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MINIREVIEW

Probing Receptor Structure/Function with Chimeric G-Protein-Coupled Receptors

Dezhong Yin, Shai Gavi, Hsien-yu Wang, and Craig C. Malbon

Departments of Molecular Pharmacology (D.Y., S.G., C.C.M.) and Physiology & Biophysics (H.W.), Diabetes & Metabolic Diseases Research Program (H.W.), University Medical Center, Stony Brook University, Stony Brook, New York

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ABSTRACT

Owing its name to an image borrowed from Greek mythology, a chimera is seen to represent a new entity created as a composite from existing creatures or, in this case, molecules. Making use of various combinations of three basic domains of the receptors (i.e., exofacial, transmembrane, and cytoplasmic segments) that couple agonist binding into activation of effectors through heterotrimeric G-proteins, molecular pharmacology has probed the basic organization, structure/function relationships of this superfamily of heptahelical receptors. Chimeric G-protein-coupled receptors obviate the need for a particular agonist ligand when the ligand is resistant to purification or, in the case of orphan receptors, is not known. Chimeric receptors created from distant members of the heptahelical receptors enable new strategies in understanding how these receptors transduce agonist binding into receptor activation and may be able to offer insights into the evolution of G-protein-coupled receptors from yeast to humans.

Chimera (chi′ · me · ra; also chi · mae · ra). *Noun*. **1.** *from Greek Mythology:* An imaginary she-monster typically displayed as a composite of grotesquely different parts, most often of lion, goat, and serpent.

Few would have suspected that the early identification of a few notable cell-surface receptors that require heterotrimeric G-proteins (GPCRs) for signal propagation (Yarden et al., 1986; Dixon et al., 1987; Hargrave and McDowell, 1992) would ultimately lead to the appreciation of GPCRs as a superfamily that constitutes ~5% of the human genome, making it the most populous family in cell signaling. Since the early characterization, isolation, purification, and molecular cloning efforts, several universal properties of GPCRs, such as their heptahelical nature, have been revealed (Morris and Malbon, 1999). Many important discoveries in the structure, function, and biology of GPCRs are the result of innovative use of chimeric receptors composed of domains of various GPCRs in an attempt to overcome formidable obstacles. The literature highlights more than 100 examples using this strategy to address issues such as the molecular nature of the ligand binding domain, the roles of specific transmembrane spanning segments (TMS), and the nature of the cytoplasmic domains in defining downstream signaling and receptor regulation/trafficking (Table 1). The central thrust of this review is to ascertain how the chimeric receptor strategy has been applied to studies of GPCRs and what we have learned about the values and limitations of the approach.

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Organization of GPCRs

The landmarks for GPCRs can be appreciated in the form of the mythological chimera (Fig. 1A). Dominant in the landmarks of a GPCR are the seven TMS or core "heptahelical" structure embedded in the lipid bilayer (here a "lily pond" bilayer). The TMS appear to be composed of 20- to 22-residue stretches of largely hydrophobic amino acids that are predicted to be α-helical in structure. The actual length of TMS might increase by two to three residues in regions of a GPCR that traverse the lipid bilayer obliquely. The N terminus (depicted as the composite of a lion/she-goat) is exofacial and includes the products of post-translational addition of *N*-linked complex carbohydrates (-CHO). The ligand binding

ABBREVIATIONS: GPCR, G-protein-coupled receptor; TMS, transmembrane segments; LH, luteinizing hormone; FSH, follicle-stimulating hormone; AC, adenylyl cyclase; Rfz2, rat Frizzled 2; β_2AR, β_2-adrenergic receptor; αMFR, yeast α mating factor receptor.

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TABLE 1
Studies of receptor structure and function by construction of chimeric G-protein-coupled receptors

Family	Parent GPCR	Donor GPCR	Donated Region				Probe			Reference
			NT	TMS	Loops	CT	Ligand Binding	Downstream Signaling	Receptor Internalization	
I	α_1-AR	β_2-AR			+			+		Cotecchia et al. (1990)
I	α_{1A}-AR	α_{1B}-AR			+			+		Chen et al. (1996)
I	α_{1A}-AR	α_{1D}-AR		+			+			Hamaguchi et al. (1996)
I	α_{1A}-AR	α_{1D}-AR		+			+			Hamaguchi et al. (1998)
I	α_{1A}-AR	α_{1B}-AR				+		+		Vazquez-Prado et al. (2000)
I	α_{1A}-AR	α_{1B}-AR		+			+			Lapinsh et al. (2001)
I	α_{1A}-AR	α_{1D}-AR			+	+	+	+		Shinoura et al. (2002)
I	α_{1B}-AR	β_2-AR			+	+	+	+		Cotecchia et al. (1992)
I	α_{1B}-AR	α_{1A}-AR		+	+		+			Zhao et al. (1996)
I	α_2-AR	mAChR3	+	+	+	+	+	+		Maggio et al. (1993)
I	α_2-AR	β_2-AR	+	+	+	+	+	+		Kobilka et al. (1988)
I	α_{2A}-AR	β_2-AR, 5-HT_{1A}			+			+		Eason and Liggett (1995)
I	α_{2A}AR	β_2-AR, 5-HT_{1A}	+		+	+	+	+		Eason and Liggett (1996)
I	α_{2A}-AR	β_2-AR, 5-HT_{1A}	+		+	+	+		+	Jewell-Motz et al. (1997)
I	α_{2A}-AR	Adenosine A_1	+	+	+	+	+		+	Saunders et al. (1998)
I	α_{2A}-AR	β_2-AR, 5-HT_{1A}	+		+	+		+		Jewell-Motz et al. (1998)
I	α_{2A}-AR	α_{2C}-AR			+		+	+		Jewell-Motz et al. (2000)
I	β_1-AR	β_2-AR		+			+	+		Frielle et al. (1988)
I	β_1-AR	β_2-AR		+		+	+	+		Rousseau et al. (1996)
I	β_1-AR	β_2-AR	+	+		+	+			Kikkawa et al. (1998)
I	β_1-AR	β_2-AR		+	+	+	+	+	+	Shiina et al. (2000)
I	β_1-AR	β_2-AR	+	+	+	+	+			Sugimoto et al. (2002)
I	β_2-AR	β_1-AR		+			+			Marullo et al. (1990)
I	β_2-AR	β_1-AR				+	+	+		Parker et al. (1995)
I	β_2-AR	β_1-AR		+			+	+		Green et al. (1996)
I	β_2-AR	VR2				+	+	+	+	Oakley et al. (1999)
I	β_2-AR	α_{2A}-AR			+		+	+		Zhao et al. (1998)
I	β_2-AR	Histamine H_2			+	+	+	+		Wang et al. (2000)
I	β_2-AR	Angiotensin II				+		+	+	Anborgh et al. (2000)
I	β_2-AR	VR2				+		+	+	Tohgo et al. (2003)
I	β_3-AR	β_2-AR			+	+		+		Nantel et al. (1993)
I	β_3-AR	β_2-AR				+	+	+		Liggett et al. (1993)
I	β_3-AR	β_2-AR	+	+	+	+	+	+		Guan et al. (1995)
I	β_3-AR	β_2-AR			+	+	+	+		Jockers et al. (1996)
I	β_3-AR	β_2-AR				+	+		+	Mostafapour et al. (1996)
I	Angiotensin I	Angiotensin II		+	+	+	+	+		Feng and Karnik (1999)
I	Angiotensin II	α_{1B}-AR, β_2-AR			+		+	+	+	Conchon et al. (1997)
I	FSH	LH			+		+	+		Dias et al. (1994)
I	FSH	LH	+				+	+		Vischer et al. (2003)
I	GnRH	TSH				+	+		+	Heding et al. (1998)
I	GnRH	TSH				+		+	+	Hanyaloglu et al. (2001)
I	5-HT_{1B}	5-HT_{2A}			+		+			Oksenberg et al. (1995)
I	5-HT_{1B}	5-HT_{2D}		+	+		+			Wurch et al. (1998)
I	5-HT_{1B}	5-HT_{2E}		+			+			Parker et al. (1996)
I	5-HT_2	5-HT_{1c}		+	+		+			Choudhary et al. (1992)
I	5-HT_2	Thrombin				+		+		Vouret-Craviari et al. (1995)
I	5-HT_{2A}	5-HT_{2C}			+	+	+	+		Alberts et al. (2003)
I	5-HT_{3A}	5-HT_{3A}			+			+		Boyd et al. (2003)
I	5-HT_{3A}	5-HT_{3B}			+			+		Kelley et al. (2003)
I	LH	FSH	+				+	+		Braun et al. (1991)
I	LH	FSH	+				+	+		Hirsch et al. (1996)
I	LH	FSH	+				+	+		Kudo et al. (1996)
I	LH	FSH	+				+	+		Osuga et al. (1997)
I	mAChR1	mAChR2		+			+	+		Kubo et al. (1988)
I	mAChR1	β_2-AR			+		+	+		Wong et al. (1990)
I	mAChR1	mAChR2		+	+		+			Lai et al. (1992)
I	mAChR2	mAChR3			+		+	+		Wess et al. (1989)
I	mAChR2	mAChR3			+			+		Wess et al. (1990a)
I	mAChR2	mAChR3		+	+	+	+	+		Wess et al. (1990b)
I	mAChR2	mAChR5		+	+		+			Wess et al. (1992)
I	mAChR2	mAChR5		+			+			Wess and Pittel (1994)
I	mAChR3	mAChR2			+			+		Felder et al. (1992)
I	mAChR3	α_{2C}-AR	+	+	+		+	+		Maggio et al. (1999)
I	mAChR3	mAChR2			+		+	+		Bluml et al. (1994)
I	Rhodopsin	β_2-AR			+			+		Marin et al. (2000)
I	Rhodopsin	mGlu1/6			+	+		+		Yamashita et al. (2001)
I	Thrombin	Substance P				+	+	+		Trejo et al. (1998)
I	Thrombin	Substance P				+	+	+		Sambrano and Coughlin (1999)
I	Thrombin	Substance P				+		+	+	Trejo and Coughlin (1999)

CT, C terminus; NT, N terminus; mAChR muscarinic acetylcholine receptor; GnRH, gonadotropin-releasing hormone; TSH, thyroid-stimulating hormone; mGluR, metabotropic glutamate receptor.

