Pharmaceutical Research, Vol. 7, No. 12, 1990

Review

Structure and Function of G Protein Coupled Receptors

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The G protein coupled receptors (GPC-Rs) comprise a large superfamily of genes encoding numerous receptors which all show common structural features, e.g., seven putative membrane spanning domains. Their biological functions are extremely diverse, ranging from vision and olfaction to neuronal and endocrine signaling. The GPC-Rs couple via multiple G proteins to a growing number of recognized second messenger pathway, e.g., cAMP and phosphatidyl inositol turnover. This review summarizes our current knowledge of the molecular mechanisms by which the GPC-Rs activate second messenger systems, and it addresses their regulation and structure.

KEY WORDS: G protein coupled receptors; neurotransmitter receptors; hormone receptors; adrenergic receptors; muscarinic cholinergic receptors; second messengers.

INTRODUCTION

Transmembrane signal transduction is crucial to the cell's ability to respond to external stimuli. Membrane receptors involved in signal transduction fall into several broad categories, including ion channels, transport proteins, growth factor receptors, and the G protein coupled receptors (GPC-R). The GPC-Rs are distinct in their molecular organization and mode of signal transduction. Whereas the growth factor receptors are anchored to the membrane by a single transmembrane domain and usually contain the effector domain in the cytoplasmic portion of the molecule (e.g., a tyrosine kinase domain), the GPC-Rs are organized into seven putative transmembrane domains (Fig. 1) and require an intermediary G protein (GTP binding protein) to activate the second messenger system (1).

Molecular cloning of a number of GPC-Rs has opened fascinating vistas of this extremely diverse family of receptors (Table I). Far from being a recent development in evolution, GPC-Rs have been identified in slime mold (cAMP receptor involved in cell division) (2) and yeast (mating factor receptor) (3). Bacteriorhodopsin, the major light-sensitive protein of the purple membrane of *Halobacterium halobium* was the first membrane protein found to be organized into seven membrane spanning domains, and it became a model for structural studies of the GPC-Rs (4). Whereas bacteriorhodopsin, which contains a retinal prosthetic group, does not represent a GPC-R—it provides energy by functioning as a light-activated proton pump—the related visual opsins, rhodopsin and blue, green and red pigment, are true GPC-Rs (1,5). They couple via a special G protein, transducin, to a cGMP phosphodiesterase upon light activation. Extensive research on the opsin family of genes has provided us with many insights into the mechanism of signal transduction and gene mutations as the basis for congenital defects (6–9).

The field of GPC-Rs took a decisive turn when it was recognized that the opsins, the adrenergic receptors, and other neurotransmitter receptors are encoded by genes with similar features, including sequence homologies and the canonical seven-membrane spanning organization (Table I, Fig. 1) (1,10). The surprising conservation of sequence, particularly in the transmembrane regions where tertiary structure folding is required to produce functional receptors, and in the spacing of key functional amino acids, suggests that this entire family may have arisen from a single ancestral gene from which a perplexing diversity of function emanated. Well over a hundred GPC-R genes may exist in the human genome (see Table II for a partial list), binding such diverse ligands as scent compounds and pheromones (olfactory receptors) (11), the neurotransmitters acetylcholine, serotonin, and catecholamines, neuropeptides and peptide hormones, and the large glycopeptide hormones FSH/LH and TSH.

Early pharmacological experiments have suggested that each of these receptors may exist in several subtypes, and indeed, the search for homologous genes by cross-hybridization techniques proved to be more rewarding than anticipated. For example, while the existence of three subtypes of the muscarinic cholinergic receptors was suspected, molecular biologists have now cloned five distinct genes

