Annu. Rev. Neurosci. 1997. 20:399–427
Copyright © 1997 by Annual Reviews Inc. All rights reserved

FUNCTIONAL AND STRUCTURAL COMPLEXITY OF SIGNAL TRANSDUCTION VIA G-PROTEIN-COUPLED RECEPTORS

Thomas Gudermann, Torsten Schöneberg, and Günter Schultz
Institut für Pharmakologie, Freie Universität Berlin, D-14195 Berlin, Germany

KEY WORDS: heptahelical receptors, receptor conformation, receptor folding and assembly, receptor–G protein interaction

ABSTRACT

A prerequisite for the maintenance of homeostasis in a living organism is fine-tuned communication between different cells. The majority of extracellular signaling molecules, such as hormones and neurotransmitters, interact with a three-protein transmembrane signaling system consisting of a receptor, a G protein, and an effector. These single components interact sequentially and reversibly. Considering that hundreds of G-protein-coupled receptors interact with a limited repertoire of G proteins, the question of coupling specificity is worth considering. G-protein-mediated signal transduction is a complex signaling network with diverging and converging transduction steps at each coupling interface. The recent realization that classical signaling pathways are intimately intertwined with growth-factor–signaling cascades adds another level of complexity. Elaborate studies have significantly enhanced our knowledge of the functional anatomy of G-protein-coupled receptors, and the concept has emerged that receptor function can be modulated with high specificity by coexpressed receptor fragments. These results may have significant clinical impact in the future.

RECEPTORS AND G PROTEINS AS INTEGRAL COMPONENTS OF TRANSMEMBRANE SIGNALING PROCESSES

Fine-tuned communication between individual cells is a prerequisite for maintaining homeostasis within a living organism. Cells have the ability to process

0147-006X/97/0301-0399$08.00

vast amounts of information conveyed to them by extracellular signals (such as hormones, neurotransmitters, autacoids, growth factors, and odorants) and by physical signals (such as light). Most of these signals do not enter the cell but affect receptors at the cell surface. In principle, transmembrane signaling processes are governed by two fundamentally different and seemingly unrelated mechanisms. Some membrane receptors (e.g. ligand-gated ion channels, protein tyrosine and serine/threonine kinase receptors, phosphoprotein phosphatases or guanylyl cyclase receptors) intramolecularly combine an extracellular ligand-binding domain and an intracellular (in the case of enzymes) or transmembrane effector domain (a pore in the case of ion channels) (Fülle et al 1995, Iismaa et al 1995, Yang et al 1995). This first transduction principle is thus characterized by a rigidly coupled receptor-effector system. Most intercellular signaling molecules, however, bind to membranous receptors that represent one element of a three-component transmembrane signaling system whose individual elements interact sequentially and reversibly. Agonist binding to a specific receptor results in activation of heterotrimeric guanine nucleotide–binding proteins (G proteins) acting as transducers and signal amplifiers. G proteins subsequently modulate the activity of effectors, such as enzymes, ion channels, and transporters, resulting in rapid alterations of second messenger (e.g. cAMP, inositol phosphates, diacylglycerol, arachidonic acid, and cytosolic ion) concentrations (Birnbaumer et al 1990, Hepler & Gilman 1992, Birnbaumer & Birnbaumer 1995, Neer 1995).

Basic Architecture of G-Protein-Coupled Receptors

G-protein-coupled receptors constitute a large and diverse superfamily (Baldwin 1994, Strader et al 1994, Gudermann et al 1995). Family members have been identified in organisms as evolutionarily distant as yeast and man. To date, several hundred G-protein-coupled receptors have been cloned. The total number, including an ever growing subgroup of odorant receptors, is assumed to exceed 1000. A comparison of the primary structures of G-protein-coupled receptors indicates that these proteins are patterned according to a common structural principle. They belong to a superfamily of integral membrane proteins characterized by seven hydrophobic stretches of amino acids that are predicted to form transmembrane α helices, connected by alternating extracellular and intracellular loops (Dohlman et al 1991, Baldwin 1994). The N terminus of these heptahelical receptors is located extracellularly; the C terminus extends into the cytoplasm.

Amino acid alignments of cloned receptors allow the construction of dendrograms that reflect the evolutionary history of receptors (Donnelly et al 1994, Vernier et al 1995), thus helping researchers decipher the puzzling molecular and pharmacological diversity of these receptors. In general, amino acid

identities are highest in the predicted transmembrane spanning regions (TM) and fall off dramatically in the N and C termini and in the extra- and intracellular loops. This pattern may suggest a molecular basis for ligand and G-protein selectivity. The construction and functional expression of receptor chimeras and of receptors with single amino acid exchanges led to the identification of specific residues important for ligand binding (Dohlman et al 1991, Strader et al 1994, Gudermann et al 1995, Wess 1995). The most crucial contact points between catecholamines and the binding pocket of adrenoceptors are thought to be an asparagine in TM 3, two serine residues in TM 5, and a phenylalanine in TM 6, all located deep in the lipid bilayer (Strader et al 1994). As potential contact points between other monoamine ligands and their respective receptors were indentified in corresponding positions, it was proposed that a binding pocket located deep in the level of the lipid bilayer represents a universal interaction and receptor activation site for all members of the seven transmembrane receptor family (Trumpp-Kallmeyer et al 1992). This model of agonist binding and receptor activation has also been applied to peptide hormone receptors. Directed mutagenesis experiments and molecular modeling of G-protein-coupled receptors indicated that the binding site for the neuropeptide vasopressin and for the tripeptide thyrotropin-releasing hormone (TRH) is situated in a deep cleft between the transmembrane helices of the respective receptors (Mouillac et al 1995, Perlman et al 1996). In particular, in the case of the TRH receptor, this view is not undisputed (Han & Tashjian 1995a,b), and a large body of experimental evidence stresses the importance of the receptor's N terminus and extracellular connecting loops for the binding of peptide ligands (Strader et al 1994, Gudermann et al 1995, Schwartz & Rosenkilde 1996). Receptor–G protein contact sites are located on cytoplasmic parts of the receptor (see below).

Molecular Diversity and Structure of G Proteins

G proteins are heterotrimers composed of α, β, and γ subunits; they are classified based upon amino acid sequence similarity of their α subunits. To date, 23 distinct α subunits, including several splice variants of G_s, G_i, and G_o, are known. These subunits are encoded by 17 different genes (Simon et al 1991, Kehlenbach et al 1995, Nürnberg et al 1995) and are subdivided into 4 families: $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$ (Simon et al 1991). Concentrations of $G_{i/o}$ proteins in the cell considerably exceed those of other families, and in brain G_o may amount to 1–2% of membrane protein (Hepler & Gilman 1992). Five β and eleven γ subunits have been described (Watson et al 1994, Morishita et al 1995, Ray et al 1995). β and γ subunits are tightly associated and can be regarded as one functional unit. The five known mammalian β subunits are characterized by a high degree of amino acid sequence identity, whereas γ subunits are considerably more diverse (Simon et al 1991, Watson et al 1994, Morishita et al

1995, Neer 1995, Ray et al 1995). Activated receptors catalyze the exchange of GDP for GTP at the α subunit's high-affinity binding site for guanine nucleotides, thus setting in motion a well-controlled dissociation-reassociation cycle of the α subunit and $\beta\gamma$ dimer (Birnbaumer & Birnbaumer 1995). Both GTP-bound α subunits and $\beta\gamma$ dimers are signaling molecules in their own rights and modulate the activity of specific effector systems (Birnbaumer 1992, Clapham & Neer 1993, Neer 1995).

Access to a new dimension of understanding G-protein structure and function has been made possible by solving crystal structures of the G-protein α subunits of transducin (Noel et al 1993, Lambright et al 1994, Sondek et al 1994) and of G_{i1} (Coleman et al 1994), of $G_t\beta\gamma$ (Sondek et al 1996), and recently of two $G\alpha\beta\gamma$ heterotrimers (Wall et al 1995, Lambright et al 1996). $G\alpha$ proteins are composed of two principal domains, a Ras-like GTPase domain consisting of a six-stranded β sheet ($\beta1$–$\beta6$) surrounded by six helices ($\alpha1$–$\alpha5$ and αG) and an α helical domain consisting of one long central helix (αA) and five shorter helices (αB–αF). The guanine nucleotide binds tightly in a deep cleft between the GTPase and α helical domains. Although current dogma states that GDP-bound α subunits associate with $\beta\gamma$ dimers to form monomeric $\alpha\beta\gamma$ heterotrimers, recent crystallographic analyses of GDP-bound $G\alpha_{i1}$ indicate the formation of $G\alpha$ multimeric complexes (Mixon et al 1995) brought about by head-to-tail contacts between N- and C-terminal microdomains. Earlier findings of large polydisperse arrays of G_i, G_o, and G_s in detergent extracts of plasma membranes lend further credence to the crystallographic results indicated above (Jahangeer & Rodbell 1993). Purification of $G\alpha_{12}$ from rat brain yielded additional evidence for the existence of multimeric $G\alpha$ complexes (Harhammer et al 1996), the biological relevance of which remains elusive.

$G\beta$ subunits fold into a highly symmetrical β propeller (Wall et al 1995, Sondek et al 1996). The characteristic amino acid sequence motif of seven repetitive WD units (Neer et al 1994, Neer 1995, Neer & Smith 1996) is reflected in the approximate sevenfold symmetry of the propeller. $G\gamma$ binds to $G\beta$ in an extended conformation devoid of intrachain tertiary interactions (Sondek et al 1996). Most noticeably, significant structural changes were not observed in $G_t\beta\gamma$ when structures of $\beta\gamma$ subunits alone or complexed with $G_t\alpha$ were determined (Lambright et al 1996, Sondek et al 1996). Thus, $\beta\gamma$ subunits may function as a rigid scaffold with pre-positioned critical residues that mediate its interaction with GDP-bound $G\alpha$ and other signaling components. Activation of $\beta\gamma$ subunits occurs as a consequence of their release from $G\alpha$, and α subunits function as negative regulators of $\beta\gamma$ dimers. There is biochemical evidence, however, that receptor–G protein interaction elicits a conformational change in the C terminus of the γ subunit to establish a high-affinity contact with the receptor (Kisselev et al 1995). Resolution of this issue awaits accomplishment

of the ultimate goal of co-crystalizing activated heptahelical receptors and G-protein heterotrimers.

Several segments in the Gα molecule undergo structural changes upon GTP hydrolysis and are designated switch regions (Lambright et al 1994, Mixon et al 1995). The most dramatic changes occur in the switch II region. Interaction between α and $\beta\gamma$ subunits takes place at two distinct interfaces. The most extensive contact area comprises or is close to the switch II region of Gα (Mixon et al 1995, Lambright et al 1996), thus providing a mechanistic explanation for subunit dissociation and reassociation depending on the guanine nucleotide bound to the α subunit. Binding of $\beta\gamma$ subunits stabilizes the flexible switch regions in the inactive complex (Mixon et al 1995, Lambright et al 1996). A variety of biochemical and mutational studies have underscored the essential role of the α subunit's N terminus for the interaction with $\beta\gamma$ (Conklin & Bourne 1993). In the heterotrimer the Gα N terminus forms a helical segment that is stabilized by specific interactions with the β propeller of the β subunit, representing a second, independent interface between α and $\beta\gamma$ subunits (Mixon et al 1995, Lambright et al 1996). The structure does not provide an obvious clue as to the specificity in the pairing of particular α and $\beta\gamma$ subunits (Lambright et al 1996). Thus, the extent to which specificity is determined at the level of Gα-G$\beta\gamma$ interaction or at the level of the interaction of the receptor with a specific heterotrimer remains unknown. Effector contact sites of Gα have been mapped to areas encompassing and surrounding the switch II region (Berlot & Bourne 1992). Therefore, G$\beta\gamma$- and effector-interacting surfaces of Gα may overlap significantly. As a consequence, Gα cannot interact with an effector unless it dissociates from $\beta\gamma$ subunits. Activation of G$\beta\gamma$ occurs as a consequence of its release from Gα. Therefore, the Gα subunit functions as a negative regulator of G$\beta\gamma$ by limiting its degree of freedom within the cell and/or by covering those surface areas of G$\beta\gamma$ that interact with downstream effectors. The regions of yeast G$\beta\gamma$ that interact with downstream signaling components have been identified genetically by dominant negative mutants and map to the N-terminal coiled coil of G$\beta\gamma$ (Leberer et al 1992). None of the residues on yeast G$\beta\gamma$ that are assumed to form part of the effector site lie within a Gα/G$\beta\gamma$ interface, so it is an unresolved issue as to how Gα would block interaction with an effector that binds there. An interaction domain of the β_1 subunit with two effectors, adenylyl cyclase 2 and the muscarinic atrial potassium channel, was allocated to an N-terminal fragment of 100 residues of Gβ_1 (Yan & Gautam 1996). As deduced from crystallographic data, the C-terminal part of this fragment is assumed to be masked by the α subunit in the G$\alpha\beta\gamma$ heterotrimer.

