G Protein Selectivity Is Regulated by Multiple Intracellular Regions of GPCRs

Stephen K.-F. Wong

Department of Neuroscience, Pfizer Global Research and Development, Groton, Conn., USA

Key Words

G protein coupled receptors · G protein · Selectivity

Abstract

GTP-binding protein coupled receptors (GPCRs) bind to a vast diversity of extracellular ligands to regulate a wide variety of physiological responses. Upon binding of extracellular ligands, these seven-transmembrane-spanning receptor molecules couple to one or several subtypes of G protein which reside at the intracellular side of the plasma membrane to trigger intracellular signaling events. Amid the large structural diversity at the intracellular regions of GPCRs, there are only 18 different subtypes of G protein belonging to four subfamilies. The question of how GPCRs select and activate a single or multiple G protein subtype(s) has been the topic of intense investigations. This review will attempt to summarize the available data on the structural determinants in GPCRs that regulate the selectivity of G protein activation. The available data suggest that G protein can be activated by structurally diverse cationic α -helical structures with no obvious homology in primary sequence. The selectivity of receptor-G protein coupling is maintained by a combination of two functional domains at the intracellular region. One is the 'activation domain' which can activate multiple G protein subtypes, while the other is the 'selectivity domain' which restricts the coupling to the desired signaling pathway(s). A slight change in the

conformation at these two functional domains can affect the fidelity of G protein selectivity. This hypothesis can account for the vast structural diversity of GPCRs which link a fascinating variety of extracellular inputs, yet couple to a limited number of intracellular signaling pathways.

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G Protein Coupled Receptors

GTP-binding protein coupled receptors (G protein coupled receptors; GPCRs) represent the largest family of cell surface receptors known, with an estimated total of 1,000 members encoded in the human genome. These transmembrane molecules can be activated by a vast diversity of extracellular inputs such as biogenic amines, amino acids, odorants, lipids, ions, protease, nucleotides, peptides, large polypeptides, and even photons. A wide variety of physiological functions are regulated by GPCRs; examples are neurotransmission, visual perception, smell, taste, growth, secretion, metabolism, and immune and inflammatory responses. GPCRs, therefore, represent major targets for drug development, with chemical entities that are agonists, partial agonists, antagonists, or inverse agonists of GPCRs being pursued as therapeutics by the pharmaceutical industry. This is reflected in that an estimated 30% of the current pharmaceutical drugs are ligands for GPCRs [reviewed in 1]. Knowledge

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Fax +41 61 306 12 34
E-Mail karger@karger.ch
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Stephen K.-F. Wong
Department of Neuroscience
Pfizer Global Research and Development, Eastern Point Road
Groton, CT 06340 (USA)
Tel. +1 860 715 0065, Fax +1 860 686 0013, E-Mail Stephen_Wong@Groton.Pfizer.com

Table 1. G protein selectivity GPCRs

Receptor	Gq/11	Gi/o	Gs	PubMed ID	Receptor	Gq/11	Gi/o	Gs	PubMed ID
5-Hydroxytryptamine 1A receptor		+		3138543	D4 dopamine receptor		+		1840645
5-Hydroxytryptamine 1B receptor		+		1351684	D5 dopamine receptor			+	1831904
5-Hydroxytryptamine 1D receptor		+		1565658	Delta-type opioid receptor		+		1335167
5-Hydroxytryptamine 1E receptor		+		1608964	EDG-1		+		9488656
5-Hydroxytryptamine 1F receptor		+		8384716	EDG-2 lysophosphatidic acid receptor		+		9070858
5-Hydroxytryptamine 2A receptor	+			1765383	EDG-3 lysophingolipid receptor	+	+		9409733
5-Hydroxytryptamine 2B receptor	+			8786115	EDG-4 lysophosphatidic acid receptor	+	+		9525886
5-Hydroxytryptamine 2C receptor	+			1302605	EDG-5 lysophingolipid receptor		+		10617617
5-Hydroxytryptamine 4 receptor			+	2849052	EDG6	+			10679247
5-Hydroxytryptamine 5A receptor		+		7988681	EDG-7 lysophosphatidic acid	+			10488122
5-Hydroxytryptamine 6 receptor			+	8522988	Endothelin B receptor	+	+		1848367
5-Hydroxytryptamine 7 receptor			+	8397408	Endothelin-1 receptor	+		+	1849646
Adenosine A1 receptor		+		1662665	Extracellular calcium-sensing receptor	+			8255296
Adenosine A2A receptor			+	1662665	FMET-LEU-PHE receptor		+		2176894
Adenosine A2B receptor			+	11048721	FMLP-related receptor 1		+		11160182
Adenosine A3 receptor		+		1323836	Follicle-stimulating hormone receptor	+	+		9139796
Alpha-1A adrenergic receptor	+			8396931	GABA-B1A receptor		+		9872315
Alpha-1B adrenergic receptor	+			1662393	GABA-B2 receptor		+		9872315
Alpha-1D adrenergic receptor	+			1656955	Galanin receptor type 1		+		7524088
Alpha-2A adrenergic receptor	+	+	+	8662784	Galanin receptor type 2	+	+		9685625
Alpha-2B adrenergic receptor		+		2164221	Galanin receptor type 3		+		9722565
Alpha-2C-1 adrenergic receptor		+		2842764	Gastric inhibitory polypeptide receptor			+	8243312
Apelin receptor		+		9792798	Gastrin-releasing peptide receptor	+			1707129
B1 bradykinin receptor	+			8063797	Glucagon receptor			+	8144028
B2 bradykinin receptor	+			1314587	Glucagon-like peptide-1 receptor			+	1326760
Beta-1 adrenergic receptor			+	2825170	Glucagon-like peptide-2 receptor			+	9990065
Beta-2 adrenergic receptor			+	3041227	Gonadotropin-releasing hormone receptor	+			1333190
Beta-3 adrenergic receptor			+	2570461	Growth hormone secretagogue receptor type 1	+			8688086
Bombesin receptor subtype 3	+			8383682	Growth hormone releasing hormone receptor			+	7680413
C3A anaphylatoxin chemotactic receptor	+	+		8702752	Histamine H1 receptor	+			1722337
C5A anaphylatoxin chemotactic receptor	+			8779720	Histamine H2 receptor			+	1703298
Calcitonin gene related peptide type 1 receptor			+	8626685	Histamine H3 receptor		+		10347254
Calcitonin receptor			+	1331173	Histamine H4 receptor		+		10973974
Cannabinoid receptor 1		+		2165569	Kappa-type opioid receptor		+		7624359
Cannabinoid receptor 2		+		7689702	Leukotriene B4 receptor	+	+		9177352
CCR10 eskine		+		10781587	Lutropin-choriogonadotropic hormone receptor	+	+	+	2502842
CCR2		+		8530354	Melanocortin-1 receptor			+	1516719
CCR3		+		8631813	Melanocortin-2 receptor			+	1325670
CCR4		+		9169480	Melanocortin-3 receptor			+	8463333
CCR5		+		8639485	Melanocortin-4 receptor			+	8392067
CCR6		+		9294137	Melanocortin-5 receptor			+	8185570
CCR7		+		9153236	Melatonin receptor type 1A		+		7946354
CCR8		+		9207005	Melatonin receptor type 1B		+		7568007
CCR9		+		10229797	Metabotropic glutamate receptor 1	+			9076744
Chemokine CCR1		+		7679328	Metabotropic glutamate receptor 2		+		1309649
Cholecystokinin type A receptor	+		+	8503909	Metabotropic glutamate receptor 3		+		1309649
Cholecystokinin type B receptor	+			1280419	Metabotropic glutamate receptor 4		+		1309649
Corticotropin-releasing factor receptor 1			+	7692441	Metabotropic glutamate receptor 5	+			7908515
Corticotropin-releasing factor receptor 2			+	8536644	Metabotropic glutamate receptor 6		+		8389366
CX3CR1 fractalkine receptor		+		9390561	Metabotropic glutamate receptor 7		+		8288585
CXCR1		+		1840701	Metabotropic glutamate receptor 8		+		9473604
CXCR2		+		1891716	Muscarinic acetylcholine receptor M1	+			3037705
CXCR3		+		9653165	Muscarinic acetylcholine receptor M2		+		3443095
CXCR4		+		8629022	Muscarinic acetylcholine receptor M3	+			11242080
CXCR5		+		11882900	Muscarinic acetylcholine receptor M4		+		3037705
Cysteinyl leukotriene receptor CysLT2	+			10913337	Muscarinic acetylcholine receptor M5	+			3037705
Cysteinyl leukotriene receptor CysLT1	+			10391245	Mu-type opioid receptor		+		8393525
D1 dopamine receptor			+	2144334	Neuromedin K receptor (NK-3)	+		+	1370820
D2 dopamine receptor		+		2974511	Neuromedin U receptor NMU1R	+			10894543
D3 dopamine receptor		+		1975644	Neuromedin U receptor NMU2R	+			10894543