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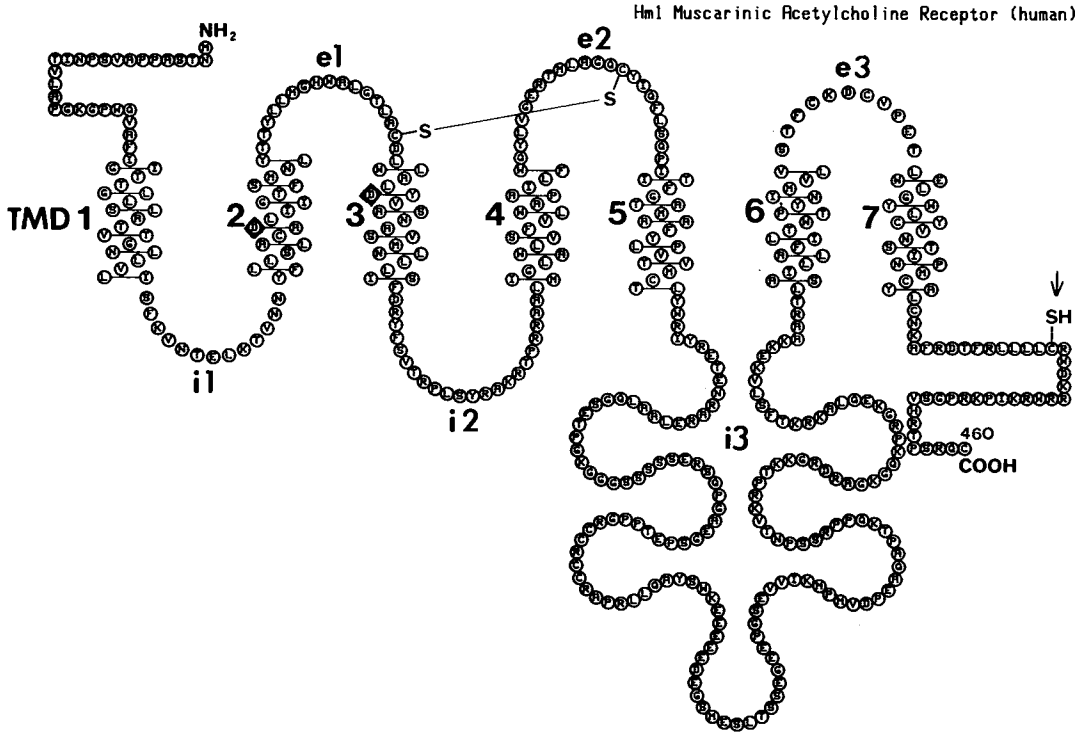


Fig. 1. Deduced amino acid sequence and putative arrangement into seven transmembrane helical domains (TMD) of Hm1. The arrow indicates a potential palmitoylation site in the C terminus. The N terminus is located outside, and the C terminus inside the cell. A putative S–S bridge between extracellular loops e1 and e2 is also indicated. Note the large intracellular loop i3 and the conserved cystein (palmitoylated?) in the C terminus.

(12,13) encoding as many receptor subtypes (Table III). Such multiplicity was presumably generated by gene duplication, and receptor diversity may be further increased by alternative splicing, as demonstrated for the dopamine D_2 receptor (14,15).

What could be the significance of multiple receptor subtypes each responsive to the same neurotransmitter? It turns out that the subtypes of the muscarinic cholinergic receptors couple via different G proteins to different second messengers; further, different concentrations of acetylcholine are required for maximum effect, providing for a greater regulatory range. Even more important, however, is the fact that each of the subtypes is under separate control of promoter and enhancer elements that specify highly tissue-selective expression. Hence, m5 appears to be expressed only in small areas of the brain, but its physiological function remains obscure at present. Nevertheless, each of the muscarinic subtypes is a separate target for more specific drugs with fewer side effects, which have limited the more widespread use of muscarinic drugs in the past. The serotonin receptor appears to emerge as the most notorious example of multiple subtypes, possibly more than a dozen, with numerous yet uncharted therapeutic possibilities. These fundamental discoveries force a merging of the disciplines of medicinal chemistry, pharmacology, and molecular biology in novel drug design.

Table I. Cloned G Protein Coupled Receptors

cAMP-R (slime mold), mating factor-R (yeast)
Opsins: rhodopsin; blue, green, red pigment
Adrenergic: *alpha*—1, 2A, 2B, 2-C4; *beta*—1, 2, 3
Muscarinic cholinergic: m—1, 2, 3, 4, 5
Serotonin-R: HT—1a, 1c, 2; dopamine-R: D_1, D_2
Substance P-R; angiotensin II-R (*mas* oncogene)
Substance K-R
Gonadotropin-R (LH-CG-R)
Thyrotropin-R (TSH-R)

GPC-R FUNCTIONS

The broad range of ligands to the GPC-Rs attests to their pervasive role in hormone and neurotransmitter signal tranduction. Let us consider here the cellular functions of these receptors, namely, their coupling to second messengers via the G proteins. Whereas the GPC-Rs number above 100, there are now nearly 20 identified mammalian G proteins that regulate possibly as many ion channels, enzymes, and transporters. The G proteins are heterotrimeric proteins consisting of a variable α subunit and the less variable tightly associated $\beta\gamma$ subunits. The receptor-activated regulatory cycle of the G protein (Fig. 2) involves GTP exchange for GDP, dissociation of α and $\beta\gamma$ subunits, activation of the second messenger pathway by GTP-G_α, and termination of activation upon GTP hydrolysis to GDP by the inherent GTPase activity of the α subunit. Some controversy remains over the question whether the $\beta\gamma$ subunit also couples to second messenger pathways upon release from the heterotrimer, for example, by activating phospholipase A_2 and thereby initiating the arachidonic acid cascade (16–18). Further, $\beta\gamma$ subunits inhibit α subunit activity by reducing its