As yet, it is not understood how an activated receptor catalyzes dissociation of GDP from a G-protein heterotrimer. Several regions in a G-protein heterotrimer are likely to be involved in this process. The most clearly defined Gα contact

sites with receptors comprise the C-terminal region of α subunits (summarized in Conklin & Bourne 1993, Gudermann et al 1996). In the structure of the heterotrimer, the C terminus of $G\alpha_{i1}$ lying in proximity to the N terminus is released from the compact microdomain observed in free GDP-liganded $G\alpha_{i1}$ (Mixon et al 1995, Lambright et al 1996) and is accessible for potential interactions with cytoplasmic receptor domains.

Evidence also exists for participation of the $G\alpha$ N terminus in receptor interaction (Hamm et al 1988, Higashijima & Ross 1991, Taylor et al 1994). In addition, Neubig and colleagues (Taylor et al 1994) demonstrated that a peptide derived from a cytoplasmic portion of the α_2 adrenergic receptor binds to a defined site of the $G\beta$ subunit, and the identity of the γ subunit in a G-protein heterotrimer has also been invoked in specifying receptor–G protein interaction (Kleuss et al 1993). Considering the observed proximity of the $G\alpha$ N terminus and $G\gamma$ C terminus on a common face of the heterotrimer (Lambright et al 1996) inserted into the plasma membrane via lipid modifications (Nürnberg et al 1995), the heterotrimer can be positioned relative to the plasma membrane to provide access of cytoplasmic receptor domains to large surface areas of α, β, and γ subunits (Wall et al 1995). A recently developed model for the receptor-$G\alpha\beta\gamma$ complex specifies interactions between the receptor's third cytoplasmic loop, the $G\alpha$ N and C termini, and the β subunit, in addition to a contact between the receptor's C-terminal tail and the $G\beta\gamma$ dimer (Lichtarge et al 1996). Because receptors cannot bind to regions of $G\beta$ now known to be covered through interactions with α and γ subunits, blades 6 and 7 of the β propeller are likely to be involved (Wall et al 1995).

SELECTIVITY OF RECEPTOR–G PROTEIN INTERACTION

Considering that hundreds of G-protein-coupled receptors transduce signals by using a fairly limited repertoire of G proteins, the issue of specificity governing the coupling of receptors to G proteins is worth considering. What are the molecular determinants funneling highly selective signals through the G-protein bottleneck? One mechanism to impart selectivity on transmembrane signaling processes is realized by compartmentalization of signal transduction components in highly specialized cells. There is evidence that certain subsets of receptors, G proteins, and effectors reside within different cellular compartments that may or may not have access to each other (Neubig 1994). In the rod photoreceptor cell, for example, all components needed for photon reception and signal transmission are contained in the outer segment; most proteins are associated with the disc membrane or with the plasma membrane (Hofmann & Heck 1996). In vitro rhodopsin was found to stimulate GTP hydrolysis by

transducin and also by structurally related G_i proteins (Cerione et al 1985). In contrast, rhodopsin in the retina interacts effectively only with transducin because of the spatial restrictions imposed on receptors and G proteins in the disc membrane. An asymmetrical distribution of some receptors and G proteins has been described in neuronal growth cones (Strittmatter et al 1990), rat Sertoli (Dym et al 1991), and madin-darby canine kidney cells (Keefer & Limbird 1993, Keefer et al 1994, Saunders et al 1996, Wozniak & Limbird 1996). Furthermore, association of G proteins with elements of the cytoskeleton (Popova et al 1994), with a certain type of coated vesicles (i.e. caveolae) (Sargiacomo et al 1993) and with secretory vesicles (Helms 1995, Nürnberg & Ahnert-Hilger 1996) may contribute to varying distribution patterns in different cells.

A given cell is endowed with a certain complement of receptors; effectors; and G-protein α, β, and γ subunits. Expression of a variety of G-protein subunits may result in a tremendous number of distinct $\alpha\beta\gamma$ heterotrimers if all subunits can associate randomly. There is evidence, however, that a degree of specificity governs $\beta\gamma$ dimer assembly and that not all possible combinations are formed (Pronin & Gautam 1992, Schmidt et al 1992, Garritsen & Simonds 1994, Katz & Simon 1995). In the yeast two-hybrid system it was shown that different β and γ subunits interact with each other with widely varying efficacies (Yan et al 1996). In conjunction with the expression level of a given subunit in a mammalian cell, this preferential $G\beta/G\gamma$ interaction may determine which $G\alpha\beta\gamma$ heterotrimers will be available and thus pave certain routes of signaling. In recent years the notion has been entertained that G-protein α subunits are not the sole determinant of selectivity in receptor–G protein interaction. The formation of interactive complexes between transducin $\beta\gamma$ and rhodopsin has been reported (Phillips & Cerione 1992), thereby confirming earlier reports of interactions between rhodopsin and $G_t\beta\gamma$ in the absence of α subunits (Kelleher & Johnson 1988, Fawzi et al 1991).

Both the isoprenoid moiety and the C-terminal primary amino acid sequence of γ subunits were highlighted as specific determinants of rhodopsin-transducin interaction (Kisselev & Gautam 1993, Kisselev et al 1994), and the prenyl group on the γ subunit appears to be an important determinant for the coupling of trimeric G proteins to the A_1 adenosine receptor (Yasuda et al 1996). Differences in the efficacy of distinct $\beta\gamma$ complexes to enhance β-adrenergic receptor kinase–mediated receptor phosphorylation were interpreted in terms of β subunits determining coupling to receptors and γ subunits specifying effector interaction (Müller et al 1993). This point of view is supported by recent findings (Taylor & Neubig 1994) that an activator peptide derived from the third cytoplasmic loop of the α_2-adrenergic receptor binds specifically to the N terminus in the G-protein α subunit and to a site in the β subunit corresponding to blade 6 or 7 of the β propeller (Wall et al 1995).

In studies on the hormonal inhibition of voltage-gated Ca^{2+} channels, nuclear injection of antisense oligonucleotides directed against mRNA sequences of G-protein α, β, and γ subunits provided compelling evidence that selectivity of receptor–G protein interaction is encoded by specific G-protein $\alpha\beta\gamma$ heterotrimers (Kleuss et al 1993, Gudermann et al 1996). The antisense approach was extended recently to studies on the regulation of another effector system, i.e. phospholipase C-β isoforms. In rat basophilic leukemia cells permanently expressing the human m1 muscarinic receptor, carbachol was shown to stimulate phospholipase C via a $\alpha_q/\alpha_{11}\beta_1/\beta_4\gamma_4$ complex (Dippel et al 1996). It is unknown whether α_q and α_{11} use common or distinct $\beta\gamma$ dimers to couple this receptor to phospholipase C-β. RBL cells endogenously express A_3 adenosine receptors, which stimulate phospholipase C-β isoforms via pertussis toxin–sensitive G proteins. Applying the antisense approach outlined above, the A_3 receptor was shown to be preferentially coupled to the effector enzyme via G_{i3} (E Dippel, F Kalkbrenner, B Wittig, G Schultz, manuscript in preparation). In the yeast two-hybrid system, different $\beta\gamma$ subunit types differ in their efficacy to interact with two downstream effectors, adenylyl cyclase 2 and the muscarinic atrial potassium channel GIRK1 (Yan & Gautam 1996). Because the antisense experiments listed above monitor a combined interaction at the receptor–G protein as well as at the G protein–effector interface, these data must not be interpreted as a mere reflection of specific interactions of a G-protein heterotrimer with a receptor.

DIVERSITY OF RECEPTOR–G PROTEIN COUPLING

Signal Processing via Receptor Subtypes and Splice Variants

The fact that interaction of a distinct G protein with a given receptor in a certain cell is governed by a high degree of selectivity imparted by specific heterotrimers contrasts with the observation that signaling molecules may give rise to a focused response in one cell while eliciting a plethora of effects in a different cell. The generation of several second messengers by one extracellular stimulus is often mediated by ligands acting at related receptor subtypes that are encoded by different genes and display distinct G-protein-coupling characteristics. A remarkable versatility of receptor subtypes prevails within the family of G-protein-coupled receptors; for example, 13 mammalian G-protein-coupled serotonin receptors have been identified by molecular cloning (Lucas & Hen 1995), and these receptors show marked differences in their expression pattern and signal transduction properties.

The first cloned genes for G-protein-coupled receptors lacked introns in their coding region. However, isoforms of various receptors were subsequently found to arise through alternative splicing of the primary transcript of a single-copy gene. Alternatively spliced receptor isoforms that differ in their seven

transmembrane domains or their intracellular C termini have been implicated in altered receptor function (Gudermann et al 1996). Insertions or deletions of short peptide sequences from cytosolic loops connecting the transmembrane domains may affect the glycosylation pattern and intracellular trafficking as well as the coupling preference of a receptor for different G proteins (e.g. D_2 dopamine receptor, Fishburn et al 1995; summarized in Gudermann et al 1996) or may allow for new coupling patterns or signaling efficacies that are specific for a certain splice variant [e.g. calcitonin and PACAP (pituitary adenylyl cyclase–activating polypeptide) type I receptors; summarized in Gudermann et al 1996, Pisegna & Wank 1996]. In some cases, altered receptor function elicited by C-terminal receptor splice variants can be related to changes in agonist-induced desensitization and down-regulation (e.g. turkey β_1-adrenoceptor, μ opioid and SSTR2 somatostatin receptors). In other instances, divergent C termini of receptors have been reported to profoundly affect ligand binding and G-protein-coupling characteristics (e.g. EP_3 prostaglandin receptor isoforms).

Two different cDNAs coding for 5-HT_4 serotonin receptors have been isolated that are the result of alternative splicing that generates a short and a long isoform diverging only in the cytoplasmic C terminus (Gerald et al 1995). Different sensitivity of these isoforms to GTPγS raises the possibility that each splice variant of the 5-HT_4 receptor displays differential G-protein coupling. A third, N-terminally truncated isoform of the human calcitonin receptor binds calcitonin with high affinity and responds to calcitonin stimulation with increases in cAMP formation but displays a nearly tenfold lower functional potency of amylin compared to the human calcitonin 1a receptor (Albrandt et al 1995).

Nakamura et al (1995) described C-terminally truncated isoforms of peptide hormone receptors that bind the respective ligand with high affinity but are incapable of signal transduction by themselves (e.g. neuropeptide Y-Y1 receptor). Truncated LH receptor isoforms lacking the transmembrane domain were reported to bind ^{125}I-hCG with high affinity and to enhance LH-stimulatable adenylyl cyclase activity when coexpressed with the full-length receptor (Vu Hai-Luu Thi et al 1992), indicating a physical interaction between the variant and wild-type receptor. In contrast, coexpression of a truncated isoform and the wild-type human receptor for gonadotropin-releasing hormone (GnRH) inhibits GnRH signaling via the wild-type receptor, probably because of specific interactions between the two receptor proteins resulting in impaired maturation of the wild-type receptor (R Grosse, T Schöneberg, G Schultz & T Gudermann, manuscript in preparation).