172 of the non-olfactory GPCRs with known G protein coupling selectivity towards G_s, G_{q/11}, and/or G_{i/o}. The references for the coupling can be found in <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed> using the PubMed ID listed in the table.

Table 1 (continued)

Receptor	Gq/11	Gi/o	Gs	PubMed ID
Neuromedin-B receptor	+			8026589
Neuropeptide Y receptor type 1	+	+		1321422
Neuropeptide Y receptor type 2		+		7559383
Neuropeptide Y receptor type 4		+		7592911
Neuropeptide Y receptor type 5		+		8700207
Neurotensin receptor type 1	+			8381365
Neurotensin receptor type 2	+			8647296
Nociceptin receptor		+		8137918
Orexin receptor type 1	+			9491897
Orexin receptor type 2	+			9491897
Oxytocin receptor	+	+		1313946
P2U purinoceptor 1	+			8159738
P2Y purinoceptor 1	+			8579591
P2Y purinoceptor 11	+		+	9405388
P2Y purinoceptor 6	+			7592819
P2Y12 platelet ADP receptor		+		11196645
Parathyroid hormone receptor			+	7797535
Parathyroid hormone related peptide receptor			+	1658941
Pituitary adenylate cyclase activating polypeptide type 1 receptor	+		+	8396727
Platelet-activating factor receptor	+	+		7876256
Protactin-releasing peptide receptor	+			11030716
Prostacyclin receptor			+	7512962
Prostaglandin D2 receptor			+	7642548
Prostaglandin E2 receptor EP1 subtype	+			8253813
Prostaglandin E2 receptor EP2 subtype			+	7910583
Prostaglandin E2 receptor EP3 subtype	+	+	+	11095474
Prostaglandin E2 receptor EP4 subtype			+	8016385
Prostaglandin F2-alpha receptor	+			8300593
Relaxin receptor LGR7			+	11809971
Relaxin receptor LGR8			+	11809971
Secretin receptor			+	1646711
Somatostatin receptor type 1		+		1346068
Somatostatin receptor type 2		+		7622071
Somatostatin receptor type 3		+		1337145
Somatostatin receptor type 4		+		8483934
Somatostatin receptor type 5		+		8373420
Substance-K receptor (NK-2)	+		+	1370820
Substance-P receptor (NK-1)	+		+	1370820
Thrombin receptor	+	+		1672265
Thromboxane A2 receptor	+			8300593
Thyrotropin receptor	+	+	+	8552586
Thyrotropin-releasing hormone receptor	+			2175902
Type 1 angiotensin II receptor	+			2041570
Type 2 angiotensin II receptor		+		7945336
Uridine nucleotide receptor	+			8537335
Urotensin II receptor	+			10499587
Vasoactive intestinal polypeptide receptor 1			+	1675791
Vasoactive intestinal polypeptide receptor 2			+	7733904
Vasopressin V1A receptor	+			1560825
Vasopressin V1B receptor	+			11091117
Vasopressin V2 receptor			+	1534150
XCR1		+		9632725

about the mechanism of signaling of GPCRs will have a great impact in understanding the physiological processes and will enhance future drug discovery efforts.

Even before the identification of the cDNA of the first GPCR, biochemical and kinetic analysis had revealed a

lot of information on the mechanisms of GPCR signaling [reviewed in 2]. An extracellular hormone agonist triggers the intracellular responses by binding to the ligand-binding site of the GPCR at the cell surface. The agonist-bound GPCR then activates GTP-binding protein (G protein) which consists of $\alpha\beta\gamma$ heterotrimer. In its basal, non-activated state, the GDP-bound G protein α subunit is tightly associated with the $\beta\gamma$ complex. Upon activation, the α subunit exchanges the bound GDP with GTP at its guanine nucleotide binding site, with subsequent dissociation from the $\beta\gamma$ complex. Both the GTP-bound α subunit and the dissociated $\beta\gamma$ subunits regulate activities of intracellular effector molecules downstream.

At least 18 different subtypes of α subunits have been identified. They can be divided into four subfamilies: (1) the ' G_s ' subfamily that stimulates adenylyl cyclase (G_s and G_{olf}); (2) the ' $G_{i/o}$ ' subfamily that inhibits adenylyl cyclase and regulates ion channels (G_{i1} , G_{i2} , G_{i3} , G_{o1} , G_{o2} , G_{o3} , G_z , G_{t1} , G_{t2} , and G_{gust}); (3) the ' $G_{q/11}$ ' subfamily that activates phospholipase C β (G_q , G_{11} , G_{14} , and $G_{15/16}$), and (4) the ' $G_{12/13}$ ' subfamily that activates the Na^+/H^+ exchanger pathway (G_{12} and G_{13}). Depending on the subtype(s) of the G protein α subunit that a given GPCR interacts with, a single or a combination of effectors can be activated. In addition, the $\beta\gamma$ complex, which anchors the α subunit to the plasma membrane, had been shown to activate ion channels. Five subtypes of β subunits and eleven subtypes of γ subunits have been identified. A combination of different subtypes of α , β , and γ subunits provides a great diversity of intracellular signaling pathways that can be regulated by GPCRs.

