residue Ser240 in ICL3 of rhodopsin is supposed to be near the N- and C-termini of Gα, as well as the α4/β6 loop; [168,170] (ii) the C-terminus of Gα was shown to interact with four non-contiguous residues in ICL3; [152] (iii) residues Leu226$^{5.61}$, Th229$^{5.64}$, and Val230$^{5.65}$ were shown to interact with the C-terminus of Gα [112]; (iv) ICL3 was cross-linked to the N-terminus of Gα and the C-terminus of Gβ; [171,172]. (v) C-terminus of Gα was mapped to a hydrophobic patch on the inner face of TM6; [112] (vi) Gα and Gγ C-termini of Gαt interact with HX8 of rhodopsin [173]. A first indication about the mutual orientation and part of the protein-interaction interface between GPCRs and G-proteins comes from the crystal structures of Table 4. Four of them are complexes formed between a GPCR and an 11 C-terminus stretch of Gα. This peptide assumed an α-helical conformation with a C-terminal reverse turn and binds in an orientation relative to rhodopsin, which is in agreement with previous transferred nuclear overhauser effect NMR studies [38,39].

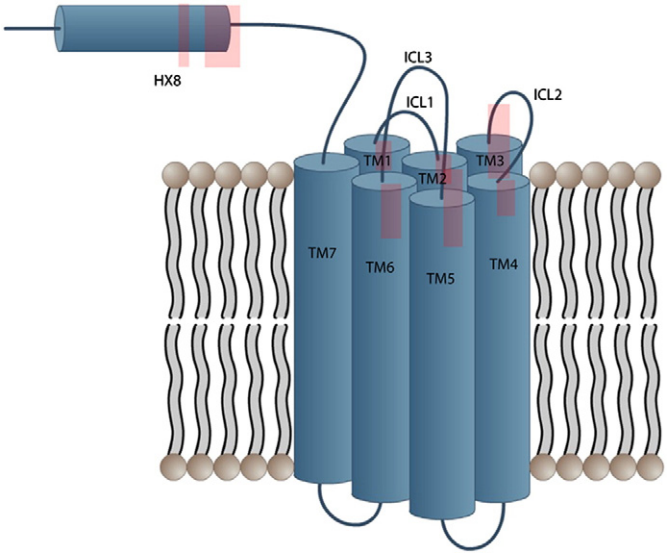

Fig. 4. Schematic representation of a G-protein coupled receptor. Key regions for GPCR/G-protein coupling are represented by a red square (different square sizes are related with the dimension of the determinant region).

5.1. G-protein determinants

5.1.1. GPCR/Gα interaction

Studies suggest that coupling selectivity involves subtle and cooperative interactions among all the various domains and Gα [215]. Experiments with a peptide corresponding to the last 55 amino acids or shorter of Gαq were able to inhibit agonist-stimulated signal transduction via the α1B-adrenergic receptor or M1 muscarinic receptor [216]. It was proposed that the N-terminus characteristic of Gaq/11 is critical for constraining the receptor coupling selectivity [217]. A peptide consisting of the 11 C-terminal amino acids of Gαi has been reported to be important for binding to α1-adenosine receptor and M2 muscarinic receptor signaling [218,219]. Various examples show that the hydrophobic residues are the essential ones for complex formation [220–222]. The leucine residues at positions corresponding to Leu344 and Leu349 in Gt are absolutely conserved in all mammalian Gα chains and among others are important for binding. Conklin et al. have also shown that the key amino acid residues are on the −3 and −4 C-terminus positions of Gα [223]. Therefore, as already mentioned it seems that the C-terminus of Gα is the main responsible for the affinity and specificity of GPCR–Gαβγ interaction [158,168,224,225]. Before binding to the receptor crevice, the 11 amino-acid Gα C-terminus presents flexibility and is always disordered in crystal not presenting a structure of its own. However, after activation it forms a helix with a C-cap structure. The nature of the conversion is not known but could be induced fit [38,39] or a conformational selection [175]. In the new rhodopsin/Gt structures (Table 4), the N terminus of Gα and the Gβγ subunits would overlap extensively with the expected plane of the lipid bilayer. Other regions of Gα adjacent to the C-terminal helix, such as the αN–α1 and α2–β3 loops at the top of the Ras-like domain important for GPCR binding would also collide with the receptor in the opsin or MetaII-Gαβγ docking models. This led to the hypothesis that the model of Gαβγ did not reflect its activated conformation [226]. Gαβγ