Coupling of Receptors to Multiple G Proteins

To channel incoming signals into different directions, cells use divergent coupling patterns at the receptor–G protein and G protein–effector interfaces.

Activation of one G protein may give rise to a bifurcating signal, since not only does the GTP-bound α subunit convey a signal to effector molecules but $\beta\gamma$ dimers can propagate part of the information (Clapham & Neer 1993). Coupling of a variety of receptors expressed primarily in neuronal and neuroendocrine cells (e.g. α_2-adrenergic, A_1 adenosine, M_2 muscarinic, opioid, and somatostatin receptors) to various G proteins belonging to one family (i.e. the $G_{i/o}$ family) has been demonstrated (summarized in Offermanns & Schultz 1994). In many cells studied, activation of this type of receptor elicits a functional triad consisting of inhibition of adenylyl cyclase (via $G\alpha_i$ and/or $\beta\gamma$), activation of K^+ currents (via $\beta\gamma$ dimers derived from $G_{i/o}$), and inhibition of voltage-gated Ca^{2+} channels (via G_o). The diversity of receptor subtypes and splice variants that might be expressed endogenously in a given cell complicates an unambiguous demonstration that agonists initiating multiple intracellular signals do so by interacting with a single receptor or by activating multiple receptor subtypes or splice variants that display a certain degree of signaling selectivity.

Several cloned, primarily $G_{i/o}$-coupled receptors have been shown to be capable of dual signaling, i.e. inhibition of adenylyl cyclase and stimulation of phospholipase C-β isoforms (see Gudermann et al 1996). Activated α subunits are thought to mediate inhibition of adenylyl cyclase, whereas $\beta\gamma$ subunit released from activated G_i affect phospholipase C activity. The biological relevance of such dual signaling is obscure because G_q-coupled receptor subtypes for many of these ligands (e.g. acetylcholine, serotonin, adrenaline/noradrenaline) stimulate phospholipase C more effectively than G_i-coupled receptors.

Another aspect of dual signaling is highlighted by primarily G_s-coupled receptors, some of which stimulate phospholipase C in addition to adenylyl cyclase (summarized in Gudermann et al 1996). In analogy to the situation with G_i-coupled receptors, phospholipase C activation by G_s-coupled receptors requires high receptor densities and high agonist concentrations when compared to stimulation of adenylyl cyclase by the same agonist, and the effect on phospholipase C is rather weak even under optimal conditions. Activation of adenylyl cyclase and phospholipase C by G_s-coupled receptors was interpreted either in terms of coupling to G proteins belonging to two different families (G_s and $G_{q/11}$) or in terms of generation of GTP-bound $G\alpha_s$ and $\beta\gamma$ subunits derived from G_s (Birnbaumer 1992).

A characteristic feature of phospholipase C stimulation via $\beta\gamma$ as opposed to α subunits is the observation that higher concentrations of $\beta\gamma$ subunits appear to be required (Camps et al 1992), thereby shifting the concentration-response curve toward higher agonist concentrations. However, the concentration of GTP-liganded α subunits required for half-maximal effector activation is a function of its rate constant for catalysis of GTP hydrolysis (k_{cat}) (Taussig et al 1993). A rise in k_{cat} resulting from the considerable activity of phospholipase

C-β1 as a GTPase-activating protein (GAP) (Berstein et al 1992) could necessitate higher concentrations of GTP-bound $\alpha_{q/11}$ as opposed to α_s for half-maximal effector activation. This mechanism provides an alternative explanation for the reduced potency of various agonists to stimulate phospholipase C via primarily G_s-coupled receptors.

Allgeier et al (1994) demonstrated in native human thyroid membranes that dual signaling through the human TSH receptor is mediated by coupling to G proteins of the G_s and G_q families. An identical coupling pattern underlying the dual signaling potential was worked out recently for the PTH/PTHrP (Offermanns et al 1996) and H_2 histamine receptors (Kühn et al 1996) (Figure 1).

As opposed to the coupling pattern introduced above, studies on the LH receptor revealed that G_s and G_i proteins are activated by the agonist-bound receptor to exert effects on adenylyl cyclase and phospholipase C (Herrlich et al 1996) (Figure 1). In consonance with the latter findings, functional coupling of the mammalian β_2-adrenoceptor to a pertussis toxin–sensitive G protein in cardiac myocytes (Xiao et al 1995) and to G_i proteins when expressed in baculovirus-infected Sf9 insect cells (B Kühn, T Wieland, KH Jakobs, T Gudermann & G Schultz, unpublished information) can be observed. The physiologic relevance of dual signaling by G_s-coupled receptors is unclear because receptor densities and hormone concentrations sufficiently high to elicit phospholipase C stimulation are rarely encountered in vivo and, in the case of the LH receptor, agonist-induced activation of G_i proteins was noted in MA-10 Leydig tumor cells in the absence of discernible phospholipase C activity (A Herrlich, G Schultz & T Gudermann, unpublished information). $\beta\gamma$ subunits released after agonist stimulation may serve additional roles such as recruiting β-adrenergic receptor kinases to the cell membrane (Lohse 1993) or mediating Ras-dependent activation of MAPKs (mitogen-activated protein kinase) belonging to the ERK (extracellular signal-regulated kinase) family (Inglese et al 1995).

Apart from G_s/G_q and G_s/G_i dual signaling, further coupling patterns have been described (Figure 1). Muscarinic (M_1 and M_3) (Offermanns et al 1994b) and A_3 adenosine receptors (Palmer et al 1995) have been shown to interact with G_q and G_i proteins, whereas the primarily G_q-coupled α_{1B}-adrenergic receptor (Horie et al 1995) also appears to activate G_s. Photolabeling of G proteins in platelet membranes in response to activation of the thromboxane A_2 receptor showed increased incorporation of [α-^{32}P]GTP azidoanilide into G_q and G_{12} proteins, whereas in the same membranes the activated thrombin receptor coupled to G proteins of three different families (i.e. G_q, G_i, and G_{12}) (Offermanns et al 1994a). When human thyroid membranes are stimulated with bovine TSH, the thyrotropin receptor is able to couple to at least ten different G proteins belonging to all four G-protein families (i.e. G_s, G_q, G_i, G_{12}) (Laugwitz

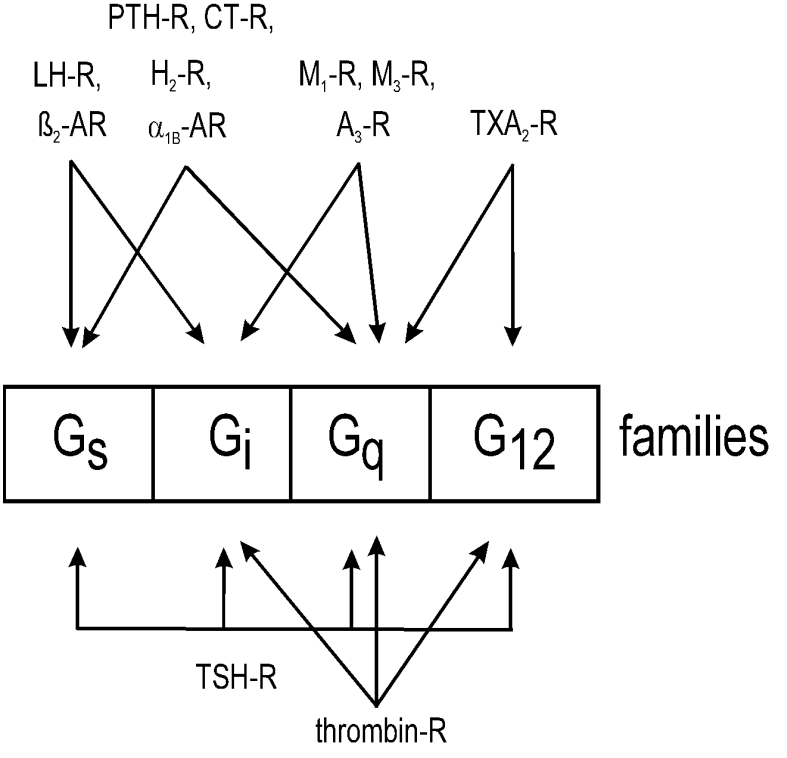

Figure 1 Examples of dual or multiple coupling of receptors to different G-protein families. α_{1B}-AR, α_{1B}-adrenergic receptor; A_3-R, A_3 adenosine receptor (Palmer et al 1995); β_2-AR, mammalian β_2-adrenergic receptor (Xiao et al 1995, B Kühn, T Wieland, KH Jakobs, T Gudermann & G Schultz, unpublished information); CT-R, calcitonin receptor (Offermanns et al 1996); H_2-R, histamine receptor (Kühn et al 1996); LH-R, lutropin/choriogonadotropin receptor (Herrlich et al 1996); M_1-R, M_1 muscarinic receptor (Offermanns et al 1994b); M_3-R, M_3 muscarinic receptor (Offermanns et al 1994b); PTH-R, parathyroid hormone receptor (Offermanns et al 1996); thrombin-R, thrombin receptor (Offermanns et al 1994b); TXA_2-R, thromboxane A_2 receptor (Offermanns et al 1994b); TSH-R, thyrotropin receptor (Laugwitz et al 1996).

et al 1996). Such a high level of diversity in receptor–G protein interaction is unprecedented and indicative of complex multifunctional signaling by the human TSH receptor.

A given receptor's signaling ability critically depends on the type of cell expressing the receptor. The G-protein α subunits $G\alpha_{15}$ and $G\alpha_{16}$, whose expression is restricted to a subset of hematopoietic cells, have been shown to functionally couple several primarily G_s-coupled receptors (e.g. β_2-adrenergic,

D_1 dopamine, V_2 vasopressin, A_{2A} adenosine receptors) and several G_i-coupled receptors (e.g. M_2 muscarinic, 5-HT_{1A} serotonin, μ opioid receptors) to β-isoforms of phospholipase C in a pertussis toxin–insensitive manner, notwithstanding these receptors' inability to interact with G proteins of the G_q family (Offermanns & Simon 1995, Wu et al 1995). However, the concept of $G\alpha_{15/16}$ as universal G-protein adaptors (Milligan et al 1996) does not appear to hold true, because the CRK-1 chemokinine receptor (Kuang et al 1996) and the human and mouse AT_2 angiotensin II receptors (T Schöneberg & T Gudermann, unpublished information) failed to couple to $G\alpha_{15/16}$ when receptors and G proteins were coexpressed in COS-7 cells. The potential for divergent cellular responses to a single agonist may depend on differential expression of signal transduction components (e.g. receptors, G proteins, effectors, and modulatory proteins such as receptor kinases) by different cells. Under ideal circumstances the expression and stoichiometry of signal transduction components should be characterized before attempting to interpret signaling data obtained from measurements of effector activity, an unsurmountable task in most instances. However, these considerations underscore the necessity to critically analyze whether responses noted in transfected cells provide useful information on the action of receptors in their native cellular background.

MOLECULAR ASPECTS OF SIGNAL PROCESSING BY HEPTAHELICAL RECEPTORS

Receptor–G Protein Contact Sites

Distinct structural receptor determinants involved in G-protein interaction have been identified by using chimeras of different G-protein-coupled receptors. Functional analysis of hybrid adrenergic receptors emphasized the importance of the third cytoplasmic loop and vicinal transmembrane portions for selective G-protein recognition (summarized in Dohlman et al 1991, Gudermann et al 1995). A recent experimental approach of expressing cytoplasmic domains together with full-length receptors in the same cell resulted in inhibition of receptor signaling, thus corroborating the coupling mechanism outlined above (Luttrell et al 1993, Hawes et al 1994).