The key event to trigger an intracellular response by GPCR is the coupling between GPCR and the α subunit of G protein. Activation of G protein by GPCR apparently does not require other molecules, as agonist-dependent activation was observed in phospholipid vesicles, where purified GPCR and G protein were reconstituted [reviewed in 3]. Understanding of the coupling specificity between GPCR and G protein thus resides at the G-protein- and receptor-coupling interface.

Table 1 summarizes the G protein coupling selectivity of 172 GPCRs, representing a major subset of all the presently known non-olfactory GPCRs. A greater part of these GPCRs couple to a single subtype of G protein, with $G_{i/o}$ -coupled GPCRs being the majority (about 43%), followed by $G_{q/11}$ -coupled receptor (33%) and G_s -coupled receptor representing 25% of the total. Despite this wealth of information on GPCRs and their cognate G protein partners, little is known about how GPCRs selectively couple to a single subtype of G protein α subunit. This is

Fig. 1. G protein coupling specificity of chimeric muscarinic:β-adrenergic receptors. The intracellular loops (I2, I3, I3N, or their combination as indicated) of β-adrenergic receptor were substituted to the corresponding region of M₁ muscarinic receptor as depicted. G protein specificities of the chimeric receptors are shown below. [Results are quoted from ref. 31.]

Fig. 2. Structure of benzalkonium chloride (alkyldimethylbenzylammonium chloride) which activates G_o [9].

particularly puzzling given that there are several thousand GPCRs of diverse primary structures [4], yet there are only four subfamilies of G protein (G_{i/o}, G_s, G_{q/11}, and G_{12/13}) to be activated by the receptors. No obvious sequence homology was observed even for GPCRs that couple to the same G protein subtype, and no consensus motif predictive of G protein coupling has been identified [reviewed in 5]. In addition, a significant number of GPCRs have been shown to couple to multiple G protein subtypes (11% of the GPCRs listed in table 1). How do these GPCRs couple to multiple G proteins and how do the other GPCRs select for a single G protein subtype? These are fundamental questions without good answers thus far. This review will focus on these questions by summarizing data on the structural determinants required to

activate G proteins and efforts to determine domains in GPCRs that regulate G protein selectivity. A hypothesis that can potentially account for the available observations is proposed.

Architecture of GPCRs

GPCRs are seven-transmembrane-spanned molecules with the N-terminus located on the extracellular side and the C-terminus on the intracellular side (fig. 1). Recent availability of the crystal structure of rhodopsin (a prototypic GPCR) [6] has confirmed the seven-transmembrane structure of GPCRs determined by hydrophobicity analysis on the primary sequences. The transmembrane spans are α-helical segments interconnected by alternating extracellular and intracellular loops bundled together to form a heptahelical bundle-like structure [6]. Information of this general architecture of GPCR is important, as it suggests that there are significant intramolecular interactions amongst different extracellular as well as intracellular loops. Rather than interacting with a single region at the intracellular loop of GPCRs, G protein, which also has a globular structure [7], is predicted to interact simultaneously with multiple intracellular regions of GPCRs.

What Does It Take to Activate G Protein?

Activation of G protein by small peptides provides clues as to how G protein can be activated. The observation that purified G_o α subunits could be activated by the wasp venom peptide mastoparan, a 14 amino acid residue peptide, suggests that G protein can be activated by short amphiphilic structures [8]. The activation highly resembles that of GPCRs. At a low concentration of Mg²⁺ (>0.1 μM), mastoparan activates G protein by stimulating the guanine nucleotide exchange of G protein, and the activation can be blocked by pertussis toxin. The peptide also blocks the ability of G_o to increase the affinity of muscarinic M₂ receptor for its agonist, suggesting that mastoparan is acting at the receptor coupling site of G protein [9]. Structure-function relationship study using a variety of synthetic peptides with sequences distinct from mastoparan suggests that G proteins can be activated by a variety of cationic and hydrophobic peptides with no obvious similarity in primary sequence [9]. G protein can be activated even by quaternary amines such as benzalkonium chloride (alkyldimethylbenzylammonium chloride; fig. 2). A common feature of these activators of G_o

appears to be amphiphilic α -helical structure with a cationic residue on one side and a hydrophobic side chain on the other [10]. Similar conformation was found for G_s -activating peptide [11]. The apparent requirement of an amphiphilic α -helical structure to activate G_q was also demonstrated in a study using alanine insertion at the N-terminal region of the third intracellular loop (I3N) of the M_3 receptor [12]. Thus conformation and charge, rather than sequence homology, appear to be the major deciding factors for G protein activation. This can explain the absence of obvious consensus sequence motifs in GPCRs which activate the same G protein subunits.

The above data suggest that one mechanism of G protein activation by GPCR is: upon binding of agonist, the receptor changes its conformation to present an α -helical amphiphilic cationic structure to the G proteins to stimulate guanine nucleotide exchange. This simple requirement for G protein activation can account for the reported ligand-dependent activation of G proteins by molecules which do not have a heptahelical structure: IGF-II/mannose 6-phosphate receptor [13], amyloid precursor protein precursor protein [14], or atrial natriuretic peptide clearance receptor [15]. Non-ligand-mediated activation of G protein by several molecules or their peptide fragments has also been reported in the literature: GAP43 [16], polycystin-1 [17], or presenilin-1 [18], although the physiological significance of these interactions *in vivo* remains to be determined.

The above results also raise cautions to interpret data using synthetic peptides derived from GPCR as an approach to locate the G protein coupling regions of GPCRs. In these studies [reviewed in 19], synthetic peptides based on the primary sequences of GPCR were shown to activate G proteins. While suggestive that the regions are the G protein activation domains in GPCRs, the activation can also be a mere coincidence, i.e., if the synthetic peptide can form cationic amphipathic helices, as in the cases of mastoparan and other cationic helical peptides. The presence of a large number of charged residues at the intracellular region of GPCRs can increase the chance for the receptor-derived peptides to activate G proteins. This is illustrated by the activation of G_s (but not $G_{i/o}$) by a synthetic peptide from the second intracellular loop of the α_2 -adrenergic receptor [20], a $G_{i/o}$ -coupled GPCR which mainly couples to $G_{i/o}$ but not G_s . In addition, the conformation of the peptide fragment in the context of the heptahelical GPCR architecture can be distinct from that of the synthetic peptide. The observation that synthetic peptides can activate G protein does not necessarily mean that the peptides will activate G protein

when inserted into the GPCR environment. Also, interpretation to conclude that a certain region of GPCR is the G protein activation region from these studies needs to reconcile with the fact that in the unliganded state, GPCRs do not activate G protein. Other ligand-mediated conformational change is necessary to induce the activation.