Table II. Partial List of Hormones and Neurotransmitters Thought to Interact with G Protein Coupled Receptors[a]

Nonpeptide	Peptide
Acetylcholine (muscarinic)	ACTH
Adenosine	Angiotensin (*mas* oncogene)
Epinephrine	Bombesin
Dopamine	Bradykinin
Histamine	Calcitonin
Norepinephrine	Calcitonin GRP
Olfactory receptors	Cholecystokinin
Platelet activating factor	CRF
Prostanoids	Dynorphin
Prostaglandins	Endothelin
Thromboxanes	β-Endorphin
Leukotrienes	Enkephalins
Prostacyclins	Formyl peptide (FMLP)
	FSH
	GABA (B receptor)
	GHRH
	GIP
	LH-CG
	LHRH
	Neuromedin
	Neurotensin
	Oxytocin
	Oxyntomodulin
	Parathyroid hormone
	PHI
	PHM
	Secretin
	Somatostatin
	Substance K
	Substance P
	Substance Y
	Substance YY
	Thrombin
	TRH
	TSH
	Vasopressin
	VIP

[a] When the receptor genes have not yet been cloned (compare to Table I), the following criteria served to suggest binding to GPC-Rs: modulation of agonist receptor binding by guanyl nucleotides, coupling to second messenger systems such as PI turnover and cAMP and its modulation by cholera or pertussis toxin, and documented interaction with G proteins. Unless all of these criteria have been documented, assignment as a GPC-R remains putative. Considering multiple subtypes for each ligand, the total number of receptor genes far exceeds the number of ligands.

affinity for GTP (1,10). Finally, the γ subunit of yeast G_m was recently shown to be polyisoprenylated to serve as a membrane anchor, and similar isoprenylation sites (CAAX) in all G_γ proteins suggest a general anchoring role for the γ subunit (19).

Apart from the coupling of the visual pigments to cGMP phosphodiesterase via the G protein transducin, GPC-R-mediated regulation of phosphatidyl inositol (PI) turnover and of adenylyl cyclase (AC) has been studied in greatest detail. Much less is known about the mechanism of coupling to ion channels (K^+ and Ca^{2+}) and to other enzymes and transporter proteins, such as guanylyl cyclase, Mg^{2+}, and glucose transporters, and the Na^+/H^+ antiporter. Figure 3 illustrates the coupling of AC, which can be stimulatory (via G_s) or inhibitory (via G_i), and consequently, the regulation of protein kinase A by cAMP. Further, the activation of PI turnover via phospholipase C stimulates two separate second messenger pathways, via diacylglycerol activating protein kinase C and via inositol 1,4,5-triphosphate to trigger the release of Ca^{2+} from intracellular stores. To appreciate the complexity of these signal transducing systems, one must consider that each of the components, receptor, G protein, and target enzyme, exists as multiple subtypes. Individual receptors may have preference for certain G proteins, but cross-talk among the various pathways has been demonstrated, e.g., for the coupling of muscarinic cholinergic receptors to AC and PI turnover (20). Further, the various second messenger systems affect each other, for example, by modulating AC activity via protein kinase C or Ca^{2+} (21), and these interactions differ from one cell type to another.

In view of the crucial importance of the GPC-R system to the cell, it is perhaps not surprising that infectious organisms have usurped its components for their own purposes. ADP ribosylation of $G_{s\alpha}$ by cholera toxin permanently activates G_s, while ADP ribosylation of $G_{i\alpha}$ by pertussis toxin blocks G_i inhibitory function, leading in each case to elevated cAMP levels in the cell. Recently, open reading frames encoding several putative GPC-Rs were detected in the genome of human cytomegalovirus, and it is speculated that these genes could serve the virus by favorably affecting the cell's response to infection (22).

We also need to consider the potential of the GPC-Rs to function as protooncogenes or oncogenes, as demonstrated for the growth factor receptors with tyrosine kinase domains (e.g., Ref. 23). Indeed, one GPC-R was originally isolated because of its transforming activity and termed *mas* oncogene, and it was only subsequently identified as a putative angiotensin-II receptor (Ref. 24 and references therein). Further, mutations of the $G_{s\alpha}$ subunit can result in receptor-independent permanent activation of G_s. As cAMP stimulates growth in certain cells, the activated G_s mutant has been proposed to function as an oncogene, termed *gsp*, in human pituitary tumors (25). Independently, the mitogenic activity of muscarinic cholinergic, serotonergic and VIP receptors coupled to PI turnover has been demonstrated in a variety of cell lines, and the mitogenic activity may be relevant during early development of the CNS (24). It is further possible that the GPC-Rs stimulate the production of less common inositol phosphates, such as 1,2-cyclic inositol phosphates and 1,3,4-phosphoinositol (rather than 1,4,5-phosphoinositol), the latter by activating PI 3-kinase, which may play a role in mitogenesis/transformation (23,26).