TABLE 1
Continued

Family	Parent GPCR	Donor GPCR	Donated Region				Probe			Reference
			NT	TMS	Loops	CT	Ligand Binding	Downstream Signaling	Receptor Internalization	
I	TSH	LH	+		+		+	+		Nagayama et al. (1990)
I	TSH	LH	+		+		+	+		Nagayama et al. (1991)
I	TSH	LH	+		+		+	+		Nagayama et al. (1991)
I	TSH	LH	+		+		+	+		Akamizu et al. (1993)
I	TSH	LH	+		+		+	+		Nagayama et al. (1994)
II	Glucagon	Dopamine D4			+	+	+	+		Cypess et al. (1999)
II	Dopamine D1	Dopamine D2		+			+	+		MacKenzie et al. (1993)
II	Dopamine D1	Dopamine D2		+	+	+	+	+		Kozell et al. (1994)
II	Dopamine D1	Dopamine D2	+	+			+	+		Kozell and Neve (1997)
II	Dopamine D2	Dopamine D3			+		+			Robinson et al. (1994)
II	Dopamine D2	Dopamine D3			+		+	+		Robinson and Caron (1996)
II	Dopamine D2	Dopamine D3			+		+	+		Lachowicz and Sibley (1997)
II	Dopamine D2	Dopamine D3				+	+	+		Scarselli et al. (2001)
II	Dopamine D2	Dopamine D3			+			+		Ilani et al. (2002)
II	Dopamine D2	Dopamine D4	+	+			+			Shih et al. (1997)
II	Dopamine D2	mAChR1			+		+	+		England et al. (1991)
II	Dopamine D2	Thrombin			+			+		Verrall et al. (1997)
II	Dopamine D3	Dopamine D1		+			+			Alberts et al. (1998)
II	Dopamine D3	Dopamine D2			+		+	+		McAllister et al. (1993)
II	Dopamine D3	Dopamine D2			+		+	+		Van Leeuwen et al. (1995)
II	Dopamine D4	Glucagon			+		+	+		Kazmi et al. (2000)
II	VPAC1	VPAC2				+	+	+		Juarranz et al. (1999)
II	VPAC1	VPAC2			+		+	+		Langer et al. (2002)
II	VPAC2	PAC1			+		+	+		McCulloch et al. (2000)
II	VPAC2	PAC1			+		+	+		McCulloch et al. (2001)
III	mGluR1	mGluR3			+			+		Gomeza et al. (1996)
III	Orphan	Ca^{2+}, mGluR1	+				+	+		Brauner-Osborne et al. (2001)
III	$GABA_B$ 1	$GABA_B$ 2				+	+	+		Calver et al. (2001)
III	$GABA_B$ 1	$GABA_B$ 2	+	+	+	+	+	+		Galvez et al. (2001)
III	$GABA_B$ 2	mGluR1		+			+			Malitschek et al. (1999)
III	$GABA_B$ 2	$GABA_B$ 1			+		+	+		Margeta-Mitrovic et al. (2001)a
III	$GABA_B$ 2	$GABA_B$ 1	+				+	+		Margeta-Mitrovic et al. (2001)b
III	$GABA_B$ 2	$GABA_B$ 1			+		+	+		Robbins et al. (2001)
III	$GABA_B$ 2	$GABA_B$ 1			+		+	+		Havlickova et al. (2002)
III	mGluR1	Ca^{2+}	+					+		Hammerland et al. (1999)
III	mGluR2	mGluR4/8			+			+		Havlickova et al. (2003)
III	mGluR3	mGluR1c		+	+	+		+		Pin et al. (1994)
III	mGluR3	mGluR1			+	+		+		Flor et al. (1996)
III	VR1a	VRR1			+	+	+	+	+	Gupte et al. (2004)
V	Frizzled-1	β_2-AR	+	+	+		+	+		Liu et al. (2001)
V	Frizzled-1	β_2-AR	+	+	+		+	+	+	DeCostanzo et al. (2002)
V	Frizzled-1	β_2-AR	+	+	+			+		Li et al. (2004)
V	Frizzled-2	β_2-AR	+	+	+		+	+		Liu et al. (1999)
V	Frizzled-2	β_2-AR	+	+	+		+	+		Ahumada et al. (2002)
V	Frizzled-2	β_2-AR	+	+	+			+		Ishitani et al. (2003)
V	Frizzled-7	Frizzled-5	+	+	+	+		+		Swain et al. (2001)

CT, C terminus; NT, N terminus; mAChR muscarinic acetylcholine receptor; GnRH, gonadotropin-releasing hormone; TSH, thyroid-stimulating hormone; mGluR, metabotropic glutamate receptor.

domain of GPCRs is not uniform with respect to the role of the N terminus, because for many GPCRs (e.g., adrenergic and serotonergic receptors), the ligand binding occurs somewhere within in the lipid bilayer (Tota and Strader, 1990), although the N terminus and exofacial "loops" must contribute to the forces that organize the binding pocket for the ligand in these receptors. Other families of GPCR make use of large N-terminal domains and exofacial domains (e.g., gonadotropin receptors and Frizzleds) that are essential for signal propagation, whereas some seem to depend mostly on the N-terminal domain itself for receptor binding and activation by agonist (e.g., glutamate metabotropic receptors). The cytoplasmic domains of a GPCR include three or four (in the case of palmitoylated GPCRs such as the β_2-adrenergic receptor and rhodopsin) intracellular loops (iLoops 1–4) and a C-terminal sequence of variable length (70+ residues for β_2-adrenergic, 20+ residues in Frizzled-1, etc.). These cytoplasmic domains are sites of post-translational modifications (including protein phosphorylation, dephosphorylation, palmitoylation, and ubiquitination), interaction with their cognate heterotrimeric G-proteins, and association with scaffolds, cytoskeletal attachments, and other protein targets.

Probing Ligand Binding Domains of GPCRs with Chimeras

Since 1988, chimeric receptors have been employed to determine which regions of a GPCR are responsible for agonist binding and for activation of the receptor. The simplest demonstration of this principle was the creation of a chimeric receptor composed of the gonadotropic leuteinizing hormone (LH) receptor, which responds poorly to the gonadotropin follicle-stimulating hormone (FSH), and the large, N-terminal exofacial domain of the FSH receptor