Table 4
Crystal structure of GPCRs/G-protein complexes retrieved from the RCSB Protein Data Bank on May of 2013. RHO: rhodopsin receptor; β2AR: β2 adrenergic receptor.

PDBid	Protein	State	Resolution/[Å]	Release date	Reference
3DQB	Gαt C-terminus/Opsin	Active	3.20	2008	[124]
2X72	Gαt C-terminus/RHO-βionone	Active	3.00	2011	[174]
3PQR	Gtα C-terminus/RHO-MetaII	Active	2.85	2011	[72]
3SN6	Gαsβ1γ2/β2AR	Active	3.20	2011	[125]
4A4M	Gαt C-terminus/RHO-MetaII	Active	3.30	2012	[181]

must undergo a significant conformational change to avoid a collision with the cell membrane. A distance change between the α5 helix and the β2 strand of Gα consistent with a rotation and translation of the C-terminal helix of Gα was predicated before, along with other structural changes [227–229]. Biased molecular dynamics simulations revealed two modes of interaction of the α5 helix with the activated form of opsin: one similar to the crystal structure of the Gα peptide and opsin and another in which the α5 helix binds to the inner surface of the TM5–TM6 helix pair and runs parallel to the membrane. The second was interpreted as a helix switch from an initial transient interaction to the final [230]. Kinetic and modeling studies were also performed to study the activated form of rhodopsin and Gt and two modes of interaction identified: (i) the S-interaction, very similar to the one seen in the opsin crystal and (ii) the I-interaction for the Rho*/GtGDP complex [231]. However, comparison of the rhodopsin–Gα peptide structure with the recently published crystal structure of the β2AR/Gs complex revealed noteworthy differences. Helix α5 of the intact Gα subunit binds to the receptor in a position tilted about 38° towards TM6 (about 26° more than that in the activated rhodopsin structure) and 2 Å away from the bottom of the crevice relative to the position of the Gα peptide in the active rhodopsin–peptide complex. Therefore, the position of the C-terminus α-helix of transducing is tilted by 30° relatively to Gs, which is probably due to the fact that in the second case a complete G-protein/GPCR was obtained [4]. It can also represent fundamental differences in the receptor–G protein interactions between these two proteins or the position of the transducin peptide in metarhodopsin II may represent the initial interaction between a GDP-bound G protein and a GPCR. In the β2AR–Gs complex a large displacement of the GasAH relative to GasRas was observed, with GasAH moving as a rigid body [125]. Although, this crystal structure reflects only one of an ensemble of possible conformations in dynamic equilibrium, it is in full agreement with other experiences. Double electron–electron resonance (DEER) spectroscopy shown large (up to 20 Å) changes in the distance between nitroxide probes positioned on the Ras and the α-helical domains of G-protein upon formation of a complex with light-activated rhodopsin [232]. It is also in agreement with results from hydrogen–deuterium exchange mass spectrometry (DXMS) experiments [233]. Westfield et al. also demonstrate by single-particle electron microscopy that the □α-helical domain undergoes a nucleotide-dependent transition from a flexible to a stabilized state [234]. Louet et al., by combining targeted molecular dynamics (TMD) and free energy profiles have found that upon forced extraction of GDP, the whole protein suffers a conformational rearrangement in agreement with the other recent findings [235].