Mutation Studies to Identify G Protein Coupling Regions of GPCRs

Despite the lack of a crystal structure information on the GPCR:G protein complex, a wealth of information had been obtained on the regions of GPCRs that are involved in G protein coupling. A large number of studies using mutations at the putative intracellular regions suggested that the intracellular loops are key regions for G protein coupling. A majority of these studies used deletion and domain swapping between GPCRs as strategies to identify the G protein coupling regions. The premise in these studies is that there is a single motif within GPCRs which is largely responsible for G protein activation and selectivity. Deletion of this domain would uncouple GPCR from G protein. This assumption, while used successfully to identify functional domains for many single membrane-spanning biomolecules (e.g., the kinase superfamily), must be taken with caution for GPCRs. The juxtaposed seven-membrane-spanning architecture of GPCR suggests that the intracellular loops can interact with each other to form functional domain(s) to interact with G protein. König et al. [21] has previously shown that synthetic peptides from I2, I3, and the C-terminus of rhodopsin, a prototypic GPCR, can inhibit the interaction between rhodopsin and transducin, its cognate G protein. Synergistic inhibition was observed when a combination of these peptides was used, suggesting that multiple intracellular loops of GPCR can interact with G protein. Deletion mutations that decrease G protein coupling can result in a change in the overall folding at the intracellular regions, rather than from the lack of a region responsible for G protein coupling. It is with these uncertainties that make interpretations from deletion studies inconclusive.

Given the above caveats of deletion studies, deletions at the 2nd and 3rd intracellular loops (I2, I3) or the N- and C-terminal regions of the 3rd intracellular loop (I3N, I3C) have resulted in GPCRs that no longer couple to G proteins while retaining their ligand-binding conformation [22, 23]. These regions have been implicated to be

Table 2. Chimeric GPCRs that have a change in G protein selectivity

Acceptor receptor (R1)		Donor receptor (R2)			Chimeric receptor R1–R2: G protein selectivity			Reference
receptor	G protein selectivity	receptor	regions substituted	G protein selectivity	R2	R1	other G proteins	
α_2 AR	G _{i/o}	β_2 AR	I3	G _s	+	ND	ND	42
mGluR ₃	G _{i/o}	mGluR ₁	I2+I3+C	G _q	+	ND	ND	43
D ₃	no coupling	D ₂	I3	G _{i/o}	+	ND	ND	44
D ₂	G _{i/o}	M ₁	I3	G _q	+	ND	ND	45
D ₂	no coupling	thrombin	I2	G _q	+	ND	no G _s	35
M ₂	G _{i/o}	M ₃	I2	G _q	+	ND	no G _s	36, 46
M ₂	G _{i/o}	M ₃	I3	G _q	+	–	ND	29
M ₃	G _q	M ₂	I3	G _{i/o}	+	–	ND	29
M ₂	G _{i/o}	M ₃	I3N	G _q	+	+	ND	29
A1A	G _{i/o}	A2A	I3, I3N	G _s	+	+	ND	47
β_2 AR	G _s	α_2 AR	I3N+I3C+C	G _{i/o}	+	+	ND	48
β_2 AR	G _s	α_1 AR	I3, I3N	G _q	+	+	ND	49
β_2 AR	G _s	thrombin	I2	G _q	+	+	ND	35
PACAPR ₂	G _s	PAFR	I3	G _q	+	+	ND	50
VP ₂	G _s	VP ₁	I2	G _q	+	+	no G _{i/o}	51
VP ₁	G _q	VP ₂	I3	G _s	+	+	no G _{i/o}	51
M ₁	G _q	β_1 AR	I3, I3N	G _s	+	+	G _{i/o} , G _z	30, 31
M ₂	G _{i/o} , G _z	β_1 AR	I3N	G _s	+	+	G _q	31
M ₁	G _q	β_1 AR	I2+I3N, I2+I3	G _s	+	–	no G _{i/o} , no G _z	30, 31
M ₂	G _{i/o} , G _z	β_1 AR	I2+I3N	G _s	+	–	no G _q , no G _z	31

Chimeric receptors (R1–R2) were constructed with the indicated intracellular region of donor receptor (R2) substituted into the corresponding region of acceptor receptor (R1). I2 = 2nd intracellular loop; I3 = 3rd intracellular loop; I3N: N-terminal region of the 3rd intracellular loop; I3C = C-terminal region of the 3rd intracellular loop, C = C-terminal tail. All of the chimeric receptors presented have acquired the G protein selectivity of R2, while a majority of them have retained the coupling to the acceptor receptor or became functionally promiscuous. No coupling = not detectable in the assay system used; ND = not determined.

involved in G protein coupling using chimeric receptors (see below). Deletion of a single amino acid at I2 of PACAP and H₂ receptors resulted in constitutively active receptors [24, 25]. While these results can indicate that the I2 loop in these receptors has a function to negatively regulate the activation of G protein, a more conservative interpretation is that these mutations can introduce a conformational change in the receptor to enable constitutive coupling. Evidence to support this precaution is that constitutive G protein activation was also observed with point mutations at regions believed not to be directly involved in G protein interaction. Examples of this include mutations at the 5th and 6th transmembrane spans of α -adrenergic receptors [26], 1st and 2nd extracellular loops of the TSH receptor [27]. The same caution should also be taken to interpret other mutations in GPCRs that resulted in constitutive activation [reviewed in 28].

Chimeric Receptors

Perhaps a more convincing strategy to identify the G protein coupling region is the use of chimeric receptors. In this approach, intracellular region(s) is(are) exchanged between GPCRs of different G protein coupling specificity. Substituting these regions of the ‘donor receptor’ onto the corresponding region in another ‘acceptor receptor’ should change the specificity of coupling and redirect the chimeric receptor to ‘abandon’ the original G protein subtype and couple with a new G protein α subunit. The assumption for this approach is that: First, G protein specificity is a result of a gain of function by having a particular sequence motif rather than a loss of selectivity (or fidelity) as a result of the mutation. Based on this rationale, the experimental design of most of the published studies involves determining the functional response

linked with the donor receptor-coupled G protein. In many cases, the coupling to the G protein of the acceptor receptor was not determined. Even fewer studies have looked into the possibility that the chimeric receptor will acquire the ability to couple a G protein subtype of which neither parent receptors were linked to. The second assumption is that specificity of G protein coupling is mainly defined by one intracellular region. When a switch in specificity of G protein coupling is observed in the chimeric receptor, it is usually assumed that the other intracellular regions do not play a major role in defining the specificity in coupling. This assumption may be valid for GPCRs that are highly homologous in other intracellular regions, but may not apply to GPCRs the primary sequences of which are distinct from each other. Below are data that may challenge the above assumptions and caution the interpretation of the published data. The available data also give us insights into how GPCRs select G protein(s).

Table 2 shows data of some studies using the chimeric receptor strategy to identify regions in G protein coupling regions of GPCRs. When the 3rd intracellular loop (I3) was exchanged between GPCRs of similar receptor subfamilies, e.g., between α_2 - and β_2 -adrenergic receptors, between dopamine D_2 and D_3 receptors, or between muscarinic M_2 and M_3 receptors, there appeared to be a switch in specificity of G protein coupling. These chimeric receptors acquired the G protein specificity of the donor receptor without retaining the G protein coupling of the acceptor receptor [29]. These results indicate the importance of I3 in G protein selectivity within closely structurally related GPCRs.