More work is needed to clarify the second messenger pathways of GPC-Rs. In particular, the possibility that mutations of the GPC-Rs could lead to genetic defects and diseases must be actively addressed. Because color blindness is easily detected, mutations of the opsin genes and their promoter regions have already been identified (6–9). Documented mutations in the genes encoding the visual pigments, and their association with color blindness, presage the discovery of similar mutations among the analogous GPC-Rs that could lead to mental illness, cardiovascular diseases, and cancer.

Table III. The Muscarinic Receptor Gene Family

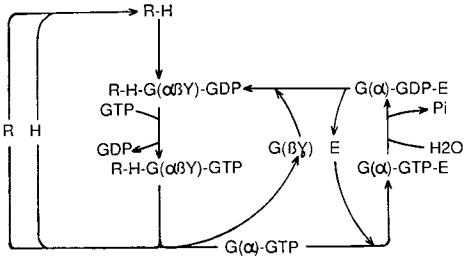
Molecular sequences	m1	m2	m3	m4	m5
Major tissue type	Cerebral	Cardiac	Glandular	Brain	Brain
Selective antagonist	Pirenzepine	AF-DX 116	*p*-Fluorohexahydro-siladifenidol		
Second messenger systems	PI ↑	cAMP ↓	PI ↑	cAMP ↓	PI ↑
Therapeutic potential					
Agonist	Alzheimer's	Tachyarrhythmias	Postop. atonia (gut, urin. bladder)	?	?
Antagonist	Peptic ulcer COPD (asthma)	Antibradycardic effects	Antispasmodic effects	?	?

RECEPTOR STRUCTURE

Direct evidence for the existence of seven transmembrane spanning domains was obtained only for bacteriorhodopsin, with the use of electron scattering and neutron diffraction analysis (4,27). Recently, a structure for rhodopsin at 3.5-Å resolution has been published by Henderson *et al.*, using high-resolution electron cryomicroscopy (28). In analogy to the structure of bacteriorhodopsin, a model for the structure of mammalian rhodopsin was constructed by Findlay *et al.* (29), and this model was subsequently expanded by G. M. Smith to the muscarinic m1 receptor (unpublished). The major justification for such an evolving model stems from hydrophobicity analysis of the deduced primary amino acid sequence, as shown in Fig. 4, which invariably documents the presence of seven hydrophobic domains of 20–25 amino acids, each capable of transversing the lipid membrane bilayer. Further, the greatest degree of similarity among the GPCRs is observed within these putative transmembrane domains (Fig. 5). However, the exact locations of the TMDs are not clearly defined. For example, the computer-calculated domain of highest lipophilicity for TMD3 in m1 is located approximately 15 amino acids closer to the N terminal than indicated for the structure in Fig. 1, which was constructed from the rhodopsin model. It is also possible that the agonist and antagonist conformations of the receptor correspond to different arrangements of the TMDs. On the basis of the structural m1 model proposed by Smith, the seven transmembrane domains are arranged in an oval shape (Fig. 6), with charged and polar amino acids pointing toward the center, small hydrophobic residues in the helix contact regions, and residues with large hydrophobic side chains toward the membrane bilayer. One can thus envisage the binding pocket in the center, with most if not all TMDs contributing to ligand binding. Helix packing and hydrophobic moment of the m1 model (Fig. 6) are consistent with the postulate that the polar residues point toward the center. However, helices TMD5 and TMD7 show hydrophobic moments in the model that deviate from the expected orientation. It remains to be seen whether such a model can serve as a guide to further experiments.

The structures of the N and C terminal tails and the e and i loops are unknown. A highly, but not universally, conserved pair of cysteins in e2 and e3 is thought to link these two loops via a disulfide bridge. Removal of one of these Cys by point mutation leads to inactivation of the receptor (see Table IV). Further, one instance of congenital color blindness has been linked to a point mutation of an opsin gene leading to the loss of Cys in e3 (6). These results suggest that the disulfide bond is necessary to maintain the receptor in a functional configuration.

A highly conserved cystein in the C terminal tail of rhodopsin (30) has been shown to be palmitoylated (see below, Receptor Regulation). The palmitoyl residue is expected to be anchored to the membrane, thereby, introducing one additional intracellular loop; however, the arrangement of this loop remains unknown. Even less is known about the N-terminal tail, which contains glycosylation sites.