The G-protein activation mechanism is not entirely understood. However, some hypotheses have been formulated. The Gαβγ heterotrimer is known to be maintained in its inactive conformation by association to GDP. Upon agonist-activated GPCR binding, GDP dissociates and the resulting nucleotide-free GPCR/G-protein complex is highly stable. The active state of a GPCR is, this way, defined as the conformation stabilized by coupling to a nucleotide-free G-protein. In this ternary complex, G-protein has a higher affinity for GTP than for GDP, and the GPCR has also higher affinity for the agonist [4]. Although, the nucleotide-free GPCR/G-protein complex is characterized by a highly flexible RasAH domain, complex formation leads to a preferentially allosteric propagation of the signal and reorientation of the RasAH domain [232]. This will facilitate GTP binding and uncoupling of G-protein and GPCR, with subsequently dissociation of Gα□ and Gβγ. However, the process by which GPCRs induce this conformational change in Gαβγ remains mysterious. So far, how the binding to the extended C-terminus of a Gα subunit coerce a global reconfiguration of the entire heterotrimer was not explained.

To better understand the interaction between the C-terminus of the G-protein and the GPCR, we have listed for the complexes in Table 4 (Supplementary Tables SI and S2) the microenvironment around the 11 amino-acid of the C-terminus of Gα. Supplementary Fig. S1 shows schematic images for these interactions. The plots in Fig. S1a from the β2AR/Gs complex, clearly demonstrate that the node Ile135$^{3.54}$ is a key residue at this interface as it is surrounded by six residues of the C-terminus of Gα. Gln239 at the ICL3 has also four edges connecting it to the G-protein. Leu394 (last residue of Gα) is the one that makes the higher number of connections to the GPCR. The 3D structure of this complex is shown in Fig. 5. As seen for rhodopsin/transducin, the main interactions within this complex are non-polar and polar with the transmembrane core (TM3, TM5 and TM6) and ICL2. So, the binding of the Gα peptide on the cytoplasmic side of rhodopsin is largely facilitated by essentially hydrophobic and polar interactions (Supplementary Fig. S2 to Fig. 5). The main interactions are in full agreement with the

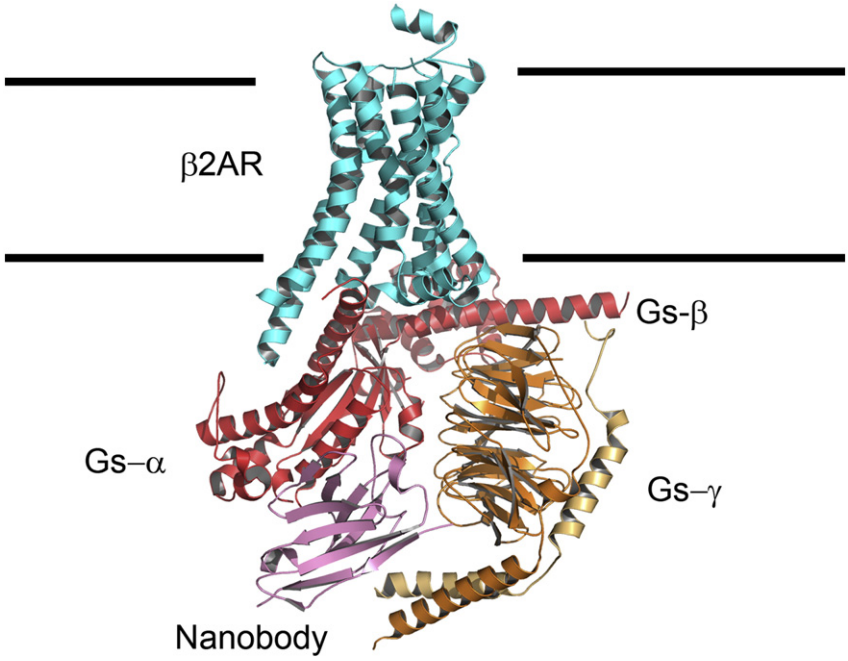

Fig. 5. Structural model of the complex between β2AR and the heterotrimeric Gs.