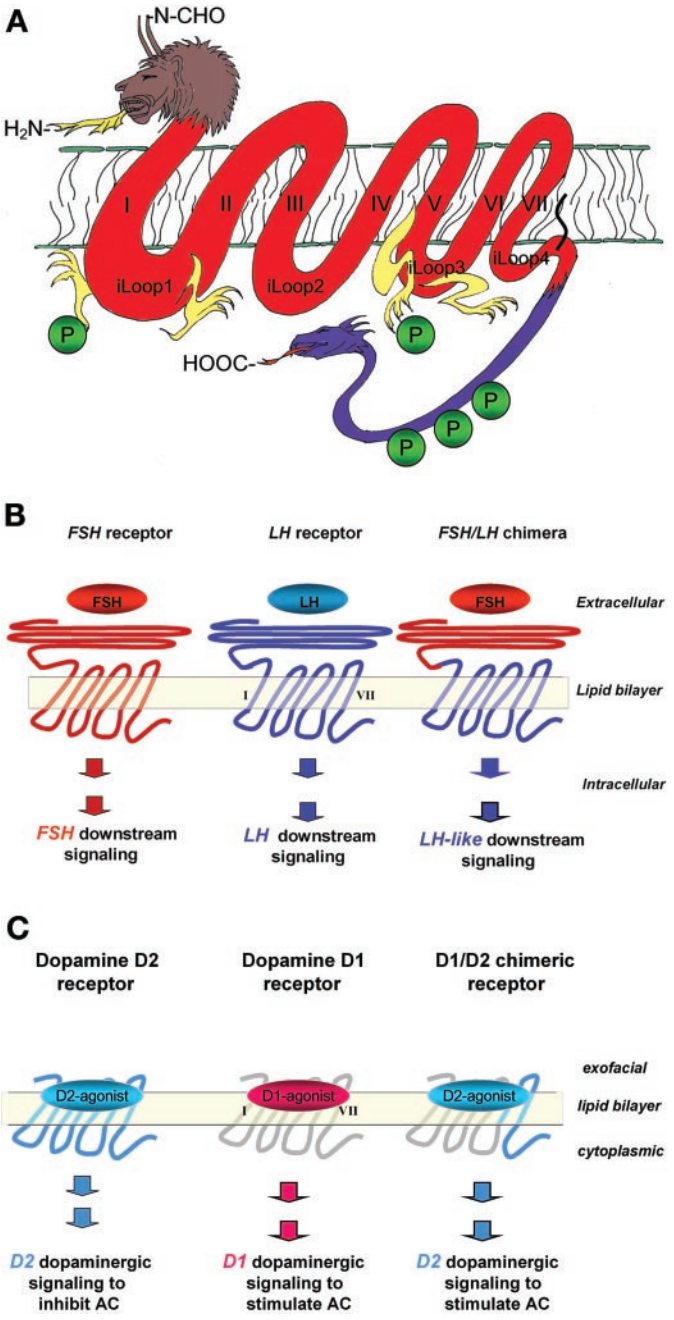

Fig. 1. GPCR chimera and analysis of ligand binding domain. The GPCR in the mythological rendition of a chimera (A). The three domains of a prototypic GPCR. The seven transmembrane segments (labeled in Roman numerals I–VII) of the GPCR constitute the lipid bilayer domain, displayed here as a lily pad. In the case of adrenergic receptors, this 7-TMS domain is the ligand-binding domain. The exofacial domain of GPCR includes the N-terminal region of the receptor (depicted as the lion head of the chimera) that is typically *N*-glycosylated with complex carbohydrate (CHO). For receptors that interact with peptide/protein ligands, the N-terminal exofacial region can be much larger and include motifs such as cysteine-rich domains (CRDs). The intracellular loops (labeled iLoop1, iLoop2, and iLoop3) and the C-terminal tail (-COOH) of a GPCR (depicted as the serpent head) constitute the cytoplasmic domains that are intimately involved in signal propagation to heterotrimeric G-proteins. In some GPCRs a cysteinyl residue in the C terminus beyond TMS VII is palmitoylated, creating a fourth loop (iLoop4). The intracellular loops are accessible to protein kinases, such as protein kinases A and C and G protein-coupled receptor kinase 2, and are subject to phosphorylation after activation by agonist. The phosphate groups are depicted as green spheres. Exofacial, transmembrane, and cytoplasmic domains of GPCR; swapping of exofacial domains dictates ligand binding/activation of LH/FSH receptors (B). This schematic demonstrates that for the GPCRs that interact with glycoprotein ligands, the exofacial domain is large, complex, and dictates the ligand binding character of the chimera. The cytoplasmic domain dictates the downstream read-outs. The 7TMS domain of many GPCRs constitutes the ligand-binding domain for small molecular weight agonists/antagonists (C). For many small ligands (catecholamines, dopamine, adenosine, etc.) that bind GPCR, the ligand binding determinants are largely transmembrane in nature and the functional nature is dictated by the cytoplasmic domains (iloops 1–3 and C-terminal tail). It has been shown that after the receptor has been expressed and incorporated into the cell membrane, exhaustive proteolytic digestion can remove the exofacial loops, whereas the 7TMS remain intact, as does the ligand binding capacity for small molecule ligands (Morris and Malbon, 1999).

(Fig. 1B). Substitution of the FSH receptor N terminus resulted in a FSH/LH chimera that bound FSH, underwent activation, and signaled similarly to the LH receptor in response to phospholipase C activation and accumulation of inositol 1,4,5-trisphosphate (Hirsch et al., 1996). Because the two gonadotropic hormone receptors do display some homology in the exofacial loops, the character of the binding of FSH and subsequent activation of the chimera cannot be ascribed solely to the presence of the N-terminal domain of the FSH receptor, but the basic observation is compelling.

For receptors with ligand binding domains embedded in the lipid bilayer, studies with chimeric GPCRs revealed new insights into what constitutes a binding site and its intrinsic ability to signal downstream. Detailed pharmacological analysis of β_1-/β_2-adrenergic receptor chimeras expressed in *Escherichia coli* revealed complex contributions of TMS and N termini to the binding character for both agonists and antagonist ligands alike (Marullo et al., 1990). Early work on chimeric α2- with β2-adrenergic receptor showed that TMS VI and especially VII were essential to agonist and antagonist binding specificity (Kobilka et al., 1988; Eason and Liggett, 1996). The dopamine D1 receptors characteristically activate adenylyl cyclase (AC), whereas the D2 receptors inhibit AC (Fig. 1C). Substitution of the region, including TMS VI, VII, and extending to the C-terminal tail of the D2 molecule into the D1 receptor, resulted in a chimera with enhanced binding of D2-agonist, diminished binding of D1 ligands, and a switch from a D1-like to a D2-like response (i.e., stimulation of AC) in response to a D2-agonist (MacKenzie et al., 1993). Many examples of making good use of TMS "swapping" to ascertain binding/activation properties of GPCRs can be found in the literature (Table 1). Most chimeras were constructed between members within a single family (family I), fewer were created from members of families II and III, and there are no reports of chimeras constructed from family IV (Wess, 1998).

Probing Downstream Signaling with Chimeric GPCRs

One of the central goals in cell signaling is to understand more completely the basis for receptor "activation" by agonist and how this signal is propagated to the downstream elements in the signaling pathways. In some cases, chimeras of GPCRs proved invaluable in sorting out the roles of specific receptor domains in receptor activation and downstream signaling. Analysis of α_1-/β_2-adrenergic chimeras demonstrated, for example, that swapping the third cyto-

plasmic loop of the α_1-receptor to the β_2-receptor resulted in a chimera with β_2-adrenergic binding character while coupling to phosphatidylinositol signaling as a α_1-adrenrgic receptor (Cotecchia et al., 1990). Most of the chimeras created were composed of domains taken from closely related members of family I of the V basic families of the GPCRs (Table 1). One very recent example, in sharp contrast, created a chimera from distant members of family I (e.g., β2-adrenergic receptor) and family V (e.g., Frizzleds) to eliminate a 20-year obstacle to the detailed analysis of the signaling of the Wnt protein, secreted glycoprotein ligands involved in many aspects of early development. Wnts bind to and activate Frizzleds, heptahelical membrane receptors found in developing embryos, and thereby regulate cell fate, proliferation, and patterning (Malbon et al., 2001). The overarching obstacle to study of the Wnt ligands and the larger Frizzled family for 20 years was the inability to purify biologically active Wnts (Cadigan, 2002). Only recently was it shown that Wnts are palmitoylated (Willert et al., 2003), which would explain the inability of standard biochemistry designed for purification of soluble proteins to succeed at isolating Wnts with full biological activity.

To obviate the need for active ligand, chimera were designed in which the exofacial and TMS of the well characterized β_2-adrenergic receptor could be used to drive the activation of the cytoplasmic domains of the Frizzleds, making use of the heptahelical nature and similarities of Frizzleds to GPCRs (Fig. 2A). The 7TMS that constitute the ligand binding domain and the exofacial N terminus and exofacial loops of the β_2-adrenergic receptor provided the core of this chimeric GPCR. The cytoplasmic domains,