For most other chimeric receptors, however, exchanging either the 2nd and 3rd intracellular loops or its N terminal region does not appear to be sufficient to exchange the specificity of G protein coupling. Most of these chimeric receptors acquired the G protein specificity of the donor GPCR, but yet retained the ability to couple to the acceptor receptor G protein (table 2). In the case of D_2 :thrombin and D_2 : M_1 chimeric receptors, the ability of coupling to $G_{i/o}$ (acceptor G protein) was not determined. It is possible that the D_2 chimera also retains coupling to $G_{i/o}$ as well. The above results suggest that coupling of chimeric receptor to one G protein subtype does not necessarily result in the loss of coupling to other G protein(s). It appears, therefore, that GPCRs are free to interact with multiple G protein subtypes.

Multiple G protein coupling of chimeric GPCRs also raises the question on the structural basis for how GPCR selects multiple G protein subtypes. There are several pos-

sibilities to account for the coupling: first, different regions of GPCR interact with different G protein subtypes, and the mutation did not prevent the interaction with the acceptor receptor G protein. Second, rather than a 'gain of function' to acquire the coupling of G protein, the mutation has resulted in a 'loss of function' and lost its ability to select a single G protein subtype. That is, the chimeric GPCR has lost its fidelity of activating only a single G protein subtype and couple nondiscriminately to more than one G protein subtype. This in turn would suggest that there are other determinants in the native donor receptor to steer the native receptor away from other G protein subtypes that are not desired.

Promiscuous GPCRs

The studies using chimeric muscarinic M_1 and M_2 receptors with various intracellular loops of β -adrenergic receptor (β AR) perhaps provide an initial clue to distinguish the above possibilities. In the initial study [30] I3 or its N-terminal region of β AR was substituted into the corresponding regions of muscarinic M_1 receptor (M_1 - β I3 and M_1 - β I3N). Similar to many other chimeric GPCRs presented in table 2, these chimeric receptors activate both G_s (donor receptor G protein) and G_q (acceptor receptor G protein) in response to muscarinic agonists. As discussed above, the dual coupling of the chimeric muscarinic: β -adrenergic receptors can either be due to other region(s) in M_1 which can activate G_q after its I3 was replaced or due to the fact that the mutation has caused the chimeric receptor to lose its ability to distinguish G_s , G_q , and perhaps other G protein subtypes as well. To discern between these possibilities, purified M_1 - β I3 was reconstituted with purified G_s , G_i , G_q , G_o , or G_z in phospholipid vesicles to determine the receptor:G protein selectivity [31]. In the case of G_i , G_o , G_z , very little functional coupling was observed with either parent receptor M_1 or β AR. However the M_1 - β I3 was observed to couple to G_i , G_o , and G_z , in addition to G_s and G_q (fig. 1). The result strongly suggests that the ability of M_1 - β I3 to couple to G_q and G_s is due to its loss of ability to discriminate between different G protein subtypes, i.e., it has become functionally promiscuous. Similar promiscuity in coupling was also observed in chimeric M_2 with I3N of β AR (M_2 - β I3N) [31].

Besides the functional promiscuous GPCRs created artificially and described above, naturally occurring functionally promiscuous GPCRs are known. For example, the TSH receptor can couple to all known G protein sub-

Table 3. GPCR splice variants with different G protein selectivity

Receptor	Splice location	G protein selectivity	References
Prostaglandin EP ₃ -A	C-terminus	G _{i/o}	38
Prostaglandin EP ₃ -B	C-terminus	G _s	38
Prostaglandin EP ₃ -C	C-terminus	G _s	38
Prostaglandin EP ₃ -D	C-terminus	G _{i/o} , G _q	38
Prostaglandin EP ₃	C-terminal truncation	constitutively activated G _{i/o}	33
PACAP-R	I3C	G _q , G _s	52
PACAP-R-hip	I3C	G _q	52
PACAP-R-hop1	I3C	G _q , G _s	52
PACAP-R-hop2	I3C	G _q , G _s	52
PACAP-R-hip-hop1	I3C	G _q , G _s	52

Naturally occurring splice variants prostaglandin EP₃ and PACAP receptors have different G protein selectivity. The location within the receptor where splice event occurs and respective G protein selectivity are presented.

families: G_s, G_{i/o}, G_q, and G_{12/13} [32]. Splice variants at the C-terminal region of I3 of the LH/GH receptor couple to a combination of G_s, G_{q11}, and G_{i/o} (table 3). Another interesting example is the splice variants at the C-terminal tail of prostaglandin EP₃ receptor which can couple one or several of all subfamilies of G protein. The C-terminal region is unlikely to be directly involved in the activation of G protein, as truncation of this region resulted in constitutively active receptor [33] (table 3). These results suggest that the C-terminal tail of the receptor constraints the receptor from activating and regulates the selectivity towards G protein. The ability of other GPCRs listed in table 1 to couple to multiple G protein subtypes can thus be a natural phenomenon, and this may be an inherent property of many GPCRs.

G Protein Selectivity Domains

The fidelity of single G protein coupling for a majority of GPCRs (table 1) suggests the presence of an intracellular region to restrict the GPCRs from interacting with more than one G protein. This was demonstrated in the M1-βI3, M1-βI3N, or M2-βI3N chimeric receptors where an additional substitution of I2 from βAR (βI2) apparently restricts the functionally promiscuous chimeric muscarinic:βAR receptors to couple to G_s only [30, 31] (fig. 1). The resulting chimeric receptors (M1-βI2-βI3; M1-βI2-

βI3N; M2-βI2-βI3N) thus have the G protein selectivity of βAR with the ligand-binding specificity of muscarinic receptor. I2 of the βAR, therefore, appears to have an uncoupling function towards G proteins which are not G_s. This is supported by a decrease of potency of G_q coupling in M1:βI2 chimeric receptor [30]. The role of I2 as a selectivity domain towards G_s was also demonstrated in other GPCRs. In the context of α₂-adrenergic receptor that couples to both G_s (in the presence of pertussis toxin) and G_{i/o} (in the presence of cholera toxin) [34] (table 2), substitution of I2 from β₂AR appears to disrupt G_{i/o} coupling while maintaining the coupling to G_s. It is important to point out that, at least in the context of M₁ and M₂, the substitution of βI2 (M1-βI2 and M2-βI2) was not sufficient to uncouple from the acceptor receptor G protein (G_q and G_{i/o}, respectively) [30, 31]. The fidelity of coupling to a single G protein coupling appears to require a combination of distinct intracellular regions (fig. 1). The specificity of G protein coupling of these GPCR mutants was demonstrated in an in vitro reconstituted system using purified receptor and G protein, further indicating that the structural determinants in GPCR have all the necessary components to define the selectivity of G protein coupling.