residues previously identified as crucial for this system. From inspection of these tables and figures we have also stress out that some interactions are common to the β2AR/Gs and to rhodopsin/Gt complexes. We are going to consider position 0 the last amino-acid residue of the C-terminus a-helix of Gα. This residue in both systems is surrounded by residues in position 6.32 and 6.33, as well as, by ICL3 residues from the two GPCRs. Residues in position (i) −1, (ii) −3, (ii) −6, (iv) −7, (v) −8 and (vi) −9 are surrounded by GPCR residues in positions 6.33, 6.36, 6.37 and ICL3 (i), 2.39, 3.50, 3.53, 3.54 (ii), 3.54 and ICL3 (iii), 3.53 and ICL2 (iv), ICL3 (v), and 3.54 and ICL3 (vi), respectively.

5.1.2. GPCR/Gβγ interaction

The Gβγ subunit also binds to GPCRs and is required to stabilize the receptor–Gα interface maybe by helping to present Gα in the appropriate conformation to the receptor [228]. GPCR ICL3 has been implicated in direct interaction with the C-terminus G-protein β subunit [172,236]. HX8 (Rho: residues 310–324) was also reported to bind Gβγ. [237] It was demonstrated that the C terminal 60 amino acids of Gβ can be crosslinked to a peptide that corresponds to the ICL3 of the α2-adrenergic receptor [172,236]. It was also observed that farnesylated C terminal 12 amino acid peptide of Gγ1 stabilizes MII74 and rhodopsin [238]. Once again, as it was seen for the Gα, this region appears to have flexibility and it appears disordered in many crystal structures [239]. Surprisedly, the new β2AR/Gs structure do not show any direct interactions with Gβ and Gγ, which is consistent with the formation of dimers in which one of the protomers interacts predominantly with Gα while the other interacts with Gβχ. This subject will be developed in more detail in the next section. In the GPCRs the main interaction regions for effective coupling to G-proteins are TM/cytoplasmic borders between TM3/ICL2, TM5/ICL3, ICL3/TM6 and also cytoplasmic tail [223,240–242]. The SM/FM NSXXNPXXY motif in TM7 and the DRY motif at the cytoplasmic border of TM3 are important not only for protein stabilization but also for G-protein activation and specificity [240,243,244]. The relative contribution of the various ICLs to G-protein selectivity varies among structurally related member of the same subfamily and within GPCR classes. The highly accessible intracellular loops are very distinct from the TMDs in that ~42% of the acid residues are strongly polar. In particular, ~29% of the loop residues are basic, which could play a special role in G protein signaling. Alternatively, these basic residues may contribute to proper membrane topology by the "basic in–acidic out" rule [245].

5.2. GPCR determinants

5.2.1. ICL2s GPCR/G-protein interaction

Several studies point out that ICL2 is very important for the selectivity of receptor/G-protein interactions and the efficiency of G-protein activation: (i) it was demonstrated that ICL2 is fundamental for G-protein coupling in the dopamine receptor; [246] (ii) investigation of ICL2 in the m5 muscarinic receptor hypothesized that ICL2 could act as a switch that enables G-protein coupling [247]; (iii) in ICL2 of the m3 muscarinic receptor specific amino acids were identified as important for coupling to the Gq/11protein by interaction with C-terminus [203]; (iv) the central portion of ICL2 was shown to be responsible for the selective recognition of the C-terminus and the αN helix of Gα of the Gα-subunit in the metabotropic receptors; [148,248] (v) for TSHR it was shown that residues 525–527 are important for agonist-induced Gαs interaction, whereas residues 528–532 were determined to be more critical for Gαq activation; [241] (vi) several residues in ICL2 were also identified as important in LH and FSH receptors [185,249].