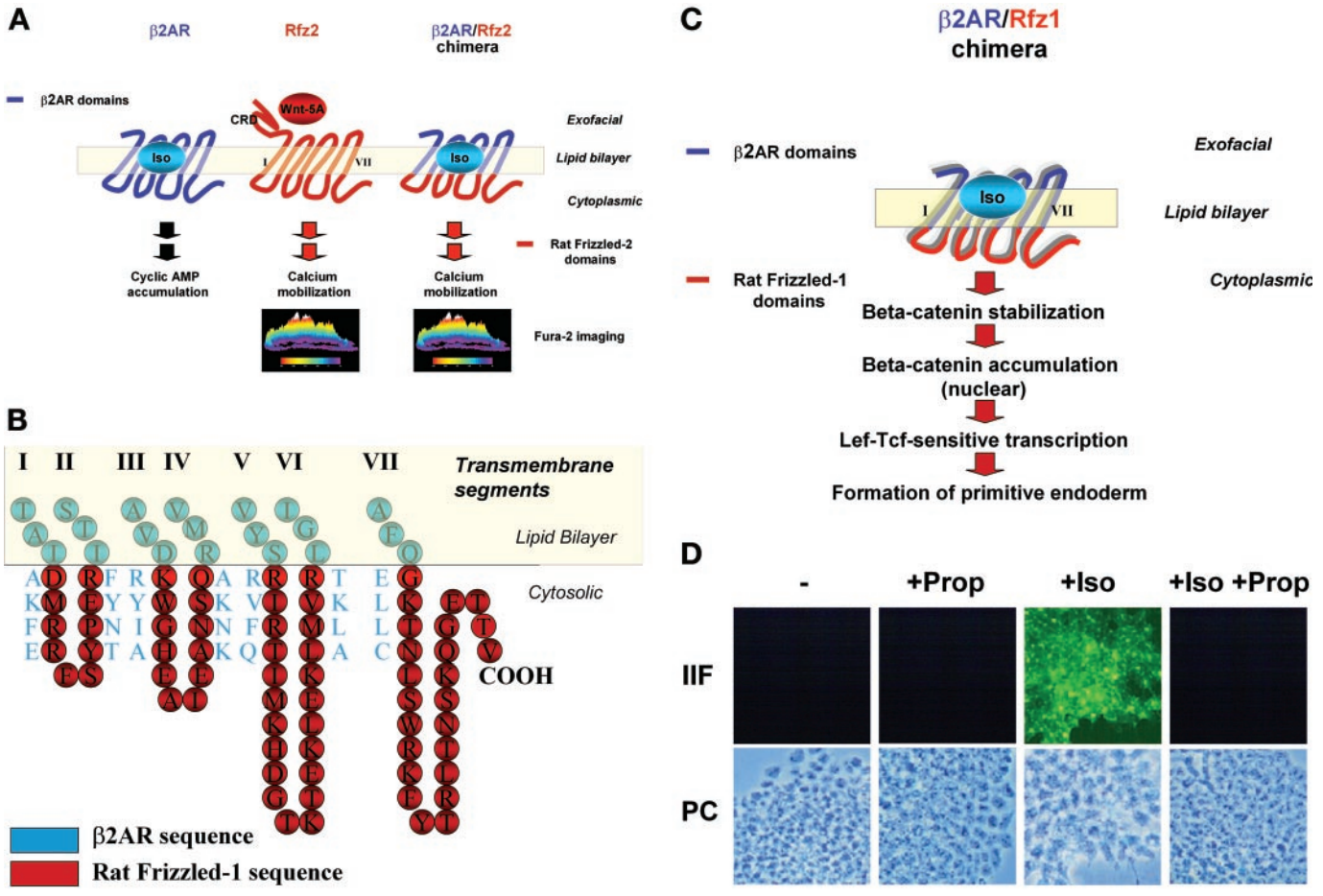

Fig. 2. Schematic representation of the β_2AR/Rfz chimeric receptor that binds β-agonist and signals via cytoplasmic, domains contributed by either Rfz2 (A) or Rfz1 (B, C). Expression of either Rfz2 or the β_2AR/Rfz2 chimera in zebrafish embryos results in appearance of agonist-stimulated calcium transients in embryos microinjected with either RNA encoding the Rfz2 and stimulated by Wnt-5A or RNA encoding the Rfz2 chimera and stimulated by β-adrenergic agoist (A; Malbon et al., 2001). Note that the wild-type Rfz2 displays a cysteine-rich domain) common to those GPCRs that bind to glycoprotein hormone ligands (e.g., FSH, LH), much like the Wnt glycoprotein ligand, which acts as an agonist for Frizzleds. The calcium imaging data are from Fura-2 fluorescence and 20-min compilation of images, then presented as pseudocolor ratio images: red denotes high values and violet denotes low values. The intracellular concentration of calcium is presented in micromolar (A). A sequence comparison is provided for the entire sequence of the intracellular loops of Rfz1 and the initial 4-amino acid regions of the intracellular loops of the β_2AR (B). Schematic of chimeric receptor composed of the exofacial and 7TMS of the β_2AR with the cytoplasmic domains of the Rfz1. The β_2AR/Rfz1 chimera, when stimulated with β-adrenergic agonist, leads to activation of the canonical Wnt-β-catenin pathway of stabilization and eventual nuclear accumulation of β-catenin, activation of Lef-Tcf-sensitive transcription, and ultimate formation of primitive endoderm (C). The β_2AR/Rfz1 chimera expressed in F9 totipotent, embryonal carcinoma cells activates primitive endoderm formation (D). F9 cells expressing the Rfz1 chimera were treated with/without the β-adrenergic agonist isoproterenol (10 μM Iso) in the presence or absence of the β-adrenergic antagonist propranolol (10 μM Pro). Phase contrast images (PC) and indirect immunofluorescence images (IIF) are displayed of the clones stained with the TROMA-1 antibody for expression of the primitive endoderm marker cytokeratin endoA. Note that Iso treatment stimulates the cells to form primitive endoderm and that this response can be blocked by treatment with the antagonist propranolol.

including the intracellular loops 1 to 3 and the cytoplasmic C terminus of the rat Frizzled-2 (Rfz2), were substituted for their corresponding sequences in the β_2-adrenergic receptor (β_2AR) (Liu et al., 1999b). Frizzled-2 was selected as the prototype for these experiments, because this receptor was shown to couple Wnt-5A signaling to calcium mobilization through a process that is pertussis toxin-sensitive in zebrafish (Slusarski et al., 1997) as well as mammalian embryonic stem cells in culture (Ahumada et al., 2002). The chimera was constructed and expressed in Chinese hamster ovary cells that lack β_2AR to ascertain whether the chimera retained the ability to bind agonist and antagonist ligands like the parent β_2AR. The affinities of the β_2AR/Rfz2 chimera for both β-adrenergic agonist and antagonist were nearly identical to those of the parent β_2AR, demonstrating that the essence of the ligand binding domain was not altered by the creation of the chimera (Ahumada et al., 2002). In addition, the β_2AR/Rfz2 chimera displayed the agonist-specific, GTP-dependent shift in receptor affinity, a hallmark for GPCRs; in this case, however, the G-proteins with which the Frizzled-2 cytoplasmic domains interact was not that of the parent β_2AR (i.e., Gs), but rather the heterotrimeric G-proteins Go and Gt2 (Wang and Malbon, 2003). Go and Gt2 were found to be essential to downstream signaling of Frizzled-2 to phosphatidylinositol signaling, Ca^{2+} mobilization, and cyclic GMP degradation (Wang and Malbon, 2003).

The success with the β_2AR/Rfz2 chimera provoked analysis of the canonical Frizzled-1 signaling that promotes activation of the phosphoprotein Disheveled, suppression of glycogen synthase kinase-3, stabilization of β-catenin, and activation of Lef/Tcf-sensitive transcription of key genes in early development (Cadigan and Nusse, 1997), a pathway for which no G-protein had been implicated. The chimera was constructed using the same successful strategy employed for the Frizzled-2 chimera (Fig. 2B). There is no homology between the cytoplasmic regions of the rat Frizzled-1 (Rfz1) and those of the parent β_2AR molecule. The β_2AR/Rfz1 chimera does not activate AC but was shown to stimulate β-catenin stabilization, accumulation, and activation of the Lef-Tcf–sensitive transcription (Fig. 2C). In mouse F9 totipotent teratocarcinoma cells, activation of the β_2AR/Rfz1 chimera leads to an activation of the Wnt-β-catenin pathway and ultimately to formation of primitive endoderm from these stem cells (Liu et al., 1999a, 2001), an early stage in vertebrate development (Fig. 2D). Isoproterenol treatment promotes formation of primitive endoderm as measured by expression of the TROMA-1 antigen, a hallmark of primitive endoderm. This activation through the β_2AR elements of the chimera can be blocked by propranolol and is insensitive to KT5720, a potent inhibitor of protein kinase A (Liu et al., 2001). In addition, the β_2AR/Rfz1 chimera displays the pharmacological properties of the β_2AR, an agonist-specific GTP-dependent shift in affinity dependent on the presence of Gq (the cognate G-protein of Rfz1) as well as sensitivity to blockade by pertussis toxin (DeCostanzo et al., 2002). These data demonstrate the ability of creating chimeric receptors between very distant members of the GPCR superfamily.

Probing Ligands and Downstream Signaling for Orphan GPCRs

The β_2-AR/Frizzled chimeras were an important advancement to a field that had labored to better define important signaling pathways in the absence of purified, active ligand. The chimeras were functional, demonstrated that Frizzleds are indeed members of the GPCR superfamily, and enabled new advances in gene profiling in which the outcome of the activation of a single, specific Frizzled could be characterized by downstream signaling and the genes that respond to Wnt activation (Li et al., 2004). A similar challenge has been stimulated by *in silico* analysis of genomic data searching for new gene products that detect a likely 7TMS motif, characteristic of a GPCR (Shaaban and Benton, 2001). Suspected GPCRs constitute a family of "orphan" receptors whose ligands and downstream signaling is not known. New 7TMS orphan receptors are identified routinely, and strategies are necessary to promote rapid identification of their ligand agonists (and antagonists) as well as the nature of the downstream signaling (Ahumada and Wang, 2002). One proposal is to make use of the chimeric GPCR in combination with the benefits of yeast genetics to screen orphan receptors for agonists (Fig. 3). Chimera can be created between the exofacial and TMSs of the orphan receptor with the cytoplasmic domains of the α-mating factor receptor (Ste2, α-MFR), a GPCR responsible for pheromone-induced mating and "schmoo" formation (Snyder, 1989). These orphan 7TMS/α-MFR chimera can be used in high-throughput screens of existing small molecule libraries (i.e., drug screens) and/or screens of combinatorial libraries of peptides. Schmoo formation can provide a facile read-out for positive clones, accelerating the efforts to "de-orphan" interesting 7TMS receptors.

Orphan receptors can be probed for downstream signaling pathways, in the absence of the knowledge of their ligands, much the way the β_2AR/Rfz chimeras overcame the absence of purified Wnt ligand to expand the knowledge of the downstream Wnt-Frizzled pathways. This approach is not as facile and requires that the investigator have available a full battery of potential read-outs, which will enhance but not guarantee the identification of the downstream signaling pathway of the orphan receptor. The downstream signaling properties of the vasopressin-related receptor 1 were elucidated recently through the application of a chimeric receptor approach (Gupte et al., 2004). Positive identification of a downstream signaling pathway for an orphan receptor can enhance ligand screens through use of sensitive downstream signaling assays as read-outs. At this point, the receptors may best be considered "homeless" rather than orphan receptors. Drawing upon information about the structure of the N terminus/exofacial and TMS sequences, the nature of the downstream signaling, and information on the expression of the receptor in vivo, it may be possible to narrow down the possibilities and to identify, with the benefit of pharmacology/physiology literature, some likely candidate ligands.

Conclusion

In mythological literature, we are shown that the chimera achieves its robustness through the incorporation of the most valued elements of other feared animals. Based upon an extensive literature compiled over more that a decade, the

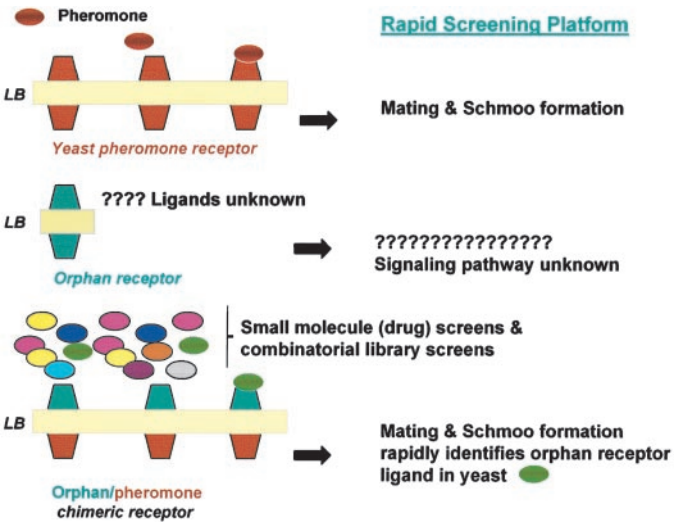

Fig. 3. Chimeric strategy to high throughput screening of active ligands for target receptors. The chimeric strategy can be used to identify ligands for orphan GPCRs or to optimize screening of new classes of compounds for existing, well known therapeutic targets. The strategy makes use of ability to rapidly identify yeast cells that have mated and formed schmoos. The 7TMS and exofacial domains of the orphan/target receptor provide the core receptor to which the cytoplasmic domains of a yeast pheromone receptor are substituted. The cytoplasmic domains of the yeast α-mating factor receptor (a GPCR) can confer the ability to signal to mating and schmoo formation. The read-out for the activation of the yeast pheromone pathway is amenable to high-throughput. It is possible, using combinatorial libraries to generate possible ligand agonists, to screen thousands of compounds per day. For optimization of existing compounds (either agonists or antagonists), the combinatorial library is designed specifically to address the target ligand structure. New, higher affinity or better efficacy compounds can be identified, taking advantage of the yeast cell read-outs and designer combinatorial libraries. LB, lipid bilayer.