Mechanism of G protein activation.
G(αβγ) : trimeric form of G protein composed of G(α)+G(βγ)
H : hormone (ligand)
R : G protein coupled receptor
E : effector

Fig. 2. G-protein cycle. Hormone–receptor complex (HR) promotes GTP-dependent dissociation of α-GTP from βγ, and effector activation by G_α-GTP. Activation is terminated by the GTPase activity of G_α.

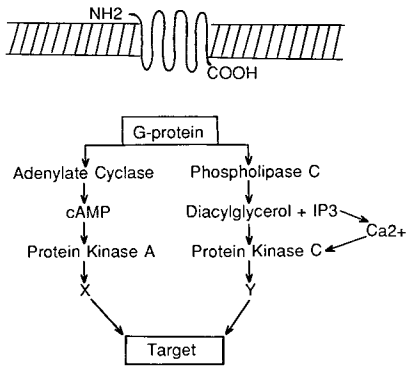


Fig. 3. Cascade of events after GPC-R activation involving cAMP and phosphatidyl inositol turnover (PI). IP_3, inositol 1,4,5-triphosphate.

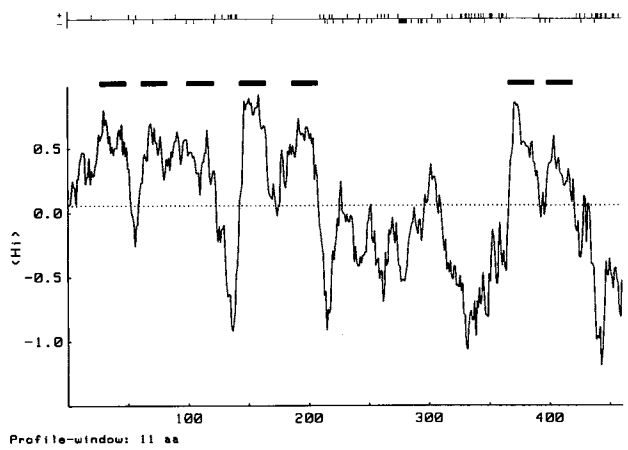


Fig. 4. Hydrophobicity analysis of the deduced amino acid sequence of Hm1. Seven hydrophobic peaks are clearly visible; however, some of the TMD are poorly defined, especially TMD3. The hydropathicity index (Hi) was calculated using Eisenberg criteria. Also shown is the distribution of positive and negative amino acids.

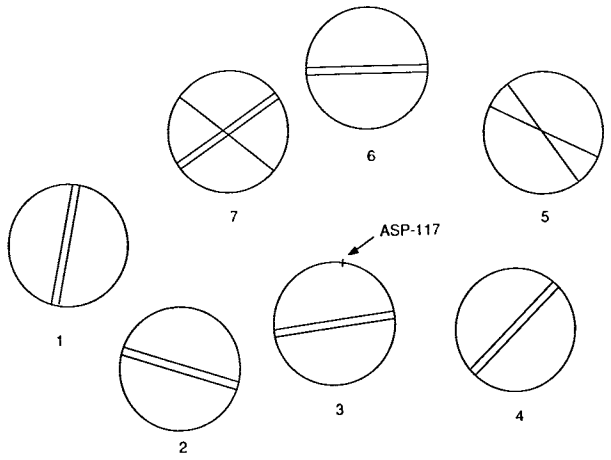


Fig. 6. Helix packing and orientation in a muscarinic receptor Hm1 model viewed from the outside cell. The lines divide the helices into hydrophobic external and hydrophilic internal faces. Nonparallel lines indicate deviation of the model from the expected orientation. Helices 1 and 7 are kinked and, therefore, divided into upper and lower segments. The lower segment of helix 7, but not of TMD1, has a distinct hydrophobic moment.

Indeed, glycosylation is thought to be important for receptor function; however, at least one of the cloned GPC-Rs, the adrenergic alpha-2B receptor, does not contain any N-terminal glycosylation site (31), suggesting that glycosylation is not an absolute requirement. A curious point mutation of a proline residue in the N-terminal tail of rhodopsin was recently linked to congenital retinitis (9). As proline bends the polypeptide chain, secondary structure of the N terminal may be important, but no hypothesis has been proposed to account for this congenital disease.

Many of the questions raised in this review could be directly addressed if the three-dimensional structure of the GPC-Rs were known. It is immensely difficult, however, to produce these membrane proteins in large quantities and to obtain crystals of sufficient quality for X-ray analysis. Recent success in crystallizing integral membrane proteins, such as the bacterial photosynthetic reaction center (32), is encouraging, and we are currently pursuing this goal with the muscarinic m1 receptor.