5.2.2. ICL3s GPCR/G-protein interaction

Various studies also support a key role of ICL3 in G-protein coupling: (i) mutating several hydrophobic residues in ICL 3 reduced the transducin activation rate by 90%; [176] (ii) upon interaction with Gαtβ1γ1 the ICL2 and ICL3 become disordered because G-protein coupling introduced a relaxation of the intracellular loops; [169] (iii) on TSHR, ICL3 and the lower portion of TM6 were identified to interact with the G-protein (residues Gln263/Gly212, Gln263/Tyr211, Thr265/Gly212, and Thr265/Tyr211); [122,183] (iv) in the m4 muscarinic and α2-adrenergic receptors two or three basic residues can also be found at the C-terminus of ICL3, which are responsible for the coupling to the Gi and Go proteins [250,251]. Hydrophobic residues at ICL3 were demonstrated in various reports to be well conserved and fundamental for G-protein interaction in GPCRs that activate PLC such as in α1-adrenergic, substance F, gastrin-releasing peptide, thyrotropin-releasing hormone, endothelin-A, CCK-A, CCK-B, and ml, m3, and m5 cholinergic receptors [205,206,252–259]. They form a XBBX or BBBX motif (B stands for a basic and X for a non-basic residue) at the C-terminus of ICL3 located near the sixth transmembrane segment. Nevertheless, there is not yet a clearly defined consensus recognition motif for specific G-protein interaction, which can be explained by the fact that ICL3 is one of the least conserved regions in GPCRs, and very heterogeneous in amino acid sequence and size. Even closely related receptors that activate the same G-protein can have very different ICLs, making it impossible to determine coupling based on primary structure alone [260]. It was found for the M5R receptor that residue Ala141 was a key determinant and was likely to reside within the structural context of a short α-helix extension of TM6 [204]. This fact suggests that secondary structure, instead of the specific regions of the 1oop, plays a critical role in G-protein activation [261]. Both N-terminus and the C-terminus tail of ICL3 as well as HX8 form an amphipathic α-helical extension of TM5 and TM6 [204,262–265]. However, the central part of ICL3 remains highly flexible [266]. As mention before, this was observed in the new X-ray structures of β2AR [92], in a few other structures of α2AR, in the squid rhodopsin [68] and β1AR [86]. The N-terminus hydrophobic character is also fundamental for G-protein recognition. In the α-factor receptor of the yeast *Saccharomyces cerevisiae* encoded by the *STE2* gene it was observed that the overall net charge of the loop is important for receptor function as the removal of increasing numbers of positively charged residues from the loop by site-directed mutagenesis caused a progressive loss of signaling [267]. Erlenbach and colleagues have shown that for V2R/Gs coupling the length of ICL3 rather than its specific sequence was the key modulator for the efficient coupling [200].

6. Specificity of GPCR/G-proteins coupling

Few G-proteins transduce signals from a large variety of GPCRs, and so each member of the G-protein family must be able to interact with many different receptors. Therefore, although preferentially linked to a certain G-protein subfamily, these receptors can also couple to other classes of G-proteins with a reduced efficiency [110]. Some G-proteins are more promiscuous than others [268]. Selectivity was seen in a number of cases. For example: (i) two splice variants of Go couple to different GPCRs despite the fact that their C-terminal eight amino acids are identical; (ii) although the last eight amino acids of Gαi and Gαt are identical, the α2 adrenergic receptor activates Gαi but not Gαt; and 5HT1B serotonin receptor activates only Gαi through a specific interaction with two amino acids in the α4 helix of this protein; [156] (iii) it was shown that replacing C-terminus of Gαq with that of Gαi results in a G-protein improved coupling to receptors that normally associate exclusively with Gαi; [167] (iv) a mutation in a highly conserved Gly in linker 1 of Gα was shown to increase the activation of Gαq by Gi- and Gs-coupled receptors; [269] (v) the occurrence of positively charged residues at position 248 in the IL3 C-terminus is more frequent in the Gi/o- or Gq/11-coupled receptors than in the Gs-coupled receptors; [198,201,270–272] (vi) in the PAR1R five residues were shown to be essential for Gq/11 interaction but not to Gi/o ou G12/13 coupling suggesting that GPCRs rely in different intracellular regions to couple to different G-proteins; [213] (vii) Kostenis et al. have also demonstrated for the M2R that receptor selectivity can be switched by single amino