The above hypothesis would also predict that disruption of I2 in βAR should lead to a loss in fidelity of G protein coupling. This is indeed what was observed. When I2 of the thrombin was substituted to βAR, the chimeric receptor lost its fidelity in G protein coupling and activated both G_s and G_q [35] (table 2). Not all substitution to I2 in βAR resulted in the loss of fidelity, as substituting I2 of D₂ into βAR did not affect the selectivity of βAR [35] (table 2). The similarity in sequence in I2 between βAR and D₂ is a likely explanation, as the tyrosine residue in the DRY motif common to all aminergic GPCR is absent in I2 of thrombin receptor, while I2 of D₂ retains this conserved DRY motif.

Another outcome from the above hypothesis is that mutations in GPCRs that disrupt the interaction between I2 and I3 will also result in a loss of fidelity to couple to a single G protein subtype. As shown in table 2, a majority of the receptors having domain swapping mutations at I2, I3, or I3N described in table 2 couple to more than one G protein subtype. Shorter mutations at I2 of M₂ or I3N of M₁ (table 4) led to a change in specificity in coupling. The coupling to the acceptor receptor G protein in several of these studies was not determined; it is possible that these receptors also couple to multiple G protein subtypes. It will also be of interest to determine the coupling to the G protein subtype which does not couple to either parent

Table 4. Other mutations of GPCRs that change G protein selectivity

Receptor	G protein selectivity	Mutation	G protein selectivity of receptor mutants	References
M ₂	G _{i/o}	4 AA mutation at I2	G _q , coupling to G _{i/o} not determined	36
M ₁	G _q	4 AA mutation at I3N	G _s , G _q	Wong and Ross unpublished
LH-CG	G _s , G _q	point mutation at extracellular loop 3	G _q , disruption of G _s coupling	53
α ₂ AR	G _i , G _k	D79 N (at 2nd span)	G _i , disruption of G _k coupling	54
α ₂ AR	G _s , G _{i/o}	substitution of I2 of βAR	G _s , disruption of G _{i/o} coupling	34
α ₂ AR	G _s , G _{i/o}	substitution of I3N, I3C of 5HT _{1A}	G _{i/o} , disruption of G _s coupling	34
Mel _{1c}	G _{i/o}	C-terminal truncation	G _{i/o} , G ₁₆	37
MT ₁ , MT ₂	G _{i/o} , G ₁₆	insertion of C terminal tail of Mel _{1c}	G _{i/o}	37
Mel _{1c}	G _{i/o}	substitution with C-terminal of MT ₁ , MT ₂	G _{i/o} , G ₁₆	37

G_k = G protein that mediates K⁺ channel activation.

receptors to determine the full G protein selectivity profile of these receptors. For those GPCRs that couple to more than one G protein subtype, additional substitution of other intracellular regions from the donor receptor would determine if these regions are involved in regulating G protein selectivity.

Not all single-domain substitutions led to a loss of fidelity in G protein coupling. Domain swapping of I3 between M₂ and M₃ appears to exchange the specificity in G protein coupling [29]. The high degree of sequence identity between M₂ and M₃ in I2 can account for the absence of coupling to more than one G protein subtype. However, when I2 of M₃ was substituted to M₂ receptor, the chimeric M₂ receptor coupled to G_q [36], indicating either I2 of M₃ directs coupling to G_q or that the mutation disrupts the ability of the M₂ chimeric receptor to select between G_q and G_i. Given the fact that a four amino acid mutation at I2 of M₂ enabled the M₂ receptor to couple to G_q [36], the second possibility is favored. Mutations at I2 of M₂ do not always lead to a change in G protein specificity, chimeric M₂ having I2 of βAR has similar G protein specificity as the native M₂ receptor [30]. Thus, the selectivity G protein coupling in the chimeric receptor is highly dependent on the context of the acceptor receptor and the sequence from the donor receptor substituted.

Besides I2 and I3, a G protein selectivity region was also demonstrated at the C-terminal region of GPCRs. Truncation of the C-terminal tail of Mel_{1c} receptor, which couples to G_{i/o} only, enabled the receptor to couple to G_{i/o} and G₁₆ [37]. Crafting this C-terminal region of Mel_{1c} to the C-terminus of MT₁ or MT₂, which couples to both G_{i/o} and G₁₆, disrupted the coupling to G₁₆ but not G_{i/o} (ta-

ble 4). The C-terminal region of Mel_{1c} thus appears to negatively regulate the coupling to G₁₆ but not G_{i/o}. The ability of the C-terminal tail to regulate G protein selectivity had also been demonstrated in the prostaglandin EP₃ receptors [38].

Conformation of GPCRs Defines G Protein Selectivity

Surprisingly little alteration in amino acid sequence for GPCR is needed for a change in G protein selectivity. Point mutations of LH/RH receptor (2nd extracellular loop) and α₂-adrenergic receptor (2nd membrane span) uncouple one G protein subtype while retaining the coupling to another subtype (table 4). These mutations are remote from the intracellular region of GPCRs and support the idea that the change in G protein selectivity in these mutant GPCRs results from a change of conformation in the intracellular domain. This hypothesis can also account for the change of G protein specificity upon changes of four amino acid residues at I3N and I2 of M₁ and M₂ [36] (table 4). These results argue against the presence of a 'consensus' G protein coupling motif to direct G protein selectivity. Rather, the change in G protein selectivity in these receptor point mutants more likely resulted from a loss in fidelity of G protein coupling due to a change in conformation at the intracellular region.

The above hypothesis is supported by the presence of naturally occurring promiscuous G proteins such as G₁₅ and G₁₆ [reviewed in 39]. Each of these two G protein subtypes couples to a variety of G_s-, G_{i/o}-, and G_q-linked

GPCRs and activates phospholipase C β . This argues that conformation of the agonist-bound GPCR to activate G protein is very similar. A subtle change in conformation of GPCR can result in a change in G protein selectivity. This can account for the difference in G protein selectivity of different splice variants of EP₃ receptor with mutations at the C-terminal tail [38] (table 3). Different C-terminal splice variants of the EP₃ receptor can modulate the conformation of the other intracellular loops to influence the selectivity of G protein coupling. Similar explanation can be extended to the splice variants at the C-terminal region of I3 of the PACAP receptor that has different selectivity in G protein coupling (table 3).

The concept of overall conformation, rather than a stretch of the sequence motif, in intracellular regions of GPCRs to define G protein selectivity can account for the lack of consensus G protein coupling sequences amongst all the GPCRs identified thus far. This concept will be important in our understanding of the structural basis of GPCR function. In the light of the recent identification of GPCR heterodimers [40, 41], it will be interesting to see whether heterodimerization occurs for GPCRs which couple to different G protein subfamilies and, if so, whether heterodimerization affects G protein selectivity.