A further refinement in the construction of chimeras and extensive mutagenesis experiments led to a closer assignment of receptor portions thought to comprise G-protein coupling sites. In the adrenergic receptor system, cytoplasmic domains in juxtaposition to the plasma membrane, in particular those close to transmembrane helices 5, 6, and 7, have been implicated in G-protein coupling (see Dohlman et al 1991, Guan et al 1995). In the design of chimeric receptors, a domain of one protein is exchanged with an analogous but preferably

functionally diverse domain of another receptor. The aim is to correlate replacement of a particular structural domain with acquisition or alteration of a particular function. Chimeras constructed from structurally closely related receptors have been most instructive. Substitution of cytoplasmic domains of the AT_1 angiotensin II receptor with analogous segments of the AT_2 angiotensin II receptor revealed that discrete amino acid residues located in the N- and C-terminal part of the third cytoplasmic loop are crucially important for receptor function (Wang et al 1995). M_3/M_2 muscarinic receptor chimeras allowed researchers to identify small stretches of amino acids in the second intracellular loop and in the N- and C-terminal portion of the third cytoplasmic loop, and these amino acids fully account for the G-protein coupling preference of the M_3 muscarinic receptor (Blin et al 1995).

Coexpression studies with hybrid M_2/M_3 muscarinic receptors and C-terminally modified G-protein α_q subunits (Conklin et al 1993) showed that a four–amino acid epitope (VITL) in the C-terminal portion of the third cytoplasmic loop specifically interacts with five C-terminal amino acids of an α_q/α_o chimeric G protein to couple the M_2 muscarinic receptor to phospholipase C (Liu et al 1995a). The insertion of alanine residues into the C-terminal portion of the third cytoplasmic loop, thus moving the critical VITL motif into the cytoplasm, led to constitutive activity of mutant M_2 muscarinic receptors (Liu et al 1996). This finding is consistent with a model in which agonist binding to the M_2 muscarinic receptor leads to conformational changes that enable critical amino acid residues in cytoplasmic receptor domains to productively interact with G proteins.

The use of chimeric V_{1A}/V_2 vasopressin receptors as research tools revealed that the second intracellular loop of the V_{1A} receptor is required for coupling to $G_{q/11}$, whereas the third intracellular loop of the V_2 receptor is responsible for coupling to G_s (Liu & Wess 1996). Thus, differential G-protein coupling profiles of individual members of a structurally closely related receptor subfamily may be mediated by different single cytoplasmic receptor domains. Systematic studies on muscarinic/β-adrenergic receptor chimeras (Wong et al 1990, Wong & Ross 1994) suggested that G-protein specificity of a given receptor may depend on the proper combination of multiple cytoplasmic receptor regions. As exemplified with rhodopsin (Ernst et al 1995) and metabotropic glutamate receptors (Gomeza et al 1996), the efficacy and selectivity of receptor–G protein interaction depends critically on multiple receptor regions being arranged to form a multiple-loop interactive conformation. However, the relative importance of a particular loop or segment must be assessed by experimental testing. There is no a priori certainty that related structural domains have similar functions in different receptors.

Several G-protein-coupled receptors have been shown to couple to more than one G protein (see above). It is unclear, however, whether contact sites

for different G proteins on cytoplasmic receptor parts can be differentiated or are identical. Mutation of a single alanine residue in the third cytoplasmic loop of the thyrotropin receptor resulted in a complete loss of coupling to phospholipase C, whereas TSH-induced cAMP formation remained unaltered (Kosugi et al 1992). Further studies revealed that the middle portion of the second cytoplasmic loop was of utmost importance for G_s-coupling. However, mutations in broadly distributed receptor portions affected TSH-stimulated inositol phosphate formation (summarized in Kosugi & Mori 1995).

Five isoforms of the PACAP type 1 receptor are generated by alternative splicing and are characterized by the absence or presence of one or two 84–base pair cassettes (hip- and hop-cassettes) inserted into the third cytoplasmic loop (Spengler et al 1993). Presence of a hip-cassette abolishes PACAP-induced phospholipase C stimulation but increases the half-maximal agonist concentration required for cAMP formation. The presence of a 16–amino acid insert in the first cytoplasmic loop of the human calcitonin receptor abolishes stimulation of phospholipase C while leaving cAMP formation unaltered (Nussenzveig et al 1994). The EC_{50} for the cAMP response, however, is 100-fold higher for the insert-positive compared with the insert-negative form (Moore et al 1995).

These examples do not necessarily indicate that structural receptor determinants governing the interaction with different G proteins were identified. A change in the conformation of cytoplasmic receptor domains may have impaired receptor–G protein coupling in general, most profoundly affecting G proteins that interact less efficiently with a certain receptor, resulting in complete abolition of only one signaling pathway. The construction and functional analysis of chimeric α_{2A}-adrenergic/5-HT_{1A} serotonin and α_{2A}-/β_2-adrenergic receptors provided the first conclusive evidence that distinct receptor contact sites can be identified for different G proteins. A discrete structural determinant for agonist-promoted α_{2A}-adrenoceptor coupling to G_s is located in the N-terminal portion of the third cytoplasmic loop. This determinant is distinct and separable from the structural requirements for α_{2A}-adrenoceptor-G_i coupling (Eason & Liggett 1995). Further detailed analysis showed that both N- and C-terminal portions of the third cytoplasmic loop are required to mediate coupling of the α_{2A}-adrenoceptor to G_s. However, each of these portions independently supports coupling to G_i (Eason & Liggett 1996). (For additional information on receptor–G protein interaction, see Strader et al 1994, Birnbaumer 1995, Gudermann et al 1995, Wess 1995.)

Folding and Assembly of G-Protein-Coupled Receptors

Transmembrane helices of G-protein-coupled receptors are thought to be arranged sequentially, in a counterclockwise fashion (as viewed from the extracellular membrane surface) to form a tightly packed ring-like structure (Baldwin 1993, 1994). Studies on the bacterial proton pump bacteriorhodopsin indicated

Table 1 Folding domains of heptahelical transmembrane proteins

Receptor	Fragments (numbers represent transmembrane regions)	Gained functions: binding (B); coupling (C)	References
Light sensitive			
Bacteriorhodopsin	1–2 + 3–7	B, C	Huang et al 1981 Liao et al 1983
	1–2 + 3–7	B, C	Popot et al 1987
	1 + 2 + 3–7	B, C	Kahn & Engelman 1992
Rhodopsin	1–3 + 4–7	B, C	Ridge et al 1995
	1–5 + 6–7	B	
	1–3 + 4–5 + 6–7	B	
Aminergic			
β_2-adrenergic	1–5 + 6–7	B, C	Kobilka et al 1988
M_2 muscarinic	1–5 + 6–7	B, C	Maggio et al 1993b
M_3 muscarinic	1–5 + 6–7	B, C	Maggio et al 1993b
	1 + 2–7	No B, no C	Schöneberg et al 1995
	1–2 + 3–7	No B, no C	
	1–3 + 4–7	B	
	1–4 + 5–7	B, C	
	1–5 + 6–7	B, C	
	1–6 + 7	No B, no C	
Peptidergic			
V_2 vasopressin	1–5 + 6–7	B, C	Schöneberg et al 1996
	1–6 + 7	No B, no C	
	1–7 (point mutation in 6) + 6–7	B, C	
GnRH	1–5 + 6–7	B, C	R Grosse, T Schöneberg, G Schultz & T Gudermann, manuscript in preparation

that the integrity of amino acid chains connecting the membranous helices does not appear to be an essential prerequisite for a functional protein (Popot & Engelman 1990). In vitro, bacteriorhodopsin could be functionally reconstituted from individual receptor fragments resulting from proteolytic cleavage of various connecting loops (Liao et al 1983, Popot et al 1987).

Evidence has accumulated from studies with "split" G-protein-coupled receptors, indicating that heptahelical receptors are composed of various independent folding units (Table 1). In particular, receptor fragments generated by cleavage within the third cytoplasmic loop are able to form functional proteins when individual fragments (TM 1–5 + TM 6–7) are coexpressed in the same cell. Functional complementation of coexpressed, mutated muscarinic and

adrenergic receptors highlighted the autonomy of individual folding domains in that the functional rescue of mutated receptor could only be explained by an intermolecular exchange of independent N- and C-terminal fragments between different receptors (Maggio et al 1993a). In the case of rhodopsin (Ridge et al 1995, 1996) and the M_3 muscarinic receptor (Schöneberg et al 1995), multiple autonomous folding domains were characterized: coexpressed polypeptide pairs TM 1–3 + TM 4–7 (rhodopsin), TM 1–4 + TM 5–7 (M_3 muscarinic receptor), and TM 1–5 + TM 6–7 were able to form a properly folded receptor capable of ligand binding and signal transduction. When expressed alone, none of the individual fragments represented a functional protein.

Using immunological techniques, researchers showed that proteins representing N- and C-terminally truncated G-protein-coupled receptors were synthesized (Pan et al 1994, Schöneberg et al 1995, Unson et al 1995) but frequently displayed the tendency to be trapped within the endoplasmic reticulum. However, truncated M_3 muscarinic (Schöneberg et al 1995) and V_2 vasopressin receptors (Pan et al 1994; D Wenkert, T Schöneberg, JJ Merendino, MSR Pena, R Vinitsky, P Goldsmith, J Wess & AM Spiegel, unpublished information) as well as truncated rhodopsin mutants (Sung et al 1993) were targeted to the plasma membrane—albeit with reduced efficacy—and inserted in proper orientation. Studies with naturally occurring mutant V_2 vasopressin receptors revealed that the exchange of a single amino acid can profoundly reduce cell surface expression of the receptor (Birnbaumer et al 1994; D Wenkert, T Schöneberg, JJ Merendino, MSR Pena, R Vinitsky, P Goldsmith, J Wess & AM Spiegel, unpublished information), thus indicating impaired trafficking and/or membrane insertion of these mutated receptors. It has been suggested that the majority of naturally occurring mutations in the rhodopsin gene give rise to misfolded proteins that appear to be less compact in their structures than the correctly folded counterparts (Liu et al 1996, Garriga et al 1996).

The so-called lipophobic effect drives transmembrane helices of G-protein-coupled receptors to be clustered in the plasma membrane (Jühnig 1983) in order to minimize the surface area of polar residues exposed to the hydrophobic environment of the lipid bilayer. Furthermore, the exact relative orientation of individual helices appears to be determined by specific intramolecular interactions. Whether the process of packing and folding of the archetypical G-protein-coupled receptor rhodopsin occurs spontaneously, as in bacteriorhodopsin, or requires the participation of various chaperones (Thomas et al 1995) involved in cellular protein folding and assembly is unclear at present. Mutational studies with aminergic (i.e. adrenergic) (Suryanarayana et al 1992), muscarinic (Liu et al 1995b), serotonin (Sealfon et al 1995), and peptide hormone receptors (GnRH receptor) (Zhou et al 1994, Arora et al 1996, Awara et al 1996, Davidson et al 1996) demonstrate a close spatial proximity of specific