acid substitutions; [160] (viii) Gαs is activated primarily by Gαs-coupled receptors, whereas G15 and G16, two members of the Gαq family, can couple indiscriminately to a large number of Gαs and Gαi binding GPCRs; (ix) some GPCRs can activate simultaneously three or four Gα subfamilies [273]; (x) replacement of the last five amino acids of Gαq with Gαo allowed Gαi coupled receptors to stimulate phospholipase C (PLC), (xi) changing the last five residues of Gαs with corresponding residues from Gαq allowed Gαs to couple to some Gαq-coupled receptors and stimulate adenylyl cyclase [223]. Furthermore, the GPCRs can couple with multiple G-proteins and activate multiple signaling pathways and signaling through these pathways can differ depending on the ligand used to stimulate the receptor [274]. These coupling is often in a cell-type-specific, agonist-specific or dose-dependent way. The compartmentalization of G-protein heterotrimers and receptors in specialized domains can also affect selectivity [110]. There are a number of parameters that affect the GPCR/G-protein coupling. As it is commonly accepted, the GPCRs are allosteric proteins with dynamic structures that can adopt different activated conformations. Each of these multiple "active" states can potentially be stabilized by a different ligand in a highly selective manner, which is the basis for functional selectivity, agonist-directed trafficking of receptor stimulus, biased agonism, differential engagement and stimulus trafficking. For example, for CXCR4 there is evidence that the active receptor can have multiple receptor conformations that leads to similar G-protein activation [190]. Therefore, different agonists can directly signal from the receptor to specific signaling cascades as a consequence of their relative affinities for different G-protein-coupled states of the same receptor [275,276]. In turn, this specific receptor conformation selectively interacts with a specific intracellular signaling complex. In the same way a specific signaling complex in a particular cell will tend to stabilize a certain receptor conformation, thereby inducing selectivity for a certain ligand. This concept was proposed by Terry Kenakin [277] and it is generally accepted [278].

Various experiments suggest that differential determinants of G-protein coupling exist in different G-protein families. Mutagenesis studies with GPCRs capable of coupling to more than one G-protein have shown that it is possible to selectively abolish receptor coupling to one class of G-proteins. Although the C-terminus of Gα seems to be the primary receptor recognition domain responsible for selectivity, [160,165] it is not the sole determinant and other regions distant from the G-protein binding interface may also be crucial for coupling through conformational change of the GPCR [279,280]. The Gγ subunit also has a role in determining receptor/G-protein specificity by the presence of different primary sequences at the C-terminus [281,282]. So, the global conformation of the receptor or changes in its dynamics may be just as important as specific side-chain interactions in determining receptor–G-protein selectivity [283,284]. The precise structure of the active receptor may also depend on the molecular properties of the activating ligand [283–285]. Agonist binding leads to a change in the receptor conformation, which could allow exposure of otherwise hidden sites to G-proteins [286]. GPCR/G-protein selectivity is still an open question.