GPCR chimera can be viewed in a similar manner. Chimeras have been created successfully from the three major domains borrowed from most of the classes that compose the GPCR superfamily. Each of these chimera provided important insight into the functional roles of specific domains in ligand recognition, receptor activation, downstream signaling, and receptor trafficking. Perhaps the most remarkable feature to emerge from all of these chimeras is that they are functional, highlighting some nearly universal properties about how GPCRs function. The exofacial and 7TMS core of the β_2AR seems to provide a "mechanosensing" device capable of converting the binding of agonist into some physical force on GPCR cytoplasmic domains or those borrowed from a distantly-related 7TMS receptor and to activate the downstream signaling pathway cognate to the donor of those cytoplasmic domains. What the nature of the physical force(s) exerted on the cytoplasmic domains of any GPCR is still largely a matter of speculation, with torsion, extension, retraction, or some combination of all three forces likely to play a role in propagating the signal to the cognate G-protein(s) involved.

One of the persistent, nagging questions about GPCRs is that of receptor retention and trafficking. The yeast αMFR provides one extreme for this discussion, a GPCR with little receptor retention or recycling after activation (Hicke et al., 1998). The αMFR undergoes activation and rapid ubiquitination /degradation, providing an example of a "one-pass" receptor. In contrast to the yeast αMFR, most mammalian GPCRs have evolved a complex biology that includes surface retention, sequestration from and re-cycling to the cell membrane, in addition to some ubiquitination and subsequent degradation by the proteosome (Shenoy et al., 2001). Some GPCRs, such as the β_2AR, are subject to internalization in response to agonist (i.e., agonist-induced sequestration) (Lefkowitz, 1998), as well as in response to growth factors such as insulin and insulin-like growth factor-I (i.e., counter-regulation) (Shumay et al., 2002). Our understanding of the receptor domains responsible for conferring spatial and regulated retention of GPCRs is fragmentary at best. Chimeric GPCRs making use of the αMFR as a target for the substitution/insertion of domains from mammalian receptors that are suspected to confer interesting retention properties may provide a powerful approach to this fundamental question. It may be profitable to explore many of the current compelling questions about GPCR retention/trafficking by making autofluorescent fusion proteins of some of the existing 100+ GPCR chimeras available that incorporate unique protein motifs and by using these tagged receptors for cellular imaging. A similar strategy may be profitable that makes use of the extensive list of GPCR chimeras to understand the receptor domains central to receptor oligomerization (Hebert and Bouvier, 1998).

The evidence is compelling that clever chimeric GPCRs can transform one type of binding domain into another (e.g., dopamine D2 to D1), from one ligand to another (e.g., FSH to LH), and from one pathway to another (e.g., AC activation to Lef-Tcf–sensitive transcription). These success stories provoke the possibility that it may be possible to create new chimeric receptors that can translate the presence of one ligand that might provoke a negative outcome (e.g., apoptosis) in its native state, to an alternative downstream pathway that might provoke a preferred outcome (e.g., cell proliferation or terminal differentiation).

Chimeric GPCRs can offer a great deal of insights on ligand binding determinants, G-protein, and effector coupling, and/or landmarks necessary for receptor internalization and trafficking. Since Kobilka et al. (1988) and Kubo et al. (1988) constructed the first chimeric GPCRs, more than several hundred studies have been published on or using chimeric GPCRs. In general, homologous domains are substituted in receptors within the same GPCR family, which probably minimizes disruption of basic receptor structure. Transmembrane spanning segments and intracellular loops are the domains most frequently exchanged, because these regions usually are responsible for determining critical aspects of ligand binding and G-protein coupling, respectively. Multiple chimeras are required to determine a minimal structural basis for ligand binding or effector coupling. With respect to G-protein selectivity and specificity, the third intracellular loop is most commonly exchanged between two GPCRs that have different effects on a downstream signaling (i.e., adenylyl cyclase versus phospholipase C activation) or couple different families of G-proteins (i.e., Gi versus Gs). It is remarkable that these exchanged domains examined so far retain most of their function with respect to ligand binding, G-protein coupling, and/or receptor internalization and trafficking. In contrast with standard mutagenesis approaches, in which the endpoint is the loss of function resulting from amino acid deletions or substitutions, the advantage of the chimeric approach is that conclusions can be drawn from qualitative changes in chimera receptor function, spanning

from the acquisition of new functions to the ability to respond to new agonists or to signal to new G-protein partners. There are limitations of the chimeric receptor approach, however; some chimeric receptors may not function because of problems in folding and/or targeting or to competing interference of endogenous receptors with which they must compete or oligomerize. Nevertheless, the chimeric approach provides an effective tool to elucidate new insights into structure/function relationships within the superfamily of GPCRs.

Targeted expression of such receptors in a failing heart or atrophic muscle may provide regulated, beneficial outcomes. Likewise, grafting onto a chimera a domain that drives the receptor to re-cycling rather than to ubiquitination/degradation may re-establish a regulatory pathway under assault by the overexpression of some growth factor or hormone that impacts negatively on a G-protein-mediated pathway. Perhaps it is time for us to think of a new generation of chimera, creating a molecular "toolbox" with a mix/match potential for GPCRs to address nagging issues in cell signaling as well as opportunities for new therapeutic strategies.

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References

Ahumada A, Slusarski DC, Liu X, Moon RT, Malbon CC, and Wang HY (2002) Signaling of rat frizzled-2 through phosphodiesterase and cyclic GMP. *Science (Wash DC)* **298:**2006–2010.

Ahumada A and Wang HY. Chimeric G-protein receptors: critical new tools in drug discovery. *Pharmaceutical News* **9:**337–345.

Akamizu T, Inoue D, Kosugi S, Ban T, Kohn LD, Imura H, and Mori T (1993) Chimeric studies of the extracellular domain of the rat thyrotropin (TSH) receptor: amino acids (268–304) in the TSH receptor are involved in ligand high affinity binding, but not in TSH receptor-specific signal transduction. *Endocr J* **40:**363–372.

Alberts GL, Chio CL, Im WB, and Slightom JL (2003) A unique phenotype of 5-HT2C, agonist-induced GTPgamma35S binding, transferable to 5-HT2A and 5-HT2B, upon swapping intracellular regions. *Br J Pharmacol* **138:**427–434.

Alberts GL, Pregenzer JF, and Im WB (1998) Identification of transmembrane regions critical for ligand binding to the human D3 dopamine receptor using various D3/D1 transmembrane chimeras. *Mol Pharmacol* **54:**379–388.

Anborgh PH, Seachrist JL, Dale LB, and Ferguson SS (2000) Receptor/beta-arrestin complex formation and the differential trafficking and resensitization of beta2-adrenergic and angiotensin II type 1A receptors. *Mol Endocrinol* **14:**2040–2053.

Bluml K, Mutschler E, and Wess J (1994) Functional role of a cytoplasmic aromatic amino acid in muscarinic receptor-mediated activation of phospholipase C. *J Biol Chem* **269:**11537–11541.

Boyd GW, Doward AI, Kirkness EF, Millar NS, and Connolly CN (2003) Cell surface expression of 5-hydroxytryptamine type 3 receptors is controlled by an endoplasmic reticulum retention signal. *J Biol Chem* **278:**27681–27687.

Braun T, Schofield PR, and Sprengel R (1991) Amino-terminal leucine-rich repeats in gonadotropin receptors determine hormone selectivity. *EMBO (Eur Mol Biol Organ) J* **10:**1885–1890.

Brauner-Osborne H, Jensen AA, Sheppard PO, Brodin B, Krogsgaard-Larsen P, and O'Hara P (2001) Cloning and characterization of a human orphan family C G-protein coupled receptor GPRC5D. *Biochim Biophys Acta* **1518:**237–248.

Cadigan KM (2002) Wnt signaling—20 years and counting. *Trends Genet* **18:**340–342.

Cadigan KM and Nusse R (1997) Wnt signaling: a common theme in animal development. *Genes Dev* **11:**3286–3305.

Calver AR, Robbins MJ, Cosio C, Rice SQ, Babbs AJ, Hirst WD, Boyfield I, Wood MD, Russell RB, Price GW, et al. (2001) The C-terminal domains of the GABA(b) receptor subunits mediate intracellular trafficking but are not required for receptor signaling. *J Neurosci* **21:**1203–1210.

Chen S, Lin F, Iismaa S, Lee KN, Birckbichler PJ, and Graham RM (1996) α1-adrenergic receptor signaling via Gh is subtype specific and independent of its transglutaminase activity. *J Biol Chem* **271:**32385–32391.

Choudhary MS, Craigo S, and Roth BL (1992) Identification of receptor domains that modify ligand binding to 5-hydroxytryptamine2 and 5-hydroxytryptamine1c serotonin receptors. *Mol Pharmacol* **42:**627–633.

Conchon S, Barrault MB, Miserey S, Corvol P, and Clauser E (1997) The C-terminal third intracellular loop of the rat AT1A angiotensin receptor plays a key role in G protein coupling specificity and transduction of the mitogenic signal. *J Biol Chem* **272:**25566–25572.