Muscarinic R	Hm1	NYFL-LSLACADL-IIGTFSMNL-YTT
	Hm2	NYFL-FSLACADL-IIGVFSMNL-YTL
	Hm3	NYFL-LSLACADL-IIGVISMNL-FTT
	Hm4	NYFL-FSLACADL-IIGAFSMNL-YTV
	Hm5	NYYL-LSLACADL-IIGIFSMNL-YTT
Adrenergic R	α-1	NYFI-VNLAIADL-LLSFTVLPFSATL
	α-2P	NLFL-VSLASADI-LVATLVIPFSLAN
	α-2K	NLFL-VSLASADI-LVATLVMPFSLAN
	β-1	NLFI-MSLASADL-VMGLLVVPFGATI
	β-2	NYFI-TSLACADL-VMGLAVVPFGAAH
	β-3	NVFV-TSLAAADL-VMGLLVVPPAATL
Angiotensin R		VYITHLSIA--DISLLFCI-FILSIDY
Substance-K R		NYFI-VNLALADLCMAA-FNAAFNFVY
Serotonin R		NYFL-MSLAIADM-LVGLLVMPLSLLA
Dopamine R	D-2	NYLI-VSLAVADL-LVATLVMPWVVYL
TSH R		RFLMC-NLAFADFCM-GMYLLLIASVD
Substance-P R		NYFL-VNLAFAEACMAA-FNTVVNFTY

Fig. 5. Sequence comparison of TMD2 among selected cloned GPC-Rs. Note the conserved aspartate (D), or glutamate (E) in the case of the substance P receptor, which is thought to contribute to agonist ligand binding. The bar indicates amino acids that are repeated in ≥50% of the receptors shown. Gaps were introduced to optimize alignment.

RECEPTOR DOMAINS

The receptor molecules mediate numerous functions, some of which we begin to understand in more detail, e.g., ligand binding, coupling of G proteins, and desensitization, while other functions remain poorly defined, e.g., receptor sequestration, internalization, and down-regulation. Mutational analysis of the cloned receptor genes has proven a powerful tool to delineate the functional domains of the receptor molecule (Table IV) (33–45). By combining the results from deletion mutations, point mutations, and chimera formation, new insights have emerged on the process of receptor activation and desensitization. The major advances are briefly discussed.

The Ligand Binding Pocket

Neutron diffraction analysis of bacteriorhodopsin in the purple membrane demonstrated that the retinal prosthetic group, which is covalently attached via an imine to a lysine residue in the seventh transmembrane domain (TMD7), is located within the core of the ring of transmembrane domains (27). Similarly, the retinal of mammalian rhodopsin binds to an equivalent lysine residue in TMD7, with a highly conserved Asp in TMD3 serving as the putative counterion to the positively charged imine function (29). It is now generally assumed that the seven-membrane spanning regions of the GPC-Rs form a binding pocket inside the membrane which can accommodate their diverse ligands. Indeed, even large deletions of several receptor domains outside the membrane did not affect ligand binding. For the positively charged ligands, such as the biogenic amines, acetyl choline, and certain peptides, negatively charged counterions inside and close to the binding pocket are crucial to receptor activation. Removing Asp in TMD3 completely abrogates the binding of agonists and antagonists to the beta-2 and the m1 receptors, whereas mutation of the Asp in the TMD2 appears to affect agonist binding selectively (33,34). The latter

Table IV. Examples of GPC-R Mutations and Resultant Changes in Receptor Function

Type of mutation	Result/conclusion	Reference No(s).
β_2-adrenergic receptor		
Point mutations		
Asp-79, 113, 130, Asn	Asp 113 (in TMD3) required for ligand binding, Asp 79 (in TMD2) plays role in agonist binding	33,34
Cys 106, 184	Disulfide bridge between e2 and e3 required for expression of functional receptor	35
Ser-204/207 → Ala	Ser-204/207 in TMD5 may contribute to catechol agonist binding *via* hydrogen bonds	36
Ser, Thr in N terminus and loop i3	Receptors lacking phosphorylations sites do not desensitize rapidly	37
Cys-341 → Gly	Palmitoylation of Cys^{341} plays crucial role in receptor coupling	38
Chimeric receptors		
β_1–β_2	TMD4 contributes to binding selectivity of agonists, TMD6 and 7 to selectivity of antagonists	39
α_2–β_2	Coupling selectivity determined by junctions of loop i3 Ligand specificity largely determined by TMD7	40
Deletions mutations		
del. 239–272 274–330	Junctions of loop i3 required for coupling	41
del. 222–229 253–270	Junctions of loop i3 required for coupling	42
Muscarinic cholinergic receptor		
Point mutation		
Asp-71, 99, 105, 122 → Asn	Asp 71 (TMD2) and Asp 105 (TMD3) are equivalent to Asp 77 and 113 in the β_2 receptor	43
Chimeric receptor		
ml–m2 loop i3	Directs selectivity of G protein coupling	44
m2–m3	17 amino acids of N-terminal junctions of loop i3 determine selectivity of G protein coupling	45