7. Oligomerization

For many years, GPCRs were thought to be monomeric. However, increasing evidence suggests that they can form dimmers or higher order oligomers, homodimers or heterodimers oligomers [287–293]. Ferre and colleagues [294] suggest that the various types of oligomerization should be classified by the resultant effects on receptor function. GPCRs inactive in binding or signaling as monomers, but which become active as oligomers, should be known as "homomeric/heteromeric receptors," whereas GPCRs intrinsically active as monomers but without activities as oligomers should be designated as "receptor homomers/heteromers [294]." Although there is strong evidence for the GPCR oligomerization, its physiological relevance is still a topic of vigorous debate [295]. Some studies suggest that a monomeric receptor is sufficient to trigger a physiological response and activate G-proteins [296–300]. However, other studies suggest that GPCRs are oligomeric allosteric machines at the ligand binding and effector activation level [301,302]. Indeed, neutron scattering revealed the formation of a GPCR dimer/Gαβγ complex composed of a leukotriene B4 receptor (BLT1) homodimer and Gi [303]. It has been also shown that the oligomeric forms of rhodopsin couple more efficiently to transducin [302]. Jastrzebska et al. suggest that a single protomer in the dimer is responsible G-protein activation and that the dimer allows a more efficient coupling to occur through multiple interactions between the two receptor protomers and different regions of the trimeric G-protein [302]. Han et al. by experimental and computational techniques have demonstrated that the minimal functional signaling unit is a complex between a dimer and Gαβγ [246]. Reconstitution experiments with BLT1 and 5HT4 receptors and their binding G-proteins [304], and functional studies on the GABAB (γ-aminobutyric acid B) receptor [305,306], metabotropic glutamate mGluR1 [307] also support the 2:1 stoichiometry. GPCRs are also suggested to be delivered as inactive dimmers/oligomers pre-associated with G-proteins [288].

Several different observations also support a role for receptor dimers in G-protein activation and that dimers might be the functional unit required for G-protein activation: (i) the relative areas of G-protein and GPCR, as the footprint of a G-protein are too large to interact with only one receptor at one time (it is compared to four GPCR molecules); (ii) the fact that native disk membrane GPCRs exist as dimers tightly packed in higher order oligomers [246,308] were seen for the rhodopsin by AFM (atomic force microscopy); (iii) the fact that Rho dimers were heterologously expressed in cell plasma membranes; [309–313] (iv) the fact that Meta II is stabilized by C-terminal fragments of Gtα and Gtγ subunits [308,314,315]; (v) β2AR is capable of forming oriented oligomer arrays probably by establishing TM1/TM8 contacts [316]. Recent work on lateral diffusion of Rho in photoreceptor membranes supports the model of binding one Gt to at least two receptors [317]. Rhodopsin was the only member of the superfamily whose oligomeric arrangement has been visualized experimentally by atomic-force microscopy (AFM) in native mouse disk membranes. It gave direct evidence of the organization of rhodopsin into two-dimensional arrays, which present several intermolecular contacts that stabilize the multimeric arrangement [318]. Strong contacts are made between TM4 and TM5 proposed to be responsible for the formation of the dimers, and TM1, TM2 and the ICL3 facilitate the formation of the rows of dimers. This contrasts with the proposal of Schertler and colleagues based on their cryo-microscopy map of squid rhodopsin, that TM4–TM4 contact is responsible for the formation of dimers. Subsequently Guo et al. reconciled the two hypotheses for the D2 receptor by proposing that the AFM and the cryo-microscopy models correspond to the inactive and active states, respectively [319]. So, a rearrangement of the dimerization interface should be a critical component of activation [292,319–324]. The possible TM–TM interfaces of various oligomers were subjected to a lot of different studies [293,320–322,325,326] but due to technical difficulties it is still very difficult to know the relevant interface. Nevertheless, new dimers were found in some of the recent X-ray structures: (i) CXCR4 involving TM4, TM5 and TM6 [327]; (ii) activated rhodopsin involving TM1, TM2 and TM8 [65]; and (iii) the β2AR involving mediation by cholesterol [78].

Oligomerization of GPCRs may influence their signaling in several ways: (i) the oligomers can couple with different Gα [290] (i.e. from the dopamine subfamily, D1 and D2 receptors, as monomers, signal through Gαs and Gαi, respectively, but D1/D2 recruit a different signaling pathway, Gαq); [328,329] (ii) different oligomer conformations can be induced by agonist binding; [298] (iii) can potentiate different extent of phosphorylation; [330] (iv) by ligand induced cross-conformational switches between the various protomers [331]. Besides complexes between G-proteins and GPCRs in their ground conformation, two different complexes could be created after activation (GPCR:GPCR*/G-protein or GPCR*:GPCR*/G-protein) because either one GPCR or two GPCRs could be activated in the dimer [2]. GPCR asymmetry and its functional role