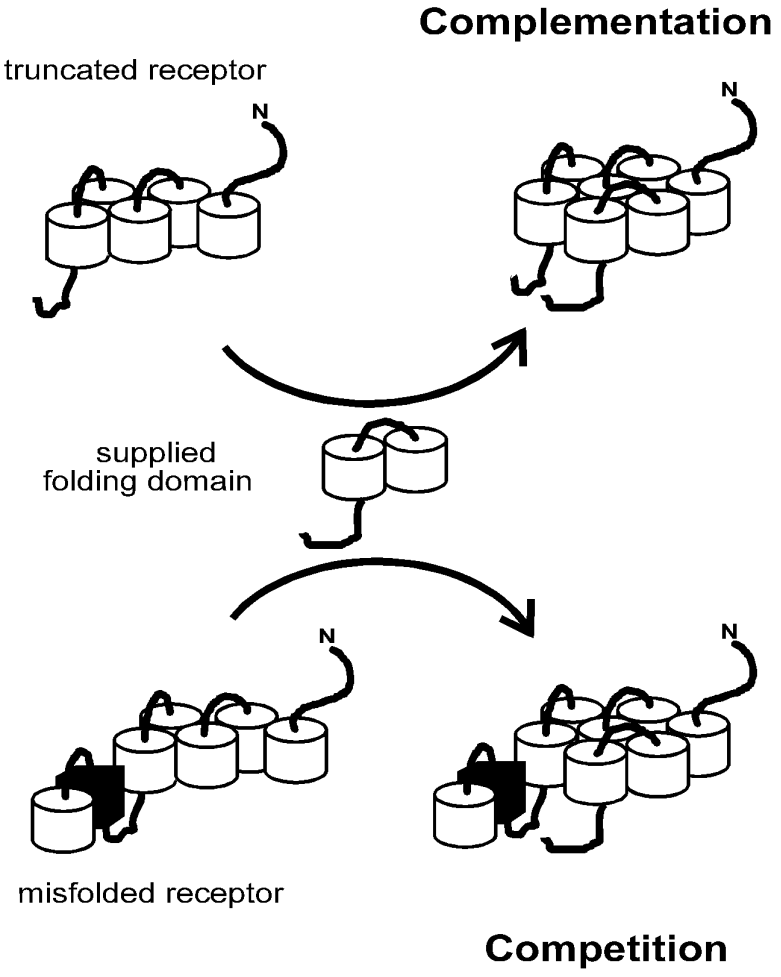

Figure 2 Hypothetical model of receptor fragment interaction and functional rescue. A truncated receptor (TM 1–5) can be functionally rescued by supplying the missing wild-type folding domain (TM 6–7) (complementation, *upper panel*). A misfolded receptor fragment resulting from a point mutation (*dark cube*) can be competed for by excess wild-type folding units, resulting in a functional receptor complex (competition, *lower panel*).

amino acid residues located within TM 1/2 and TM 7, which are remarkably distant in the primary protein stucture. The creation of artificial metal ion–binding sites in the tachykinin NK-1 receptor and the κ opioid receptor (Elling et al 1995, Thirstrup et al 1996) allowed identification of distinct amino acid residues in TM 5 and 6 that are involved in helix-helix interactions. Folding and assembly of integral membrane proteins such as G-protein-coupled receptors may be governed by a two-step process (Popot & Engelman 1990): In step 1, individual α helices are assumed to be inserted into the plasma membrane in proper orientation, and in step 2, they may specifically interact with each other to form a functional protein. An analogous multi-step mechanism of protein folding and packing has explicitly been postulated for one of the best-studied model receptors, rhodopsin (Khorana 1992).

Based on the finding that G-protein-coupled receptors are assembled from multiple independently stable folding units, Wess and colleagues (Schöneberg et al 1996) devised a coexpression strategy to functionally rescue inactive mutant V_2 vasopressin receptors harboring nonsense, frameshift, deletion, or missense mutations in the third cytoplasmic loop or the last two transmembrane helices. The mutant receptors not only displayed severely impared ligand-binding and signal transduction properties, but they were not trafficked properly to the cell surface. Several mutant receptors, however, regained functional activity and significant plasma membrane insertion upon coexpression with a C-terminal receptor peptide covering the amino acid sequence harboring the various mutations. Depending on the receptor defect, two general mechanisms of functional receptor rescue can be considered (Figure 2): (*a*) A prematurely terminated polypeptide chain caused by nonsense mutations or frameshifts can be functionally complemented by an analogous wild-type folding domain. (*b*) In the case of misfolded receptor proteins resulting from point mutations, the autonomous wild-type folding domain is thought to compete with the misfolded receptor segment for assembly into a tightly packed bundle of α helices. As expected, the latter mechanism is functionally less efficient than the former (Schöneberg et al 1996). These findings may allow the rational design of novel strategies aimed at the treatment of human disease caused by inactivating mutations of G-protein-coupled receptors (Birnbaumer 1995).

CONCLUSIONS AND PERSPECTIVES

Recent experimental results have modified profoundly our understanding of G-protein-mediated transmembrane signaling. The conceptualization of signal transduction pathways in a linear fashion, i.e. one receptor coupling to one G protein that activates one effector, is inadequate to account for recent findings. The majority of heptahelical receptors interact with diverse G proteins and elicit

multiple intracellular signals. Interaction of a single G protein with a given receptor in a certain cell, however, may be governed by a high degree of selectivity. G-protein-mediated signal transduction can be regarded as a complex, highly organized signaling network (Offermanns & Schultz 1994) with diverging and converging transduction steps at the ligand-receptor, receptor–G protein, and G protein–effector interfaces. Coincidence detection (Bourne & Nicoll 1993) and gating (Iyengar 1996) allow for some crosstalk without masking cell-specific dominant pathways guiding the main flow of information.

The recent realization that classical signaling pathways are tightly intertwined with growth-factor–signaling cascades has added yet another level of complexity to this field (see Inglese et al 1995, Gudermann et al 1996). The transduction of G-protein-mediated mitogenic signals involves the Ras/mitogen-activated protein kinase (ERK-MAPK) cascade common to both G-protein-coupled receptors and receptor tyrosine kinases (van Biesen et al 1995). There is evidence, however, that in certain cells these signaling cascades are not activated independently. Receptor tyrosine kinases may function as downstream mediators in G-protein-initiated mitogenic signaling via ligand-independent receptor tyrosine kinase activation through intracellular crosstalk (Linseman et al 1995, Rao et al 1995, Daub et al 1996). The involvement of the small GTP-binding proteins Rac and Cdc42 in the cytokine-mediated regulation of c-Jun N-terminal kinases (JNKs) has been demonstrated (Coso et al 1995, Minden et al 1995). Furthermore, human leukocyte p21-activated kinases (PAKs) can be regulated through G-protein-coupled receptors (Knaus et al 1995). Because PAKs are direct targets of Rac and Cdc42, these observations suggest a functional link between heterotrimeric G proteins and small GTPases belonging to the Rho subfamily.

Our knowledge about physiological roles of the pertussis toxin–insensitive G proteins of the G_{12} family is still vague. Those receptors shown to activate G_{12} and G_{13} (Offermanns et al 1994a, Laugwitz et al 1996) were characterized as coupling primarily to G_q (thromboxane A_2 and thrombin receptors) or to G_s (thyrotropin receptor). It may be argued, therefore, that the lack of known receptors coupling primarily to G_{12} proteins is one major reason for the limited insight we have into physiological processes involving G_{12} and G_{13}. In addition to differential regulation of a Na^+/H^+ exchanger (Dhanesekaran et al 1994), G_{12} class proteins stimulate Rho-dependent stress fiber formation and focal adhesion assembly (Buhl et al 1995). In NIH3T3 and HEK293 cells, an activated mutant of $G\alpha_{12}$ has been reported to stimulate c-Jun N-terminal kinase via the small G proteins Ras and Rac (Collins et al 1996), whereas $G\alpha_{13}$ failed to do so in transfected COS-7 cells (Coso et al 1996). The small GTPase Rho (Machesky & Hall 1996) has been identified as a regulator of TCF (ternary complex factor)-independent, SRF (serum response factor)-dependent signaling at the c-Fos

promoter (Hill et al 1995), and it is tempting to speculate that in analogy to Rac1 and Cdc42, RhoA will also couple to a MAPK cascade whose elements still need to be defined. The above-mentioned findings suggest that such a pathway would also be regulated through heterotrimeric G proteins. The functional interplay between heterotrimeric and low-molecular-weight GTPases thus emerges as an exciting field of future investigation.

Numerous elaborate studies have enhanced markedly our knowledge of the functional anatomy of G-protein-coupled receptors. Researchers speculated that the association of N- and C-terminal receptor domains may occur not only intra- but also intermolecularly, thus indicating a molecular basis for receptor dimerization. Thus, studies with cotransfected chimeric α_2-adrenergic and M_3 muscarinic receptors (Maggio et al 1993a) and with two binding-defective angiotensin II receptor mutants (Monnot et al 1996) demonstrated that intermolecular interactions can occur between heptahelical receptors. A dimerization domain for β_2-adrenergic receptors has been allocated to TM 6, and the functional importance of receptor dimerization was emphasized by the observation that peptide-mediated inhibition of dimerization also inhibited agonist-induced adenylyl cyclase activity (Hebert et al 1996). The interconversion of G-protein-coupled receptors between monomeric and dimeric states may therefore be important for biological activity. Functionally inactive receptor mutants can specifically be rescued by cotransfection of receptor fragments. In theory, constitutively active receptor could in turn be silenced by specific receptor fragments. Such a finding may lead to the development of novel therapeutic strategies for the treatment of human diseases caused by constitutively active receptors (Parma et al 1994) for which inverse agonists are not available.

The basic GTPase cycle is extrinsicly controlled by three regulatory proteins: Guanine nucleotide exchange factors (GEFs) accelerate the release of bound GDP, whereas guanine nucleotide dissociation inhibitors (GDIs) counteract this process. GTPase-activating proteins (GAPs) accelerate the rate of intrinsic GTPase reaction and shorten the life span of the protein in its active, GTP-bound conformation. In the case of heterotrimeric G proteins, the receptor fulfills the role of a GEF, and $\beta\gamma$ subunits functionally behave as GDIs. Two downstream effectors have been described as GAPs: phospholipase C-β and the γ subunit of a retinal cGMP-specific phosphodiesterase (summarized in Ross 1995). The GAP activity of a given effector may contribute to the specificity of signaling because a receptor has to be a highly effective exchange catalyst and have a high affinity for the G protein to remain stably bound in a proposed highly active receptor–G protein–effector complex (Biddlecome et al 1996). Yeast genetics has recently allowed identification a novel family of regulators of G-protein signaling (RGS) (Siderovski et al 1996). Three out of 15 isolated mammalian gene products (i.e. GAIP, RGS4, and RGS10) have been shown to

function as GAPs for the G_i subfamily (Berman et al 1996, Hunt et al 1996, Watson et al 1996). At present it is unknown whether GAPs also exist for G proteins belonging to the G_s and G_{12} subfamilies and whether RGS family members function as effectors or adaptors in addition to their role as GAPs. The molecular basis for the selective GAP activity toward G_i family members is also not understood. The controlled expression of RGS family members, however, represents a versatile tool for the biological regulation of G-protein signaling activity in the cell.

ACKNOWLEDGMENTS

The authors are indebted to KP Hofmann and W Rosenthal for critically reading the manuscript. We would like to thank many colleagues for helpful discussions and for making preprints available to us. The authors' own research reported herein was supported by the Deutsche Forschungsgemeinschaft (DFG) and Fonds der Chemischen Industrie.

Visit the *Annual Reviews home page* at http://www.annurev.org.