may be located more toward the cytoplasmic side of the membrane, so that these results suggest different sites and modes of receptor binding for agonists and antagonists. Further, agonist binding may introduce conformational changes that are as yet poorly understood. Nevertheless, because of their circular arrangement, all of the seven transmembrane helices could be involved with forming the ligand binding pocket. Binding profiles of alpha-2/beta-2 chimeric receptors indicate that the receptor selectivity may reside largely in TMD2, 3, and 7; however, serine residues in TMD4 and 5 are thought to provide hydrogen bonding for catechol ligands, and thus, also contribute to selectivity (36,46). The large size of the glycoprotein hormones, LH-FSH-CG and TSH (MW above 30,000 daltons), precludes these ligands from fitting into the small intramembrane binding pocket. The recent cloning of their respective receptor genes revealed an unusually large extracellular N terminal, which was hence postulated as the putative ligand binding domain (e.g., Ref. 47). It remains to be determined whether these receptors prefer a completely different mode of activation or whether ligand binding to the N terminal allows interaction of a small ligand domain to fit into the putative binding pocket in the membrane.

Coupling to G Proteins

The exact mechanism of receptor–G protein interactions remains unclear, since we do not understand the structural changes during receptor activation and no clear consensus sequence has emerged for the G protein binding domains. All of the cytoplasmic loops and the C terminus must be considered, but most work has concentrated on the loop i3 and the C terminus. Loops i1 and i2, which are more stringently conserved than the other cytoplasmic receptor domains, may contribute to G protein coupling. However, attempts to create receptor mutants that still bind agonists but fail to couple have been mostly unsuccessful, possibly because any changes in these two regions are deleterious to the overall receptor structure. In contrast, mutations of i3 and the C terminus have yielded receptors displaying ligand binding but no coupling (Table IV). On the basis of deletions within i3 of the beta-2 adrenergic and muscarinic receptors, it was concluded that only the junctions of i3 near the transmembrane helices are involved in coupling. We have recently found that deletion mutants of the muscarinic Hm1 receptor with only 11 and 9 amino acids at the N- and C-terminal junctions of i3 were still fully active in stimulating PI turnover, while the remainder of this large loop (Fig. 1) was dispensable. Indeed, the significance of the i3 loop remains enigmatic, as there is very little sequence conservation even among closely related receptor subtypes (Fig. 7). Selectivity of coupling to different G proteins was also shown to reside in i3, on the basis of chimeric receptor mutants. By exchanging the i3 loops of m1, which couples to PI turnover, and of m2, which inhibits adenylyl cyclase, the selectivity of m1 and m2 could be interchanged (44). Finally, point mutation of a single lysine in i3 of rhodopsin abrogated receptor coupling of transducin (48). These results strongly

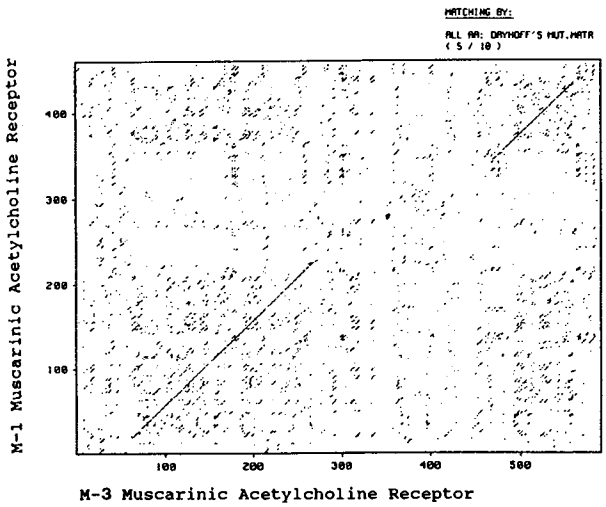


Fig. 7. Sequence comparison between muscarinic receptors Hm1 and Hm3. Matching employed Dayhoff's mutation matrix (window 5/10). Note the large stretch of no identity, which represents the large i3 loop (except for the junctions of i3 which are similar).

implicate the junctions of i3 in the coupling process, at least in determining coupling specificity to select G proteins.