Cotecchia S, Exum S, Caron MG, and Lefkowitz RJ (1990) regions of the Alpha 1-adrenergic receptor involved in coupling to phosphatidylinositol hydrolysis and enhanced sensitivity of biological function. *Proc Natl Acad Sci USA* **87:**2896–2900.

Cotecchia S, Ostrowski J, Kjelsberg MA, Caron MG, and Lefkowitz RJ (1992) Discrete amino acid sequences of the α1-adrenergic receptor determine the selectivity of coupling to phosphatidylinositol hydrolysis. *J Biol Chem* **267:**1633–1639.

Cypess AM, Unson CG, Wu CR, and Sakmar TP (1999) Two cytoplasmic loops of the glucagon receptor are required to elevate cAMP or intracellular calcium. *J Biol Chem* **274:**19455–19464.

DeCostanzo AJ, Huang XP, Wang HY, and Malbon CC (2002) The frizzled-1/(beta$_2$)-adrenergic receptor chimera: pharmacological properties of a unique G protein-linked receptor. *Naunyn Schmiedeberg's Arch Pharmacol* **365:**341–348.

Dias JA, Zhang Y, and Liu X (1994) Receptor binding and functional properties of chimeric human follitropin prepared by an exchange between a small hydrophilic intercysteine loop of human follitropin and human lutropin. *J Biol Chem* **269:**25289–25294.

Dixon RA, Sigal IS, Rands E, Register RB, Candelore MR, Blake AD, Strader CD (1987) Ligand binding to the beta-adrenergic receptor involves its rhodopsin-like core. *Nature (Lond)* **326:**73–77.

Eason MG and Liggett SB (1995) Identification of a Gs coupling domain in the amino terminus of the third intracellular loop of the α2A-adrenergic receptor. Evidence for distinct structural determinants that confer Gs versus Gi coupling. *J Biol Chem* **270:**24753–24760.

Eason MG and Liggett SB (1996) Chimeric mutagenesis of putative G-protein coupling domains of the α2A-adrenergic receptor. Localization of two redundant and fully competent Gi coupling domains. *J Biol Chem* **271:**12826–12832.

England BP, Ackerman MS, and Barrett RW (1991) A chimeric D2 dopamine/M1 muscarinic receptor with D2 binding specificity mobilizes intracellular calcium in response to dopamine. *FEBS Lett* **279:**87–90.

Felder CC, Poulter MO, and Wess J (1992) Muscarinic receptor-operated Ca^{2+} influx in transfected fibroblast cells is independent of inositol phosphates and release of intracellular Ca^{2+}. *Proc Natl Acad Sci USA* **89:**509–513.

Feng YH and Karnik SS (1999) Role of transmembrane helix IV in G-protein specificity of the angiotensin II type 1 receptor. *J Biol Chem* **274:**35546–35552.

Flor PJ, Gomeza J, Tones MA, Kuhn R, Pin JP, and Knopfel T (1996) The C-terminal domain of the mGluR1 metabotropic glutamate receptor affects sensitivity to agonists. *J Neurochem* **67:**58–63.

Frielle T, Daniel KW, Caron MG, and Lefkowitz RJ (1988) Structural basis of beta-adrenergic receptor subtype specificity studied with chimeric beta 1/beta 2-adrenergic receptors. *Proc Natl Acad Sci USA* **85:**9494–9498

Galvez T, Duthey B, Kniazeff J, Blahos J, Rovelli G, Bettler B, Prezeau L, and Pin JP (2001) Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *EMBO (Eur Mol Biol Organ) J* **20:**2152–2159.

Gomeza J, Joly C, Kuhn R, Knopfel T, Bockaert J, and Pin JP (1996) The second intracellular loop of metabotropic glutamate receptor 1 cooperates with the other intracellular domains to control coupling to G-proteins. *J Biol Chem* **271:**2199–2205.

Green SA, Spasoff AP, Coleman RA, Johnson M, and Liggett SB (1996) Sustained activation of a G protein-coupled receptor via "anchored" agonist binding. Molecular localization of the salmeterol exosite within the 2-adrenergic receptor. *J Biol Chem* **271:**24029–24035.

Guan XM, Amend A, and Strader CD (1995) Determination of structural domains for G protein coupling and ligand binding in β 3-adrenergic receptor. *Mol Pharmacol* **48:**492–498.

Gupte J, Cutler G, Chen JL, and Tian H (2004) Elucidation of signaling properties of vasopressin receptor-related receptor 1 by using the chimeric receptor approach. *Proc Natl Acad Sci USA* **101:**1508–1513.

Hamaguchi N, True TA, Goetz AS, Stouffer MJ, Lybrand TP, and Jeffs PW (1998) Alpha 1-adrenergic receptor subtype determinants for 4-piperidyl oxazole antagonists. *Biochemistry* **37:**5730–5737.

Hamaguchi N, True TA, Saussy DL Jr, and Jeffs PW (1996) Phenylalanine in the second membrane-spanning domain of alpha 1a-adrenergic receptor determines subtype selectivity of dihydropyridine antagonists. *Biochemistry* **35:**14312–14317.

Hammerland LG, Krapcho KJ, Garrett JE, Alasti N, Hung BC, Simin RT, Levinthal C, Nemeth EF, and Fuller FH (1999) Domains determining ligand specificity for Ca^{2+} receptors. *Mol Pharmacol* **55:**642–648.

Hanyaloglu AC, Vrecl M, Kroeger KM, Miles LE, Qian H, Thomas WG, and Eidne KA (2001) Casein kinase II sites in the intracellular C-terminal domain of the thyrotropin-releasing hormone receptor and chimeric gonadotropin-releasing hormone receptors contribute to β-arrestin-dependent internalization. *J Biol Chem* **276:**18066–18074.

Hargrave PA and McDowell JH (1992) Rhodopsin and phototransduction: a model system for G protein-linked receptors. *FASEB J* **6:**2323–2331.

Havlickova M, Blahos J, Brabet I, Liu J, Hruskova B, Prezeau L, and Pin JP (2003) The second intracellular loop of metabotropic glutamate receptors recognizes C termini of G-protein α-subunits. *J Biol Chem* **278:**35063–35070.

Havlickova M, Prezeau L, Duthey B, Bettler B, Pin JP, and Blahos J (2002) The intracellular loops of the GB2 Subunit are crucial for G-protein coupling of the heteromeric γ-aminobutyrate b receptor. *Mol Pharmacol* **62:**343–350.

Hebert TE and Bouvier M (1998) Structural and functional aspects of G protein-coupled receptor oligomerization. *Biochem Cell Biol* **76:**1–11

Heding A, Vrecl M, Bogerd J, McGregor A, Sellar R, Taylor PL, and Eidne KA (1998) Gonadotropin-releasing hormone receptors with intracellular carboxyl-terminal tails undergo acute desensitization of total inositol phosphate production and exhibit accelerated internalization kinetics. *J Biol Chem* **273:**11472–11477.

Hicke L, Zanolari B, and Riezman H (1998) Cytoplasmic tail phosphorylation of the alpha-factor receptor is required for its ubiquitination and internalization. *J Cell Biol* **141:**349–358.

Hirsch B, Kudo M, Naro F, Conti M, and Hsueh AJ (1996) The C-terminal third of the human luteinizing hormone (LH) receptor is important for inositol phosphate release: analysis using chimeric human LH/follicle-stimulating hormone receptors. *Mol Endocrinol* **10:**1127–1137.

Ilani T, Fishburn CS, Levavi-Sivan B, Carmon S, Raveh L, and Fuchs S (2002)