Literature Cited

Albrandt K, Brady EMG, Moore CX, Mull E, Sierzega ME, Beaumont K. 1995. Molecular cloning and functional expression of a third isoform of the human calcitonin receptor and partial characterization of the calcitonin receptor gene. *Endocrinology* 136:5377–84

Allgeier A, Offermanns S, Van Sande J, Spicher K, Schultz G, Dumont JE. 1994. The human thyrotropin receptor activates G-proteins G_s and $G_{q/11}$. *J. Biol. Chem.* 269:13733–35

Arora KK, Cheng Z, Catt KJ. 1996. Dependence of agonist activation on an aromatic moiety in the DPLIY motif of the gonadotropin-releasing hormone receptor. *Mol. Endocrinol.* 10:979–86

Awara WM, Guo C-H, Conn PM. 1996. Effects of Asn^{318} and Asp^{87} Asn^{318} mutations on signal transduction by the gonadotropin-releasing hormone receptor and receptor regulation. *Endocrinology* 137:655–62

Baldwin JM. 1993. The probable arrangement of the helices in G protein-coupled receptors. *EMBO J.* 12:1693–703

Baldwin JM. 1994. Structure and function of receptors coupled to G proteins. *Curr. Opin. Cell Biol.* 6:180–90

Berlot CH, Bourne HR. 1992. Identification of effector-activating residues of $G_{s\alpha}$. *Cell* 68:911–22

Berman DM, Wilkie TM, Gilman AG. 1996. GAIP and RGS4 are GTPase-activating proteins for the G_i subfamily of G protein α subunits. *Cell* 86:445–52

Berstein G, Blank JL, Jhon D-Y, Exton JH, Rhee SG, Ross EM. 1992. Phospholipase C-β1 is a GTPase-activating protein for $G_{q/11}$, its physiologic regulator. *Cell* 70:411–18

Biddlecome GH, Berstein G, Ross EM. 1996. Regulation of phospholipase C-β1 by G_q and m1 muscarinic receptor. Steady-state balance of receptor-mediated activation and GTPase-activating protein-promoted deactivation. *J. Biol. Chem.* 271:7999–8007

Birnbaumer L. 1992. Receptor-to-effector signaling through G proteins: roles for $\beta\gamma$ dimers as well as α subunits. *Cell* 71:1069–72

Birnbaumer L, Abramowitz J, Brown AM. 1990. Receptor-effector coupling by G proteins. *Biochim. Biophys. Acta* 1031:163–224

Birnbaumer L, Birnbaumer M. 1995. Signal transduction by G proteins: 1994 edition. *J. Rec. Signal Transduct. Res.* 15:213–52

Birnbaumer M. 1995. Mutations and diseases of G protein coupled receptors. *J. Rec. Signal Transduct. Res.* 151:131–60

Birnbaumer M, Gilbert S, Rosenthal W. 1994. An extracellular congenital nephrogenic diabetes insipidus mutation of the vasopressin receptor reduces cell surface expression, affinity for ligand, and coupling to

the G_s/adenylyl cyclase system. *Mol. Endocrinol.* 8:886–94
Blin N, Yun J, Wess J. 1995. Mapping of single amino acid residues required for selective activation of $G_{q/11}$ by the m3 muscarinic acetylcholine receptor. *J. Biol. Chem.* 270:17741–48
Bourne HR, Nicoll R. 1993. Molecular machines integrate coincident synaptic signals. *Cell* 72:65–75
Buhl AM, Johnson NL, Dhanasekaran N, Johnson GL. 1995. $G\alpha_{12}$ and $G\alpha_{13}$ stimulate Rho-dependent stress fiber formation and focal adhesion assembly. *J. Biol. Chem.* 270:24631–34
Camps M, Carozzi A, Schnabel P, Scheer A, Parker P, Gierschik P. 1992. Isozyme-selective stimulation of phospholipase C-β_2 by G protein $\beta\gamma$ subunits. *Nature* 60:684–86
Cerione RA, Staniszewski C, Benovic JL, Lefkowitz RJ, Caron M, et al. 1985. Specificity of the functional interactions of the β-adrenergic receptor and rhodopsin with guanine nucleotide regulatory proteins reconstituted in phospholipid vesicles. *J. Biol. Chem.* 260:1493–500
Clapham DE, Neer EJ. 1993. New roles for G protein $\beta\gamma$-dimers in transmembrane signalling. *Nature* 365:403–6
Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR. 1994. Structures of active conformations of $G_{i\alpha 1}$ and the mechanism of GTP hydrolysis. *Science* 265:1405–12
Collins LR, Minden A, Karin M, Heller Brown J. 1996. $G\alpha_{12}$ stimulates c-Jun NH_2- terminal kinase through the small G proteins Ras and Rac. *J. Biol. Chem.* 271:17349–53
Conklin B, Bourne HR. 1993. Structural elements of $G\alpha$ subunits that interact with $G\beta\gamma$, receptors, and effectors. *Cell* 73:631–41
Conklin BR, Farfel Z, Lustig KD, Julius D, Bourne HR. 1993. Substitution of three amino acids switches receptor specificity of $G_q\alpha$ to that of $G_i\alpha$. *Nature* 363:274–76
Coso OA, Chiariello M, Yu J-C, Teramoto H, Crespo P, et al. 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81:1137–46
Coso OA, Teramoto H, Simonds WF, Gutkind JS. 1996. Signaling from G protein-coupled receptors to c-Jun kinase involves $\beta\gamma$ subunits of heterotrimeric G proteins acting on a Ras and Rac1-dependent pathway. *J. Biol. Chem.* 271:3963–66
Daub H, Weiss FU, Wallasch C, Ullrich A. 1996. Role of transactivation of the EGF receptor in signaling by G-protein-coupled receptors. *Nature* 379:557–60
Davidson JS, McArdle CA, Davies P, Elario R, Flanagan CA, Millar RP. 1996. Asn^{102} of the gonadotropin-releasing hormone receptor is a critical determinant of potency for agonists containing C-terminal glycinamide. *J. Biol. Chem.* 271:15510–14
Dhanesekaran N, Vara Prasad MVVS, Wadsworth SJ, Dermott JM, van Rossum G. 1994. Protein kinase C-dependent and -independent activation of Na^+/H^+ exchanger by $G\alpha_{12}$ class of G proteins. *J. Biol. Chem.* 269:11802–6
Dippel E, Kalkbrenner F, Wittig B, Schultz G. 1996. A heterotrimeric G-protein complex couples the muscarinic m1 receptor to phospholipase C-β. *Proc. Natl. Acad. Sci. USA* 93:1391–96
Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ. 1991. Model systems for the study of seven-transmembrane-segment receptors. *Annu. Rev. Biochem.* 60:653–88
Donnelly D, Findlay JBC, Blundell TL. 1994. The evolution and structure of aminergic G protein-coupled receptors. *Recept. Channels* 2:61–78
Dym M, Lamsam-Casalotti S, Jia MC, Kleinman HK, Papadopoulos V. 1991. Basement membrane increases G-protein levels and follicle-stimulating hormone responsiveness of sertoli cell adenylyl cyclase activity. *Endocrinology* 128:1167–76
Eason MG, Liggett SB. 1995. Identification of a G_s coupling domain in the amino terminus of the third intracellular loop of the α_{2A}-adrenergic receptor. Evidence for distinct structural determinants that confer G_s versus G_i coupling. *J. Biol. Chem.* 270:24753–60
Eason MG, Liggett SB. 1996. Chimeric mutagenesis of putative G-protein coupling domain of the α_{2A}-adrenergic receptor. Localization of two redundant and fully competent G_i coupling domains. *J. Biol. Chem.* 271:12826–32
Elling CE, Nielsen SM, Schwartz TW. 1995. Conversion of antagonist-binding site to metal-ion site in the tachykinin NK-1 receptor. *Nature* 374:74–77
Ernst OP, Hofmann KP, Sakmar TP. 1995. Characterization of rhodopsin mutants that bind transducin but fail to induce GTP nucleotide uptake. Classification of mutant pigments by fluorescence, nucleotide release, and flash-induced light-scattering assays. *J. Biol. Chem.* 270:10580–86
Fawzi AB, Fay DS, Murphy EA, Tamir H, Erdos JJ, Northup JK. 1991. Rhodopsin and the retinal G-protein distinguish among G-protein $\beta\gamma$ subunit forms. *J. Biol. Chem.* 266:12194–200
Fishburn CS, Elazar Z, Fuchs S. 1995. Differential glycosylation and intracellular trafficking for the long and short isoforms of

the D_2 dopamine receptor. *J. Biol. Chem.* 270:29819–24

Fülle HJ, Vassar R, Foster DC, Yang RB, Axel R, Garbers DL. 1995. A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. *Proc. Natl. Acad. Sci. USA* 92:3571–75

Garriga P, Liu X, Khorana HG. 1996. Structure and function in rhodopsin: correct folding and misfolding in point mutants at and in proximity to the site of the retinitis pigmentosa mutation Leu-125 → Arg in the transmembrane helix C. *Proc. Natl. Acad. Sci. USA* 93:4560–64

Garritsen A, Simonds WF. 1994. Multiple domains of G protein β confer subunit specificity in $\beta\gamma$ interaction. *J. Biol. Chem.* 269: 24418–23

Gerald C, Adham N, Kao HT, Olsen MA, Laz TM, et al. 1995. The 5-HT_4 receptor: molecular cloning and pharmacological characterization of two splice variants. *EMBO J.* 14:2806–15

Gomeza J, Joly C, Kuhn R, Knöpfel T, Bockaert J, Pin J-P. 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.* 271:2199–205

Guan X-M, Amend A, Strader CD. 1995. Determination of structural domains for G protein coupling and ligand binding in β_3-adrenergic receptor. *Mol. Pharmacol.* 48:492–98

Gudermann T, Kalkbrenner F, Schultz G. 1996. Diversity and selectivity of receptor–G protein interaction. *Annu. Rev. Pharmacol. Toxicol.* 36:429–59

Gudermann T, Nürnberg B, Schultz G. 1995. Receptors and G proteins as primary components of transmembrane signal transduction. 1. G-protein-coupled receptors: structure and function. *J. Mol. Med.* 73:51–63

Hamm HE, Deretic D, Arendt A, Hargrave PA, Koenig B, Hofmann KP. 1988. Site of G protein binding to rhodopsin mapped with synthetic peptides from the α subunit. *Science* 241:832–35

Han B, Tashjian AH Jr. 1995a. Importance of extracellular domains for ligand binding in the thyrotropin-releasing hormone receptor. *Mol. Endocrinol.* 9:1708–19

Han B, Tashjian AH Jr. 1995b. Identification of Asn289 as a ligand binding site in the rat thyrotropin-releasing hormone (TRH) receptor as determined by complementary modifications in the ligand and receptor: A new model for TRH binding. *Biochemistry* 34:13412–22

Harhammer R, Nürnberg B, Harteneck C, Leopoldt D, Exner T, Schultz G. 1996. Distinct biochemical properties of the native members of the G_{12} G-protein subfamily. *Biochem. J.* In press

Hawes BE, Luttrell LM, Exum ST, Lefkowitz RJ. 1994. Inhibition of G protein-coupled receptor signaling by expression of cytoplasmic domains of the receptor. *J. Biol. Chem.* 269:15776–85

Hebert TE, Moffet S, Morello J-P, Loisel TP, Bichet DG, et al. 1996. A peptide derived from a β_2-adrenergic receptor transmembrane domain inhibits both receptor dimerization and activation. *J. Biol. Chem.* 271:16384–92

Helms JB. 1995. Role of heterotrimeric GTP binding proteins in vesicular protein transport: indications for both classical and alternative G protein cycles. *FEBS Lett.* 369:84–88

Hepler JR, Gilman AG. 1992. G proteins. *Trends Biochem. Sci.* 17:383–87

Herrlich A, Kühn B, Grosse R, Schmid A, Schultz G, Gudermann T. 1996. Involvement of G_s and G_i proteins in dual coupling of the luteinizing hormone receptor to adenylyl cyclase and phospholipase C. *J. Biol. Chem.* 271:16764–72

Higashijima T, Ross EM. 1991. Mapping of the mastoparan-binding site on G proteins. *J. Biol. Chem.* 266:12655–61

Hill CS, Wynne J, Treisman R. 1995. The Rho family GTPases RhoA, Rac1, and CDC42Hs regulate transcriptional activation by SRF. *Cell* 81:1159–70

Hofmann KP, Heck M. 1996. Light-induced protein-protein interactions on the rod photoreceptor disc membrane. In *Biomembranes II*, ed. AG Lee. Greenwich, CT: JAI. In press

Horie K, Itoh H, Tsujimoto G. 1995. Hamster α_{1B}-adrenergic receptor directly activates G_s in the transfected chinese hamster ovary cells. *Mol. Pharmacol.* 48:392–400

Huang KS, Baylay H, Liao MJ, London E, Khorana HG. 1981. Refolding of an integral membrane protein. *J. Biol. Chem.* 256:3802–9

Hunt TW, Fields TA, Casey PJ, Peralta EG. 1996. RGS10 is a selective activator of $G\alpha_i$ GTPase activity. *Nature* 383:175–77

Iismaa TP, Biden TJ, Shine J, eds. 1995. G *Protein-Coupled Receptors.* New York: Springer. 180 pp.