A New View on the Structural Basis for G Protein Selectivity of GPCRs

This review provides a new view on the structural basis for G protein selectivity of GPCRs. G protein selectivity is defined by the conformation at the intracellular region of GPCRs. This conformation is regulated by the interaction between several intracellular regions. Rather than one receptor region directing the specificity of G protein coupling, the selectivity is a result of a combination of one 'activation domain' which can activate multiple G protein subtypes and another 'selectivity domain' to restrict the promiscuous coupling. This hypothesis is supported by: (1) the presence of naturally occurring GPCRs which can couple to multiple G protein subtypes; (2) the activation of G protein by small cationic helical structure; (3) no consensus G protein coupling motif amongst GPCRs; (4) naturally occurring splice variants that restrict or alter the selectivity of G protein coupling; (5) change of G protein selectivity by point mutations at nonintracellular domain and intracellular loops; (6) loss of fidelity of coupling to a single G protein subtype in many GPCR mutants, and (7) promiscuous coupling of chimeric muscarinic: β AR, the fidelity of selectivity of which towards

G_s can be rescued by an additional substitution of I2 of β AR.

The structural basis for G protein selectivity proposed above can also explain the purpose of the heptahelical structure and the sequence diversity of GPCR. The seven-membrane spans form the ligand-binding pocket and provide the necessary architecture to the three intracellular loops and the C-terminal tail to form a three-dimensional 'pocket' to regulate agonist-mediated G protein activation and selectivity amongst G protein subtypes. This region is designed in fascinating sequence diversity amongst GPCRs, yet having similar conformation and mode of action to couple to limited subtypes of G protein. This provides a platform for the GPCR superfamily to bind an amazing variety of extracellular receptor ligands and allows it to couple to a few intracellular signaling pathways. A slight change of conformation can alter efficacy and selectivity of G protein activation, and this enables the receptor-G protein interaction to be fine-tuned by different extracellular ligands as well as by the intracellular environment. Deeper understanding of how GPCR interacts with G protein awaits the crystal structure of the GPCR:G protein complex, more mutation analysis to validate the proposed model, and the development of bioinformatic algorithms to predict the three-dimensional structure at the receptor-G protein interface.

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References

- Hopkins AL, Groom CR: The druggable genome. *Nat Rev Drug Discov* 2002;1:727–730.
- Ross EM, Gilman AG: Biochemical properties of hormone-sensitive adenylate cyclase. *Annu Rev Biochem* 1980;49:533–564.
- Cerione RA, Ross EM: Reconstitution of receptors and G proteins in phospholipid vesicles. *Methods Enzymol* 1991;195:329–342.
- Probst WC, Snyder LA, Schuster DI, Brosius J, Sealfon SC: Sequence alignment of the G-protein-coupled receptor superfamily. *DNA Cell Biol* 1992;11:1–20.
- Moller S, Vilo J, Croning MD: Prediction of the coupling specificity of G protein coupled receptors to their G proteins. *Bioinformatics* 2001;17(suppl 1):174–181.
- Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M: Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* 2000;289:739–745.
- Mixon MB, Lee E, Coleman DE, Berghuis AM, Gilman AG, Sprang SR: Tertiary and quaternary structural changes in Gi alpha 1 induced by GTP hydrolysis. *Science* 1995;270:954–960.
- Higashijima T, Uzu S, Nakajima T, Ross EM: Mastoparan, a peptide toxin from wasp venom, mimics receptors by activating GTP-binding regulatory proteins (G proteins). *J Biol Chem* 1988;263:6491–6494.
- Higashijima T, Burnier J, Ross EM: Regulation of G_i and G_o by mastoparan, related amphiphilic peptides, and hydrophobic amines; Mechanism and structural determinants of activity. *J Biol Chem* 1990;265:14176–14186.
- Wakamatsu K, Okada A, Miyazawa T, Ohya M, Higashijima T: Membrane-bound conformation of mastoparan-X, a G-protein-activating peptide. *Biochemistry* 1992;31:5654–5660.
- Sukumar M, Ross EM, Higashijima T: A G_s-selective analog of the receptor-mimetic peptide mastoparan binds to G_s alpha in a kinked helical conformation. *Biochemistry* 1997;36:3632–3639.
- Blüml K, Mutschler E, Wess J: Insertion mutagenesis as a tool to predict the secondary structure of a muscarinic receptor domain determining specificity of G-protein coupling. *Proc Natl Acad Sci USA* 1994;91:7980–7984.
- Okamoto T, Katada T, Murayama Y, Ui M, Ogata E, Nishimoto I: A simple structure encodes G protein-activating function of the IGF-II/mannose 6-phosphate receptor. *Cell* 1990;62:709–717.
- Okamoto T, Takeda S, Murayama Y, Ogata E, Nishimoto I: Ligand-dependent G protein coupling function of amyloid transmembrane precursor. *J Biol Chem* 1995;270:4205–4208.
- Murthy KS, Teng BQ, Zhou H, Jin JG, Grider JR, Makhlof GM: G(i-1)/G(i-2)-dependent signaling by single-transmembrane natriuretic peptide clearance receptor. *Am J Physiol Gastrointest Liver Physiol* 2000;278:G974–G980.
- Strittmatter SM, Valenzuela D, Kennedy TE, Neer EJ, Fishman MC: G_o is a major growth cone protein subject to regulation by GAP-43. *Nature* 1990;344:836–841.
- Parnell SC, Magenheimer BS, Maser RL, Rankin CA, Smine A, Okamoto T, Calvet JP: The polycystic kidney disease-1 protein, polycystin-1, binds and activates heterotrimeric G-proteins in vitro. *Biochem Biophys Res Commun* 1998;251:625–631.
- Smine A, Xu X, Nishiyama K, Katada T, Gambetti P, Yadav SP, Wu X, Shi YC, Yasuhara S, Homburger V, Okamoto T: Regulation of brain G-protein by Alzheimer's disease gene presenilin-1. *J Biol Chem* 1998;273:16281–16288.
- Hedin KE, Duerson K, Clapham DE: Specificity of receptor-G protein interactions: Searching for the structure behind the signal. *Cell Signal* 1993;5:505–518.
- Okamoto T, Nishimoto I: Detection of G protein-activator regions in M4 subtype muscarinic, cholinergic, and alpha 2-adrenergic receptors based upon characteristics in primary structure. *J Biol Chem* 1992;267:8342–8346.
- König B, Arendt A, McDowell JH, Kahlert M, Hargrave PA, Hofmann KP: Three cytoplasmic loops of rhodopsin interact with transducin. *Proc Natl Acad Sci USA* 1989;86:6878–6882.
- Cheung AH, Dixon RA, Hill WS, Sigal IS, Strader CD: Separation of the structural requirements for agonist-promoted activation and sequestration of the beta-adrenergic receptor. *Mol Pharmacol* 1990;37:775–779.
- Chicchi GG, Graziano MP, Koch G, Hey P, Sullivan K, Vicario PP, Cascieri MA: Alterations in receptor activation and divalent cation activation of agonist binding by deletion of intracellular domains of the glucagon receptor. *J Biol Chem* 1997;272:7765–7769.
- Alewinse AE, Timmerman H, Jacobs EH, Smit MJ, Roovers E, Cotechia S, Leurs R: The effect of mutations in the DRY motif on the constitutive activity and structural instability of the histamine H(2) receptor. *Mol Pharmacol* 2000;57:890–898.
- Cao YJ, Gimpl G, Fahrenholz F: A mutation in the second intracellular loop of the pituitary adenylate cyclase activating polypeptide type I receptor confers constitutive receptor activation. *FEBS Lett* 2000;469:142–146.
- Hwa J, Graham RM, Perez DM: Chimeras of alpha1-adrenergic receptor subtypes identify critical residues that modulate active state isomerization. *J Biol Chem* 1996;271:7956–7964.
- Parma J, Van Sande J, Swillens S, Tonacchera M, Dumont J, Vassart G: Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: Identification of additional mutations activating both the cyclic adenosine 3',5'-monophosphate and inositol phosphate-Ca²⁺ cascades. *Mol Endocrinol* 1995;9:725–733.
- Parnot C, Miserey-Lenkei S, Bardin S, Corvol P, Clauser E: Lessons from constitutively active mutants of G protein-coupled receptors. *Trends Endocrinol Metab* 2002;13:336.
- Wess J, Bonner TI, Dorje F, Brann MR: Delineation of muscarinic receptor domains conferring selectivity of coupling to guanine nucleotide-binding proteins and second messengers. *Mol Pharmacol* 1990;38:517–523.
- Wong SK, Parker EM, Ross EM: Chimeric muscarinic cholinergic:beta-adrenergic receptors that activate G_s in response to muscarinic agonists. *J Biol Chem* 1990;265:6219–6224.
- Wong SK, Ross EM: Chimeric muscarinic cholinergic:beta-adrenergic receptors that are functionally promiscuous among G proteins. *J Biol Chem* 1994;269:18968–18976.
- Laugwitz KL, Allgeier A, Offermanns S, Spicher K, Van Sande J, Dumont JE, Schultz G: The human thyrotropin receptor: A heptahelical receptor capable of stimulating members of all four G protein families. *Proc Natl Acad Sci USA* 1996;93:116–120.
- Hasegawa H, Negishi M, Ichikawa A: Two isoforms of the prostaglandin E receptor EP3 subtype different in agonist-independent constitutive activity. *J Biol Chem* 1996;271:1857–1860.
- Eason MG, Liggett SB: Chimeric mutagenesis of putative G-protein coupling domains of the alpha2A-adrenergic receptor: Localization of two redundant and fully competent G coupling domains. *J Biol Chem* 1996;271:12826–12832.
- Verrall S, Ishii M, Chen M, Wang L, Tram T, Coughlin SR: The thrombin receptor second cytoplasmic loop confers coupling to Gq-like G proteins in chimeric receptors: Additional evidence for a common transmembrane signaling and G protein coupling mechanism in G protein-coupled receptors. *J Biol Chem* 1997;272:6898–6902.
- Blin N, Yun J, Wess J: Mapping of single amino acid residues required for selective activation of Gq/11 by the m3 muscarinic acetylcholine receptor. *J Biol Chem* 1995;270:17741–17748.
- Lai FP, Mody SM, Yung LY, Kam JY, Pang CS, Pang SF, Wong YH: Molecular determinants for the differential coupling of Galpha(16) to the melatonin MT1, MT2 and Xenopus Mel1c receptors. *J Neurochem* 2002;80:736–745.
- Namba T, Sugimoto Y, Negishi M, Irie A, Ushikubi F, Kakizuka A, Ito S, Ichikawa A, Narumiya S: Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP3 determines G-protein specificity. *Nature* 1993;365:166–170.
- Milligan G, Marshall F, Rees S: G16 as a universal G protein adapter: Implications for agonist screening strategies. *Trends Pharmacol Sci* 1996;17:235–237.
- Jordan BA, Devi LA: G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* 1999;399:697–700.