Coupling of dopamine receptors to G Proteins: studies with chimeric D2/D3 dopamine receptors. *Cell Mol Neurobiol* **22:**47–56.
Ishitani T, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, Shibuya H, Moon RT, Ninomiya-Tsuji J, and Matsumoto K (2003) The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca^{2+} pathway to antagonize Wnt/beta-catenin signaling. *Mol Cell Biol* **23:**131–139.
Jewell-Motz EA, Donnelly ET, Eason MG, and Liggett SB (1997) Role of the amino terminus of the third intracellular loop in agonist-promoted downregulation of the alpha2a-adrenergic receptor. *Biochemistry* **36:**8858–8863.
Jewell-Motz EA, Donnelly ET, Eason MG, and Liggett SB (1998) Agonist-mediated downregulation of G alpha i via the alpha 2-adrenergic receptor is targeted by receptor-Gi interaction and is independent of receptor signaling and regulation. *Biochemistry* **37:**15720–15725.
Jewell-Motz EA, Small KM, Theiss CT, and Liggett SB (2000) α2A/α2C-adrenergic receptor third loop chimera show that agonist interaction with receptor subtype backbone establishes G protein-coupled receptor kinase phosphorylation. *J Biol Chem* **275:**28989–28993.
Jockers R, Da Silva A, Strosberg AD, Bouvier M, and Marullo S (1996) New Molecular and structural determinants involved in β2-adrenergic receptor desensitization and sequestration. Delineation using chimeric β3/β2-adrenergic receptors. *J Biol Chem* **271:**9355–9362.
Juarranz MG, Van Rampelbergh J, Gourlet P, De Neef P, Cnudde J, Robberecht P, and Waelbroeck M (1999) Different vasoactive intestinal polypeptide receptor domains are involved in the selective recognition of two $VPAC_2$-selective ligands. *Mol Pharmacol* **56:**1280–1287.
Kazmi MA, Snyder LA, Cypess AM, Graber SG, and Sakmar TP (2000) Selective reconstitution of human D4 dopamine receptor variants with Gi alpha subtypes. *Biochemistry* **39:**3734–3744.
Kelley SP, Dunlop JI, Kirkness EF, Lambert JJ, and Peters JA (2003) A cytoplasmic region determines single-channel conductance in 5-HT3 receptors. *Nature (Lond)* **424:**321–324.
Kikkawa H, Isogaya M, Nagao T, and Kurose H (1998) The role of the seventh transmembrane region in high affinity binding of a β2-Selective agonist TA-2005. *Mol Pharmacol* **53:**128–134.
Kobilka BK, Kobilka TS, Daniel K, Regan JW, Caron MG, and Lefkowitz RJ (1988) Chimeric alpha 2-,beta 2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science (Wash DC)* **240:**1310–1316.
Kozell LB, Machida CA, Neve RL, and Neve KA (1994) Chimeric D1/D2 dopamine receptors. Distinct determinants of selective efficacy, potency and signal transduction. *J Biol Chem* **269:**30299–30306.
Kozell LB and Neve KA (1997) Constitutive activity of a chimeric D2/D1 dopamine receptor. *Mol Pharmacol* **52:**1137–1149.
Kubo T, Bujo H, Akiba I, Nakai J, Mishina M, and Numa S (1988) Location of a region of the muscarinic acetylcholine receptor involved in selective effector coupling. *FEBS Lett* **241:**119–125.
Kudo M, Osuga Y, Kobilka BK, and Hsueh AJ (1996) Transmembrane regions V and VI of the human luteinizing hormone receptor are required for constitutive activation by a mutation in the third intracellular loop. *J Biol Chem* **271:**22470–22478.
Lachowicz JE and Sibley DR (1997) Chimeric D2/D3 dopamine receptor coupling to adenylyl cyclase. *Biochem Biophys Res Commun* **237:**394–399.
Lai J, Nunan L, Waite SL, Ma SW, Bloom JW, Roeske WR, and Yamamura HI (1992) Chimeric M1/M2 muscarinic receptors: correlation of ligand selectivity and functional coupling with structural modifications. *J Pharmacol Exp Ther* **262:**173–180.
Langer I, Vertongen P, Perret J, Waelbroeck M, and Robberecht P (2002) A small sequence in the third intracellular loop of the VPAC(1) receptor is responsible for its efficient coupling to the calcium effector. *Mol Endocrinol* **16:**1089–1096.
Lapinsh M, Prusis P, Gutcaits A, Lundstedt T, and Wikberg JE (2001) Development of proteo-chemometrics: a novel technology for the analysis of drug-receptor interactions. *Biochim Biophys Acta* **1525:**180–190.
Lefkowitz RJ (1998) G Protein-coupled receptors. III. New roles for receptor kinases and β-arrestins in receptor signaling and desensitization. *J Biol Chem* **273:**18677–18680.
Li H, Malbon CC, and Wang HY (2004) Gene profiling of Frizzled-1 and Frizzled-2 signaling: expression of G-protein-coupled receptor chimeras in mouse F9 teratocarcinoma embryonal cells. *Mol Pharmacol* **65:**45–55.
Liggett SB, Freedman NJ, Schwinn DA, and Lefkowitz RJ (1993) Structural basis for receptor subtype-specific regulation revealed by a chimeric beta 3/beta 2-adrenergic receptor. *Proc Natl Acad Sci USA* **90:**3665–3669.
Liu T, DeCostanzo AJ, Liu X, Wang H, Hallagan S, Moon RT, and Malbon CC (2001) G protein signaling from activated rat frizzled-1 to the beta-catenin-Lef-Tcf pathway. *Science (Wash DC)* **292:**1718–1722.
Liu T, Liu X, Wang H, Moon RT, and Malbon CC (1999a) Activation of rat frizzled-1 promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via pathways that require $G\alpha_q$ and $G\alpha_o$ function. *J Biol Chem* **274:**33539–33544.
Liu X, Liu T, Slusarski DC, Yang-Snyder J, Malbon CC, Moon RT, and Wang H (1999b) Activation of a frizzled-2/beta-adrenergic receptor chimera promotes wnt signaling and differentiation of mouse F9 teratocarcinoma cells via Galphao and Galphat. *Proc Natl Acad Sci USA* **96:**14383–14388.
MacKenzie RG, Steffey ME, Manelli AM, Pollock NJ, and Frail DE (1993) A D1/D2 chimeric dopamine receptor mediates a D1 response to a D2-selective agonist. *FEBS Lett* **323:**59–62.
Maggio R, Barbier P, Colelli A, Salvadori F, Demontis G, and Corsini GU (1999) G protein-linked receptors: pharmacological evidence for the formation of heterodimers. *J Pharmacol Exp Ther* **291:**251–257.
Maggio R, Vogel Z, and Wess J (1993) Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular "cross-talk" between G-protein-linked receptors. *Proc Natl Acad Sci USA* **90:**3103–3107.
Malbon CC, Wang H, and Moon RT (2001) Wnt signaling and heterotrimeric G-proteins: strange bedfellows or a classic romance? *Biochem Biophys Res Commun* **287:**589–593.
Malitschek B, Schweizer C, Keir M, Heid J, Froestl W, Mosbacher J, Kuhn R, Henley J, Joly C, Pin JP, et al. (1999) The N-terminal domain of γ-aminobutyric $acid_B$ receptors is sufficient to specify agonist and antagonist binding. *Mol Pharmacol* **56:**448–454.
Margeta-Mitrovic M, Jan YN, and Jan LY (2001a) Function of GB1 and GB2 subunits in G protein coupling of GABA(B) receptors. *Proc Natl Acad Sci USA* **98:**14649–14654.
Margeta-Mitrovic M, Jan YN, and Jan LY (2001b) Ligand-induced signal transduction within heterodimeric GABA(B) receptor. *Proc Natl Acad Sci USA* **98:**14643–14648.
Marin EP, Krishna AG, Zvyaga TA, Isele J, Siebert F, and Sakmar TP (2000) The amino terminus of the fourth cytoplasmic loop of rhodopsin modulates rhodopsin-transducin interaction. *J Biol Chem* **275:**1930–1936.
Marullo S, Emorine LJ, Strosberg AD, and Delavier-Klutchko C (1990) Selective binding of ligands to beta 1, beta 2 or chimeric beta 1/beta 2-adrenergic receptors involves multiple subsites. *EMBO (Eur Mol Biol Organ) J* **9:**1471–1476.
McAllister G, Knowles MR, Patel S, Marwood R, Emms F, Seabrook GR, Graziano M, Borkowski D, Hey PJ, and Freedman SB (1993) Characterisation of a chimeric HD3/D2 dopamine receptor expressed in CHO cells. *FEBS Lett* **324:**81–86.
Morris AJ and Malbon CC (1999) Physiological regulation of G protein-linked signaling. *Physiol Rev* **79:**1373–1430.
Mostafapour S, Kobilka BK, and von Zastrow M (1996) Pharmacological sequestration of a chimeric beta 3/beta 2 adrenergic receptor occurs without a corresponding amount of receptor internalization. *Recept Signal Transduct* **6:**151–163.
Nagayama Y, Chazenbalk GD, Takeshita A, Kimura H, Ashizawa K, Yokoyama N, Rapoport B, and Nagataki S (1994) Studies on homologous desensitization of the thyrotropin receptor in 293 human embryonal kidney cells. *Endocrinology* **135:**1060–1065.
Nagayama Y, Russo D, Chazenbalk GD, Wadsworth HL, and Rapoport B (1990) Extracellular domain chimeras of the TSH and LH/CG receptors reveal the mid-region (amino acids 171–260) to play a vital role in high affinity TSH binding. *Biochem Biophys Res Commun* **173:**1150–1156.
Nagayama Y, Wadsworth HL, Russo D, Chazenbalk GD, and Rapoport B (1991) Binding domains of stimulatory and inhibitory thyrotropin (TSH) receptor autoantibodies determined with chimeric TSH-lutropin/chorionic gonadotropin receptors. *J Clin Investig* **88:**336–340.
Nantel F, Bonin H, Emorine LJ, Zilberfarb V, Strosberg AD, Bouvier M, and Marullo S (1993) The human β3-adrenergic receptor is resistant to short term agonist-promoted desensitization. *Mol Pharmacol* **43:**548–555.
Oakley RH, Laporte SA, Holt JA, Barak LS, and Caron MG (1999) Association of β-arrestin with G protein-coupled receptors during clathrin-mediated endocytosis dictates the profile of receptor resensitization. *J Biol Chem* **274:**32248–32257.
Oksenberg D, Havlik S, Peroutka SJ, and Ashkenazi A (1995) The third intracellular loop of the 5-hydroxytryptamine2a receptor determines effector coupling specificity. *J Neurochem* **64:**1440–1447.
Osuga Y, Hayashi M, Kudo M, Conti M, Kobilka B, and Hsueh AJ (1997) Co-expression of defective luteinizing hormone receptor fragments partially reconstitutes ligand-induced signal generation. *J Biol Chem* **272:**25006–25012.
Parker EM, Izzarelli DG, Lewis-Higgins L, Palmer D, and Shapiro RA (1996) Two amino acid differences in the sixth transmembrane domain are partially responsible for the pharmacological differences between the 5-HT1D beta and 5-HT1E 5-hydroxytryptamine receptors. *J Neurochem* **67:**2096–2103.
Parker EM, Swigart P, Nunnally MH, Perkins JP, and Ross EM (1995) Carboxyl-terminal domains in the avian β1-adrenergic receptor that regulate agonist-promoted endocytosis. *J Biol Chem* **270:**6482–6487.
Pin JP, Joly C, Heinemann SF, and Bockaert J (1994) Domains involved in the specificity of G protein activation in phospholipase C-coupled metabotropic glutamate receptors. *EMBO (Eur Mol Biol Organ) J* **13:**342–348.
Pittel Z and Wess J (1994) Intramolecular interactions in muscarinic acetylcholine receptors studied with chimeric M2/M5 receptors. *Mol Pharmacol* **45:**61–64.
Robbins MJ, Calver AR, Filippov AK, Hirst WD, Russell RB, Wood MD, Nasir S, Couve A, Brown DA, Moss SJ, et al. (2001) GABA(B2) is essential for G-protein coupling of the GABA(B) receptor heterodimer. *J Neurosci* **21:**8043–8052.
Robinson SW and Caron MG (1996) Chimeric D2/D3 dopamine receptors efficiently inhibit adenylyl cyclase in HEK 293 cells. *J Neurochem* **67:**212–219.
Robinson SW, Jarvie KR, and Caron MG (1994) High affinity agonist binding to the dopamine D3 receptor: chimeric receptors delineate a role for intracellular domains. *Mol Pharmacol* **46:**352–356.
Rousseau G, Nantel F, and Bouvier M (1996) Distinct receptor domains determine subtype-specific coupling and desensitization phenotypes for human β1- and β2-adrenergic receptors. *Mol Pharmacol* **49:**752–760.
Sambrano GR and Coughlin SR (1999) The carboxyl tail of protease-activated receptor-1 is required for chemotaxis. Correlation of signal termination and directional migration. *J Biol Chem* **274:**20178–20184.
Saunders C, Keefer JR, Bonner CA, and Limbird LE (1998) Targeting of G protein-coupled receptors to the basolateral surface of polarized renal epithelial cells involves multiple, non-contiguous structural signals. *J Biol Chem* **273:**24196–24206.
Scarselli M, Novi F, Schallmach E, Lin R, Baragli A, Colzi A, Griffon N, Corsini GU, Sokoloff P, Levenson R, et al. (2001) D2/D3 dopamine receptor heterodimers exhibit unique functional properties. *J Biol Chem* **276:**30308–30314.
Shaaban S and Benton B (2001) Orphan G protein-coupled receptors: from DNA to drug targets. *Curr Opin Drug Discov Devel* **4:**535–547.
Shenoy SK, McDonald PH, Kohout TA, and Lefkowitz RJ (2001) Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. *Science (Wash DC)* **294:**1307–1313.
Shih YH, Chung FZ, and Pugsley TA (1997) Cloning, expression and characteriza-