Inglese J, Koch WJ, Touhara K, Lefkowitz RJ. 1995. $G_{\beta\gamma}$ interactions with PH domains and Ras-MAPK signaling pathways. *Trends Biochem. Sci.* 20:151–56

Iyengar R. 1996. Gating by cyclic AMP: expanded role for an old signaling pathway. *Science* 271:461–63

Jahangeer S, Rodbell M. 1993. The disaggregation theory of signal transduction revisited: further evidence that G proteins are

multimeric and disaggregate to monomers when activated. *Proc. Natl. Acad. Sci. USA* 90:8782–86

Jähnig F. 1983. Thermodynamics and kinetics of protein incorporation into membranes. *Proc. Natl. Acad. Sci. USA* 80:3691–95

Kahn TW, Engelman DM. 1992. Bacteriorhodopsin can be refolded from two independently stable transmembrane helices and the complementary five-helix fragment. *Biochemistry* 31:6144–51

Katz A, Simon MI. 1995. A segment of the C-terminal half of the G-protein β_1 subunit specifies its interaction with the γ_1 subunit. *Proc. Natl. Acad. Sci. USA* 92:1998–2002

Keefer JR, Kennedy ME, Limbird LE. 1994. Unique structural features important for stabilization versus polarization of the α_{2A}-adrenergic receptor on the basolateral membrane of madin-darby canine kidney cells. *J. Biol. Chem.* 269:16425–33

Keefer JR, Limbird LE. 1993. The α_{2A}-adrenergic receptor is targeted directly to the basolateral membrane domain of madin-darby canine kidney cells independent of coupling to pertussis toxin-sensitive GTP-binding proteins. *J. Biol. Chem.* 268:11340–47

Kehlenbach RH, Matthey J, Huttner WB. 1995. Xlαs is a new type of G protein. *Nature* 372:804–9

Kelleher DJ, Johnson GL. 1988. Transducin inhibition of light-dependent rhodopsin phosphorylation: evidence for $\beta\gamma$ subunit interaction with rhodopsin. *Mol. Pharmacol.* 34:452–60

Khorana HG. 1992. Rhodopsin, photoreceptor of the rod cell. An emerging pattern for structure and function. *J. Biol. Chem.* 267:1–4

Kisselev O, Ermolaeva MV, Gautam N. 1994. A farnesylated domain in the G protein γ subunit is a specific determinant of receptor coupling. *J. Biol. Chem.* 269:21399–402

Kisselev O, Gautam N. 1993. Specific interaction with rhodopsin is dependent on the γ subunit type in a G protein. *J. Biol. Chem.* 268:24519–22

Kisselev O, Pronin A, Ermolaeva M, Gautam N. 1995. Receptor-G protein coupling is established by a potential conformational switch in the $\beta\gamma$ complex. *Proc. Natl. Acad. Sci. USA* 92:9102–6

Kleuss C, Scherübl H, Hescheler J, Schultz G, Wittig B. 1993. Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. *Science* 259:832–34

Knaus UG, Morris S, Dong H-J, Chernoff J, Bokoch GM. 1995. Regulation of human leukocyte p21-activated kinases through G protein-coupled receptors. *Science* 269:221–23

Kobilka BK, Kobilka TS, Daniel K, Regan JW, Caron MG, Lefkowitz RJ. 1988. Chimeric α2-, β2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science* 240:1310–16

Kosugi S, Mori T. 1995. TSH receptor and LH receptor, 1995. *Endocrinol. J.* 42:587–606

Kosugi S, Okajima F, Ban T, Hidaka A, Shenker A, Kohn LD. 1992. Mutation of alanine 623 in the third cytoplasmic loop of the rat thyrotropin (TSH) receptor results in a loss in the phosphoinositide but not cAMP signal induced by TSH and receptor autoantibodies. *J. Biol. Chem.* 267:24153–56

Kühn B, Schmid A, Harteneck C, Gudermann T, Schultz G. 1996. G proteins of the G_q family couple the H_2 histamine receptor to phospholipase *C. Mol. Endocrinol.* In press

Kuang Y, Wu Y, Jiang H, Wu D. 1996. Selective G protein coupling by C-C chemokine receptors. *J. Biol. Chem.* 271:3975–78

Lambright DG, Noel JP, Hamm HE, Sigler PB. 1994. Structural determinants for activation of a heterotrimeric G protein. *Nature* 369:621–28

Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB. 1996. The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature* 379:311–19

Laugwitz K-L, Allgeier A, Offermanns S, Spicher K, Van Sande J, et al. 1996. The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G-protein families. *Proc. Natl. Acad. Sci. USA* 93:116–20

Leberer E, Dignard D, Hougan L, Thomas DY, Whiteway M. 1992. Dominant-negative mutants of a yeast G-protein β subunit identify two functional regions involved in pheromone signaling. *EMBO J.* 11:4805–13

Liao MJ, London E, Khorana HG. 1983. Regeneration of the native bacteriorhodopsin structure from two chymotrypic fragments. *J. Biol. Chem.* 258:9949–55

Lichtarge O, Bourne HR, Cohen FE. 1996. Evolutionary conserved $G_{\alpha\beta\gamma}$ binding surfaces support a model of the G protein-receptor complex. *Proc. Natl. Acad. Sci. USA* 93:7507–11

Linseman DA, Benjamin CW, Jones DA. 1995. Convergence of angiotensin II and platelet-derived growth factor signaling cascades in vascular smooth muscle cells. *J. Biol. Chem.* 270:12563–68

Liu J, Blin N, Conklin BR, Wess J. 1996. Molecular mechanisms involved in muscarinic acetylcholine receptor-mediated G protein activation studied by insertion mutagenesis. *J. Biol. Chem.* 271:6172–78

Liu J, Conklin BR, Blin N, Yun J, Wess J. 1995a. Identification of a receptor/G-protein contact

site critical for signaling specificity and G-protein activation. *Proc. Natl. Acad. Sci. USA* 92:11642–46

Liu J, Schöneberg T, van Rhee M, Wess J. 1995b. Mutational analysis of the relative orientation of transmembrane helices I and VII in G protein-coupled receptors. *J. Biol. Chem.* 270:19532–39

Liu J, Wess J. 1996. Different single receptor domains determine the distinct protein coupling profiles of members of the vasopressin receptor family. *J. Biol. Chem.* 271:8772–78

Liu X, Garriga P, Khorana HG. 1996. Structure and function in rhodopsin: correct folding and misfolding in two point mutants in the intradiscal domain of rhodopsin identified in retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* 93:4554–59

Lohse MJ. 1993. Molecular mechanisms of membrane receptor desensitization. *Biochim. Biophys. Acta* 1179:171–88

Lucas JJ, Hen R. 1995. New players in the 5-HT receptor field: genes and knockouts. *Trends Pharmacol. Sci.* 16:246–52

Luttrell LM, Ostrowski J, Cotecchia S, Kendall H, Lefkowitz RJ. 1993. Antagonism of catecholamine receptor signaling by expression of cytoplasmic domains of the receptors. *Science* 259:1453–57

Machesky LM, Hall A. 1996. Rho: a connection between membrane receptor signalling and the cytoskeleton. *Trends Cell Biol.* 6:304–10

Maggio R, Vogel Z, Wess J. 1993a. Coexpression studies with mutant muscarinic/adrenergic receptors provide evidence for intermolecular 'cross-talk' between G-protein-linked receptors. *Proc. Natl. Acad. Sci. USA* 90:3103–7

Maggio R, Vogel Z, Wess J. 1993b. Reconstitution of functional muscarinic receptors by coexpression of amino- and carboxyl-terminal receptor fragments. *FEBS Lett.* 319:195–200

Milligan G, Marshall F, Rees S. 1996. G_{16} as a universal G protein adaptor: implications for agonist screening strategies. *Trends Pharmacol. Sci.* 17:235–37

Minden A, Lin A, Claret F-X, Abo A, Karin M. 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81:1147–57

Mixon MB, Lee E, Coleman DE, Berghuis AM, Gilman AG, Sprang SR. 1995. Tertiary and quarternary structural changes in $G_{i\alpha 1}$ induced by GTP hydrolysis. *Science* 270:954–60

Monnot C, Bihoreau C, Conchon S, Curnow KM, Corvol P, Clausner E. 1996. Polar residues in the transmembrane domains of the type I angiotensin II receptor are required for binding and coupling. Reconstitution of the binding site by co-expression of two deficient mutants. *J. Biol. Chem.* 271:1507–13

Moore EE, Kuestner RE, Stroop SD, Grant FJ, Matthewes SL, et al. 1995. Functionally different isoforms of the human calcitonin receptor result from alternative splicing of the gene transcript. *Mol. Endocrinol.* 9:959–68

Morishita R, Nakayama H, Isobe T, Matsuda T, Hashimoto Y, et al. 1995. Primary structure of a γ subunit of G protein, γ_{12}, and its phosphorylation by protein kinase C. *J. Biol. Chem.* 270:29469–75

Mouillac B, Chini B, Balestre M-N, Elands J, Trumpp-Kallmeyer S, et al. 1995. The binding site of neuropeptide vasopressin V1a receptor. Evidence for a major localization within transmembrane regions. *J. Biol. Chem.* 270:25771–77

Müller S, Hekman M, Lohse MJ. 1993. Specific enhancement of β-adrenergic receptor kinase activity by defined G-protein β and γ subunits. *Proc. Natl. Acad. Sci. USA* 90:10439–43

Nakamura M, Sakanaka C, Aoki Y, Ogasawara H, Tsuji T, et al. 1995. Identification of two isoforms of mouse neuropeptide Y-Y1 receptor generated by alternative splicing. Isolation, genomic structure, and functional expression of the receptors. *J. Biol. Chem.* 270: 30102–10

Neer EJ. 1995. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* 80:249–57

Neer EJ, Schmidt CJ, Nambudripad R, Smith TF. 1994. The ancient regulatory-protein family of WD-repeat proteins. *Nature* 371:297–300

Neer EJ, Smith TF. 1996. G protein heterodimers: new structures propel new questions. *Cell* 84:175–78

Neubig RR. 1994. Membrane organization in G-protein mechanisms. *FASEB J.* 8:939–46

Noel JP, Hamm HE, Sigler PB. 1993. The 2.2 Å crystal structure of transducin-α complexed with GTPγS. *Nature* 366:654–63

Nürnberg B, Ahnert-Hilger G. 1996. Potential roles of heterotrimeric G proteins of the endomembrane system. *FEBS Lett.* 389:61–65

Nürnberg B, Gudermann T, Schultz G. 1995. Receptors and G proteins as primary components of transmembrane signal transduction. Part 2. G proteins: structure and function. *J. Mol. Med.* 73:123–32, Correction: 73:379

Nussenzveig DR, Thaw CN, Gershengorn MC. 1994. Inhibition of inositol phosphate second messenger formation by intracellular loop one of a human calcitonin receptor. *J. Biol. Chem.* 269:28123–29