in G-protein activation are supported by studies on BLT1 (leukotriene B4 receptor), $GABA_B$, taste receptor T1R, the metabotropic glutamate receptor mGluR and the dopamine receptor D2 [246,290] These can be in "cis-activation" in which the agonist-coupled receptor interacts with the G-protein or "trans-activated" in which the agonist-coupled receptor activates the empty receptor that by each turn activates the G-protein. "Cis-activation" was seen for BLT1 and melatonin MT1 and MT2 receptors [332,333]. "Transactivation" can also occur in which oligomerization of two defective receptors is able to restore receptor functionality. For example, it has been shown that coexpression of two mutant LH receptors in cells (one which cannot signal but can bind LH and a second that can signal but cannot bind LH) allows the receptors to complement each other so that LH is bound and receptor signaling is restored [334]. It has also shown for the D2R [246] and $GABA_B$ receptors [306]. Recently it was shown that the pentameric (2:1:1:1) complex of the rhodopsin dimer and the trimeric transducing is asymmetrical, with 50% of RHO trapped in a Meta-II conformation while the others are in an opsin conformation [335]. In the past, I and colleagues have demonstrated that for the human D2R the minimal signaling unit is constituted by a dimer of receptors and a single G-protein, which forms an asymmetrical activated dimmer. This study involved an innovative functional complementation assay and a molecular model of the oligomer organization of the interaction between GPCRs and G-protein [246]. Weinstein's lab has also demonstrated for the serotonin 2A receptor that binding to different ligands produces a differential reorganization of the receptor environment, which promotes ligand-dependent oligomerization patterns [336]. The asymmetrical nature of this type of complexes is even more enhanced by the binding of other partners besides the G-protein. For example, there is increasing evidence of asymmetry for the complexes formed between GPCR dimers, G-proteins and regulators of G-protein signaling (RGS) [333,337–341]. The molecular composition of oligomer/G-protein complexes might trap the receptor in different confirmations that can potentially bind to different ligands and have different physiological or pathological consequences. Although the physiological importance of oligomerization and the communication between the protomers is not entirely clear, the prospect of developing drugs that target them is a new exciting field.

8. Conclusion

The crucial importance of the GPCR signaling mechanism was revealed by the exceptionally profound and rapid progress that is now being achieved, and which has been recognized by the 2012 Nobel Prize awarded to leaders in the field. G-protein/GPCR complex formation leads to a spectrum of various cellular responses, which are triggered differentially according to the type of G-protein recognized by the active receptor. It is fundamental to comprehensively examine the structure–function relationships of receptor–G protein coupling. Although the C-terminus of Gα seems to be the primary receptor recognition domain responsible for selectivity, probably it is not the sole determinant and other regions distant from the G-protein binding interface may also be crucial for coupling through conformational change of the GPCR. Therefore, the coupling is nowadays thought to be not only thorough specific pair interactions but also by global changes in the conformation of the receptor or environment or ligand-dependent changes in its dynamics. However, a general principle that emerged is that the relative contribution of different intracellular receptor domains to the selectivity of G-protein recognition varies among structurally closely related members of the same receptor subfamily. In this review, we have focused on the key determinants for specificity as well as in new structural insights derived from the recent X-ray structures. The review illustrates that despite an abundance of information in the literature several key questions remain unanswered: (i) the atomistic understanding of the structural differences between the different GPCR/G-protein complexes; (ii) the mechanism of functional selectivity, how different ligands can affect the structure and/or dynamics of a GPCR; and (iii) the understanding of the effect of GPCR oligomerization in GPCR/G-protein coupling. These are key aspects of understanding needed to push forward the fields of GPCR signaling, functional selectivity, and drug development. Hopefully, with the rapid growth of the number of structures available and with a collaborative effort of experimental and computational approaches, it will be possible to understand the mechanism of GPCR activation and GPCR/G-protein specificity.

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Appendix A. Supplementary data

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