- 41 Rocheville M, Lange DC, Kumar U, Patel SC, Patel RC, Patel YC: Receptors for dopamine and somatostatin: Formation of hetero-oligomers with enhanced functional activity. *Science* 2000;288:154–157.
- 42 Kobilka BK, Kobilka TS, Daniel K, Regan JW, Caron MG, Lefkowitz RJ: Chimeric alpha 2-, beta 2-adrenergic receptors: Delineation of domains involved in effector coupling and ligand binding specificity. *Science* 1988;240:1310–1316.
- 43 Gomeza J, Joly C, Kuhn R, Knopfel T, Bockaert J, Pin JP: The second intracellular loop of metabotropic glutamate receptor 1 cooperates with the other intracellular domains to control coupling to G-proteins. *J Biol Chem* 1996;271:2199–2205.
- 44 Van Leeuwen DH, Eisenstein J, O'Malley K, MacKenzie RG: Characterization of a chimeric human dopamine D₃/D₂ receptor functionally coupled to adenylyl cyclase in Chinese hamster ovary cells. *Mol Pharmacol* 1995;48:344–351.
- 45 England BP, Ackerman MS, Barrett RW: A chimeric D₂ dopamine/m1 muscarinic receptor with D2 binding specificity mobilizes intracellular calcium in response to dopamine. *FEBS Lett* 1991;279:87–90.
- 46 Kostenis E: Is Galpha16 the optimal tool for fishing ligands of orphan G-protein-coupled receptors? *Trends Pharmacol Sci* 2001;22:560–564.
- 47 Olah ME: Identification of A2a adenosine receptor domains involved in selective coupling to Gs: Analysis of chimeric A1/A2a adenosine receptors. *J Biol Chem* 1997;272:337–344.
- 48 Liggett SB, Caron MG, Lefkowitz RJ, Hnatowich M: Coupling of a mutated form of the human beta 2-adrenergic receptor to Gi and Gs: Requirement for multiple cytoplasmic domains in the coupling process. *J Biol Chem* 1991;266:4816–4821.
- 49 Cotecchia S, Ostrowski J, Kjelsberg MA, Caron MG, Lefkowitz RJ: Discrete amino acid sequences of the alpha 1-adrenergic receptor determine the selectivity of coupling to phosphatidylinositol hydrolysis. *J Biol Chem* 1992;267:1633–1639.
- 50 Carlson SA, Chatterjee TK, Fisher RA: The third intracellular domain of the platelet-activating factor receptor is a critical determinant in receptor coupling to phosphoinositide phospholipase C-activating G proteins: Studies using intracellular domain minigenes and receptor chimeras. *J Biol Chem* 1996;271:23146–23153.
- 51 Liu J, Wess J: Different single receptor domains determine the distinct G protein coupling profiles of members of the vasopressin receptor family. *J Biol Chem* 1996;271:8772–8778.
- 52 Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, Journot L: Differential signal transduction by five splice variants of the PACAP receptor. *Nature* 1993;365:170–175.
- 53 Gilchrist RL, Ryu KS, Ji I, Ji TH: The luteinizing hormone/chorionic gonadotropin receptor has distinct transmembrane conductors for cAMP and inositol phosphate signals. [Erratum in *J Biol Chem* 1996;271:26444.] *J Biol Chem* 1996;271:19283–19287.
- 54 Surprenant A, Horstman DA, Akbarali H, Limbird LE: A point mutation of the alpha 2-adrenoceptor that blocks coupling to potassium but not calcium currents. *Science* 1992;257:977–980.