Offermanns S, Iida-Klein A, Segre GV, Simon MI. 1996. $G\alpha_q$ family members couple PTH/PTHrP and calcitonin receptors to phos-

pholipase C in COS-7 cells. *Mol. Endocrinol.* 10:566–74
Offermanns S, Laugwitz KL, Spicher K, Schultz G. 1994a. G proteins of the G_{12} family are activated via thromboxane A_2 and thrombin receptors in human platelets. *Proc. Natl. Acad. Sci. USA* 91:504–8
Offermanns S, Schultz G. 1994. Complex information processing by the transmembrane signaling system involving G proteins. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 350:329–38
Offermanns S, Simon MI. 1995. $G\alpha_{15}$ and $G\alpha_{16}$ couple a wide variety of receptors to phospholipase C. *J. Biol. Chem.* 270:15175–80
Offermanns S, Wieland T, Homann D, Sandmann J, Bombien E, et al. 1994b. Transfected muscarinic acetylcholine receptors selectively couple to G_i-type G proteins and $G_{q/11}$. *Mol. Pharmacol.* 45:890–98
Palmer TM, Gettys TW, Stiles GL. 1995. Differential interaction with and regulation of multiple G-proteins by the rat A_3 adenosine receptor. *J. Biol. Chem.* 270:16895–902
Pan Y, Wilson P, Gitschier J. 1994. The effect of eight V2 vasopressin receptor mutations on stimulation of adenylyl cyclase and binding to vasopressin. *J. Biol. Chem.* 269:31933–37
Parma J, Dupret L, Van Sande J, Paschke R, Tonacchera M, et al. 1994. Constitutively active receptors as a disease-causing mechanism. *Mol. Cell. Endocrinol.* 100:159–62
Perlman JH, Laakkonen LJ, Guarnieri F, Osman R, Gershengorn MC. 1996. A refined model of the thyrotropin-releasing hormone (TRH) receptor binding pocket. Experimental analysis and energy minimization of the complex between TRH and TRH receptor. *Biochemistry* 35:7643–50
Phillips WJ, Cerione RA. 1992. Rhodopsin/transducin interactions. I. Characterization of the binding of the transducin-$\beta\gamma$ subunit complex to rhodopsin using fluorescence spectroscopy. *J. Biol. Chem.* 267:17032–39
Pisegna JR, Wank SA. 1996. Cloning and characterization of the signal transduction of four splice variants of the human pituitary adenylate cyclase activating polypeptide receptor. Evidence for dual coupling to adenylate cyclase and phospholipase C. *J. Biol. Chem.* 271:17267–74
Popot JL, Engelman DM. 1990. Membrane protein folding and oligomerization: the two-stage model. *Biochemistry* 29:4031–37
Popot JL, Gerchman SE, Engelman DM. 1987. Refolding of bacteriorhodopsin in lipid bilayers. A thermodynamically controlled two-stage process. *J. Mol. Biol.* 198:655–76
Popova JS, Johnson GL, Rasenick MM. 1994. Chimeric $G_{\alpha s}/G_{\alpha i2}$ proteins define domains on $G_{\alpha s}$ that interact with tubulin for β-adrenergic activation of adenylyl cyclase. *J. Biol. Chem.* 269:21748–54
Pronin AN, Gautam N. 1992. Interaction between G protein β and γ subunit types is selective. *Proc. Natl. Acad. Sci. USA* 89:6220–24
Rao GN, Delafontaine P, Runge MS. 1995. Thrombin stimulates phosphorylation of insulin-like growth factor-1 receptor, insulin receptor substrate-1, and phospholipase C-γ1 in rat aortic smooth muscle cells. *J. Biol. Chem.* 270:27871–75
Ray K, Kunsch C, Bonner LM, Robishaw JD. 1995. Isolation of cDNA clones encoding eight different human G protein γ subunits, including three novel forms designated the γ_4, γ_{10}, and γ_{11} subunits. *J. Biol. Chem.* 270:21765–71
Ridge KD, Lee SSJ, Yao LL. 1995. In vivo assembly of rhodopsin from expressed polypeptide fragments. *Proc. Natl. Acad. Sci. USA* 92:3204–8
Ridge KD, Lee SSJ, Abdulaev NG. 1996. Examining rhodopsin folding and assembly through expression of polypeptide fragments. *J. Biol. Chem.* 271:7860–67
Ross EM. 1995. G protein GTPase-activating proteins: regulation of speed, amplitude, and signaling selectivity. *Rec. Progr. Horm. Res.* 50:207–21
Sargiacomo M, Sudol M, Tang ZL, Lisanti MP. 1993. Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. *J. Cell. Biol.* 122:789–807
Saunders C, Keefer JR, Kennedy AP, Wells JN, Limbird LE. 1996. Receptors coupled to pertussis toxin–sensitive G-proteins traffic to opposite surfaces in Madin-Darby canine kidney cells. A_1 adenosine receptors achieve basolateral localization. *J. Biol. Chem.* 271:995–1002
Schmidt CJ, Thomas TC, Levine MA, Neer EJ. 1992. Specificity of G protein β and γ subunit interactions. *J. Biol. Chem.* 267:13807–10
Schöneberg T, Liu J, Wess J. 1995. Plasma membrane localization and functional rescue of truncated forms of a G protein-coupled receptor. *J. Biol. Chem.* 270:18000–6
Schöneberg T, Yun J, Wenkert D, Wess J. 1996. Functional rescue of mutant V2 vasopressin receptors causing nephrogenic diabetes insipidus by a co-expressed receptor polypeptide. *EMBO J.* 15:1283–91
Schwartz TW, Rosenkilde MM. 1996. Is there a 'lock' for all agonist 'keys' in 7TM receptors? *Trends Pharmacol. Sci.* 17:213–16
Sealfon SC, Chi L, Ebersole BJ, Rodic V, Zhang D, et al. 1995. Related contribution of specific helix 2 and 7 residues to conformational ac-

tivation of the serotonin 5-HT_{2A} receptor. *J. Biol. Chem.* 270:16683–88
Siderovski DP, Hessel A, Chung S, Mak TW, Tyers M. 1996. A new family of regulators of G-protein-coupled receptors? *Curr. Biol.* 6:211–12
Simon MI, Strathmann MP, Gautam N. 1991. Diversity of G proteins in signal transduction. *Science* 252:802–8
Sondek J, Bohm A, Lambright DG, Hamm HE, Sigler PB. 1996. Crystal structure of a G_A protein $\beta\gamma$ dimer at 2.1 Å resolution. *Nature* 379:369–74
Sondek J, Lambright DG, Noel JP, Hamm HE, Sigler PB. 1994. GTPase mechanism of G protein from the 1.7 Å crystal structure of transducin $\alpha\bullet GDP\bullet AlF_4^-$. *Nature* 372:276–79
Spengler D, Waeber C, Pantaloni C, Holsboer F, Bockaert J, et al. 1993. Differential signal transduction by five splice variants of the PACAP receptor. *Nature* 365:170–75
Strader CD, Fong TM, Tota MR, Underwood D, Dixon RAF. 1994. Structure and function of G protein-coupled receptors. *Annu. Rev. Biochem.* 63:101–32
Strittmatter SM, Valenzuela D, Kennedy TE, Neer EJ, Fishman MC. 1990. G_o is a major growth cone protein subject to regulation by GAP-43. *Nature* 344:833–41
Sung CH, Davenport CM, Nathans J. 1993. Rhodopsin mutations responsible for autosomal dominant retinitis pigmentosa: clustering of functional classes along the polypeptide chain. *J. Biol. Chem.* 268:26645–49
Suryanarayana S, von Zastrow M, Kobilka BK. 1992. Identification of intramolecular interactions in adrenergic receptors. *J. Biol. Chem.* 267:21991–94
Taussig R, Iñiguez-Lluhi JA, Gilman AG. 1993. Inhibition of adenylyl cyclase by $G_{i\alpha}$. *Science* 261:218–21
Taylor JM, Jacob-Mosier GG, Lawton RG, Remmers AE, Neubig RR. 1994. Binding of an α_2 adrenergic receptor third intracellular loop peptide to $G\beta$ and the amino terminus of $G\alpha$. *J. Biol. Chem.* 269:27618–24
Taylor JM, Neubig RR. 1994. Peptides as probes for G protein signal transduction. *Cell. Signal.* 6:841–49
Thirstrup K, Elling CE, Hjorth SA, Schwartz TW. 1996. Construction of a high affinity zinc switch in the κ-opioid receptor. *J. Biol. Chem.* 271:7875–78
Thomas PJ, Qu B-H, Pedersen PL. 1995. Defective protein folding as a basis of human disease. *Trends Biochem. Sci.* 20:456–59
Trumpp-Kallmeyer S, Hoflach J, Bruinvels A, Hibert M. 1992. Modeling of G-protein-coupled receptors: application to dopamine, adrenaline, serotonin, acetylcholine, and mammalian opsin receptors. *J. Med. Chem.* 35:3448–62
Unson CG, Cypress AM, Kim HN, Goldsmith PK, Carruthers CJL, et al. 1995. Characterization of deletion and truncation mutants of the rat glucagon receptor. *J. Biol. Chem.* 270:27720–27
van Biesen T, Hawes BE, Luttrell DK, Krueger KM, Touhara K, et al. 1995. Receptor-tyrosine-kinase- and $G\beta\gamma$-mediated MAP kinase activation by a common signalling pathway. *Nature* 376:781–84
Vernier P, Cardinaud B, Valdenaire O, Philippe H, Vincent J-D. 1995. An evolutionary view of drug-receptor interaction: the bioamine receptor family. *Trends Pharmacol. Sci.* 16:375–81
Vu Hai-Luu Thi MT, Misrahi M, Houllier A, Jolivet A, Milgrom E. 1992. Variant forms of the pig lutropin/choriogonadotropin receptor. *Biochemistry* 31:8377–83
Wall MA, Coleman DE, Lee E, Iñiguez-Lluhi JA, Posner BA, et al. 1995. The structure of the G protein heterotrimer $G_{i\alpha1}\beta_1\gamma_2$. *Cell* 83:1047–58
Wang C, Jayadev S, Escobedo JA. 1995. Identification of a domain in the angiotensin II type 1 receptor determining G_q coupling by the use of receptor chimeras. *J. Biol. Chem.* 270:16677–82
Watson N, Linder ME, Druey KM, Kehrl JH, Blumer KJ. 1996. RGS family members: GTPase-activating proteins for heterotrimeric G-protein α-subunits. *Nature* 383:172–75
Watson AJ, Katz A, Simon MI. 1994. A fifth member of the mammalian G protein β-subunit family. *J. Biol. Chem.* 269:22150–56
Wess J, ed. 1995. *Molecular Mechanisms of Muscarinic Acetylcholine Receptor Function*. New York: Springer. 272 pp.
Wong SK-F, Parker EM, Ross EM. 1990. Chimeric muscarinic cholinergic:β-adrenergic receptors that activate G_s in response to muscarinic agonists. *J. Biol. Chem.* 265:6219–24
Wong SK-F, Ross EM. 1994. Chimeric muscarinic cholinergic:β-adrenergic receptors that are functionally promiscuous among G proteins. *J. Biol. Chem.* 269:18969–76
Wozniak M, Limbird LE. 1996. The three α2-adrenergic receptor subtypes achieve basolateral localization in Madin-Darby canine kidney II cells via different targeting mechanisms. *J. Biol. Chem.* 271:5017–24
Wu D, Kuang Y, Wu Y, Jiang H. 1995. Selective coupling of β_2-adrenergic receptor to hematopoietic-specific G proteins. *J. Biol. Chem.* 270:16008–10

Xiao R-P, Ji X, Lakatta EG. 1995. Functional coupling of the β_2-adrenoceptor to a pertussis toxin–sensitive G protein in cardiac myocytes. *Mol. Pharmacol.* 47:322–29

Yan K, Gautam N. 1996. A domain on the G protein β subunit interacts with both adenylyl cyclase 2 and the muscarinic atrial potassium channel. *J. Biol. Chem.* 271:17597–600

Yan K, Kalyanaraman V, Gautam N. 1996. Differential ability to form G protein $\beta\gamma$ complex among members of the β and γ subunit families. *J. Biol. Chem.* 271:7141–46

Yang RB, Foster DC, Garbers DL, Fülle HJ. 1995. Two membrane forms of guanylyl cyclase found in the eye. *Proc. Natl. Acad. Sci. USA* 92:602–6

Yasuda H, Lindorfer MA, Woodfork KA, Fletcher JE, Garrison JC. 1996. Role of the prenyl group on the G protein γ subunit in coupling trimeric G proteins to A1 adenosine receptors. *J. Biol. Chem.* 271:18588–95

Zhou W, Flanagan C, Ballesteros JA, Konvicka K, Davidson JS, et al. 1994. A reciprocal mutation supports helix 2 and helix 7 proximity in the gonadotropin-releasing hormone receptor. *Mol. Pharmacol.* 45:165–70