tion of a human dopamine D4.2 receptor (CHO K1 Cells) and various D4.2/D2L chimeras (COS-7 cells). *Prog Neuropsychopharmacol Biol Psychiatry* **21:**153–167.
Shiina T, Kawasaki A, Nagao T, and Kurose H (2000) Interaction with β-arrestin determines the difference in internalization behavor between β1- and β2-adrenergic receptors. *J Biol Chem* **275:**29082–29090.
Shinoura H, Shibata K, Hirasawa A, Tanoue A, Hashimoto K, and Tsujimoto G (2002) Key amino acids for differential coupling of alpha1-adrenergic receptor subtypes to Gs. *Biochem Biophys Res Commun* **299:**142–147.
Shumay E, Song X, Wang HY, and Malbon CC (2002) Pp60Src mediates insulin-stimulated sequestration of the beta(2)-adrenergic receptor: insulin stimulates Pp60Src phosphorylation and activation. *Mol Biol Cell* **13:**3943–3954.
Slusarski DC, Yang-Snyder J, Busa WB, and Moon RT (1997) Modulation of embryonic intracellular Ca^{2+} signaling by Wnt-5A. *Dev Biol* **182:**114–120.
Snyder M (1989) The SPA2 protein of yeast localizes to sites of cell growth. *J Cell Biol* **108:**1419–1429.
Sugimoto Y, Fujisawa R, Tanimura R, Lattion AL, Cotecchia S, Tsujimoto G, Nagao T, and Kurose H (2002) β_1-selective agonist (−)-1-(3,4-dimethoxyphenetylamino)-3-(3,4-dihydroxy)-2-propanol [(−)-RO363] differentially interacts with key amino acids responsible for β_1-selective binding in resting and active states. *J Pharmacol Exp Ther* **301:**51–58.
Swain RK, Medina A, and Steinbeisser H (2001) Functional analysis of the *Xenopus* frizzled 7 protein domains using chimeric receptors. *Int J Dev Biol* **45:**259–264.
Tohgo A, Choy EW, Gesty-Palmer D, Pierce KL, Laporte S, Oakley RH, Caron MG, Lefkowitz RJ, and Luttrell LM (2003) The stability of the G protein-coupled receptor-β-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J Biol Chem* **278:**6258–6267.
Tota MR and Strader CD (1990) Characterization of the binding domain of the β-adrenergic receptor with the fluorescent antagonist carazolol. Evidence for a buried ligand binding site. *J Biol Chem* **265:**16891–16897.
Trejo J and Coughlin SR (1999) The cytoplasmic tails of protease-activated receptor-1 and substance P receptor specify sorting to lysosomes versus recycling. *J Biol Chem* **274:**2216–2224.
Trejo J, Hammes SR, and Coughlin SR (1998) Termination of signaling by protease-activated receptor-1 is linked to lysosomal sorting. *Proc Natl Acad Sci USA* **95:**13698–13702.
Van Leeuwen DH, Eisenstein J, O'Malley K, and MacKenzie RG (1995) Characterization of a chimeric human dopamine D3/D2 receptor functionally coupled to adenylyl cyclase in chinese hamster ovary cells. *Mol Pharmacol* **48:**344–351.
Vazquez-Prado J, Medina LC, Romero-Avila MT, Gonzalez-Espinosa C, and Garcia-Sainz JA (2000) Norepinephrine- and phorbol ester-induced phosphorylation of α_{1a}-adrenergic receptors. Functional aspects. *J Biol Chem* **275:**6553–6559.
Verrall S, Ishii M, Chen M, Wang L, Tram T, and Coughlin SR (1997) 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* **272:**6898–6902.
Vischer HF, Granneman JC, Noordam MJ, Mosselman S, and Bogerd J (2003) Ligand selectivity of gonadotropin receptors. Role of the β-strands of extracellular leucine-rich repeats 3 and 6 of the human luteinizing hormone receptor. *J Biol Chem* **278:**15505–15513.
Vouret-Craviari V, Auberger P, Pouyssegur J, and Obberghen-Schilling E (1995) Distinct mechanisms regulate 5-HT2 and thrombin receptor desensitization. *J Biol Chem* **270:**4813–4821.
Wang HY and Malbon CC (2003) Wnt signaling, Ca^{2+} and cyclic GMP: visualizing frizzled functions. *Science (Wash DC)* **300:**1529–1531.
Wang LD, Gantz I, Butler K, Hoeltzel M, and Del Valle J (2000) Histamine H2 receptor mediated dual signaling: mapping of structural requirements using beta2 adrenergic chimeric receptors. *Biochem Biophys Res Commun* **276:**539–545.
Wess J (1998) Molecular basis of receptor/G-protein-coupling selectivity. *Pharmacol Ther* **80:**231–264.
Wess J, Bonner TI, and Brann MR (1990a) Chimeric M2/M3 Muscarinic receptors: role of carboxyl terminal receptor domains in selectivity of ligand binding and coupling to phosphoinositide hydrolysis. *Mol Pharmacol* **38:**872–877.
Wess J, Bonner TI, Dorje F, and Brann MR (1990b) Delineation of muscarinic receptor domains conferring selectivity of coupling to guanine nucleotide-binding proteins and second messengers. *Mol Pharmacol* **38:**517–523.
Wess J, Brann MR, and Bonner TI (1989) Identification of a small intracellular region of the muscarinic M3 receptor as a determinant of selective coupling to PI turnover. *FEBS Lett* **258:**133–136.
Wess J, Gdula D, and Brann MR (1992) Structural basis of the subtype selectivity of muscarinic antagonists: a study with chimeric M2/M5 muscarinic receptors. *Mol Pharmacol* **41:**369–374.
Willert K, Brown JD, Danenberg E, Duncan AW, Weissman IL, Reya T, Yates JR, and Nusse R (2003) Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature (Lond)* **423:**448–452.
Wong SK, Parker EM, and Ross EM (1990) Chimeric muscarinic cholinergic: β-adrenergic receptors that activate gs in response to muscarinic agonists. *J Biol Chemi* **265:**6219–6224.
Wurch T, Colpaert FC, and Pauwels PJ (1998) Chimeric receptor analysis of the ketanserin binding site in the human 5-hydroxytryptamine1D receptor: importance of the second extracellular loop and fifth transmembrane domain in antagonist binding. *Mol Pharmacol* **54:**1088–1096.
Yamashita T, Terakita A, and Shichida Y (2001) The Second cytoplasmic loop of metabotropic glutamate receptor functions at the third loop position of rhodopsin. *J Biochem (Tokyo)* **130:**149–155.
Yarden Y, Rodriguez H, Wong SK, Brandt DR, May DC, Burnier J, Harkins RN, Chen EY, Ramachandran J, and Ullrich A (1986) The Avian beta-adrenergic receptor: primary structure and membrane topology. *Proc Natl Acad Sci USA* **83:**6795–6799.
Zhao MM, Gaivin RJ, and Perez DM (1998) The third extracellular loop of the β2-adrenergic receptor can modulate receptor/G protein affinity. *Mol Pharmacol* **53:**524–529.
Zhao MM, Hwa J, and Perez DM (1996) Identification of critical Extracellular loop residues involved in α1-adrenergic receptor subtype-selective antagonist binding. *Mol Pharmacol* **50:**1118–1126.

Address correspondence to: Craig Malbon, Department of Pharmacology, HSC, Stony Brook University, Stony Brook, NY 11794-8651. E-mail: craig@pharm.sunysb.edu

Correction to "Probing receptor structure/function with chimeric G-protein-coupled receptors"

In the above Minireview [Yin D, Gavi S, Wang H, and Malbon CC (2003) *Mol Pharmacol* **65:**1323–1332], the names of some GPCRs in Table 1 were incorrect. The correct names are Frizzled-1 through Frizzled-7. The online version has been corrected in departure from print.

We regret this error and apologize for any confusion or inconvenience it may have caused.