The critical function of the C terminus in receptor coupling was, again, first suggested for rhodopsin. Two vicinal Cys residues in this region, conserved among the opsins, were shown to be palmitoylated, suggesting the existence of one additional cytoplasmic loop if this site is anchored into the membrane (30). More importantly, these palmitoyl moieties appear to be hydrolyzed upon photoactivation of the receptor, resulting in increased susceptibility of the C terminus to peptidase cleavage and to phosphorylation by receptor kinases (see below, Desensitization). One of these Cys residues is universally conserved among the cloned GPC-Rs, and palmitoylation has been recently documented for the beta-2 adrenergic receptor (38). Further, point mutation of this Cys residue yielded a receptor with little coupling activity, thereby indicating that it plays an important role in the coupling process (38). The equivalent Cys in Hm1 is located in position 435 (Fig. 1, arrow). It remains to be shown whether deacylation of the C terminus represents a critical event which allows the receptor to couple and to interact with additional components of the G protein–receptor complex.

The discovery of an unrelated protein that also couples to and activates the major G protein present in neuronal growth cones, G_o, sheds new light on the receptor domain that may directly activate G proteins. Strittmatter *et al.* (49) have shown that GAP-43, a growth-associated protein, not only activates G_o, but also contains a C-terminal sequence similar to the C-terminus domain of GPC-Rs containing the palmitoylated Cys (GAP-43, MLCCMRR; Hm1, LLCRWKR). GAP-43 appears to be anchored to the membrane solely through the palmitoylated Cys residue, and its 20-amino acid terminal peptide fragment is equally capable of activating G_o (49). These results support the hypothesis that the C terminus of the GPC-Rs may be similarly involved in G protein activation.

Receptor Regulation

Modulation of receptor activity can occur either through changes in gene transcription and translation or by post-translational events. Very little is known about the promoter and enhancer regions that control GPC-R transcription, although the regulatory elements of each gene are likely candidates for genetically determined abnormal gene activity. Mutation of a single promoter element upstream of several opsin genes led to the inactivation of these genes and to monochromasie (double color blindness) (6). However, the promoter regions of other GPC-Rs remain largely unexplored.

In contrast, the regulation of receptor activity at the cell membrane has been studied in detail. We differentiate several distinct processes, namely, desensitization (inability of the receptor to couple), internalization (removal of active or desensitized receptor from the cell surface to the interior, with possible recycling of intact receptor to the surface), and down-regulation (permanent loss of receptors from the cell). Other processes may include receptor aggregation and patch formation, possibly as a prelude to receptor mediated endocytosis, and receptor sequestration. In the latter process, receptors may show a high affinity for agonists but fail to couple; hence, sequestration is equivalent to desensitization. There are vast differences in how the various GPC-Rs are regulated, ranging from the dopamine D2 or the adrenergic α_2 receptors (46), which are largely resistant to regulation in some tissues (50), to the beta-adrenergic receptors, which rapidly desensitize upon agonist activation and subsequently internalize and down-regulate more slowly. Other receptors again rapidly internalize, such as the muscarinic cholinergic receptors (51) and the VIP receptor (52). In the latter cases, it may often be difficult to determine whether desensitization occurs simultaneously, because internalization should also terminate functional coupling.

Rhodopsin again served as the model for subsequent studies on the desensitization of neurotransmitter and hormone receptors. Upon light activation, rhodopsin not only activates transducin, and hence, cGMP phosphodiesterase, but also becomes a substrate for a rhodopsin-specific kinase which phosphorylates multiple serine and threonine residues in its C terminus (53). Phosphorylation of Ser and Thr appears to be a crucial step in receptor desensitization; however, yet another protein of 48 kD, termed arrestin, is required to suppress active receptor coupling (Ref. 54 and references therein). Recent evidence points to the possibility that depalmitoylation of the Cys residues in the C terminal may be required to allow phosphorylation, and hence desensitization, to proceed (30). Analogous processes have now been confirmed by Caron, Lefkowits and associates for the beta adrenergic receptor and other GPC-Rs (38). The beta-adrenergic receptor kinase, bARK, was first thought to be specific for the beta-adrenergic receptor, but it was soon shown to phosphorylate also muscarinic receptors, the somatostatin receptor, and others (55). Despite this lack of receptor selectivity, the receptor kinase mediates homologous desensitization, since only the agonist-activated receptor serves as an efficient substrate. Mutational replacement of multiple Ser and Thr residues in the C terminus of the beta-2 receptor indeed abolishes the rapid receptor desensi-

tization, supporting the hypothesis that homologous desensitization proceeds via receptor kinase mediated phosphorylation (37). Further, a 48-kD protein akin to arrestin which is necessary to inactivate the phosphorylated receptor has been identified (54), again emphasizing the close similarity of the visual pigments and the other GPC-Rs.

Heterologous receptor desensitization, i.e., the desensitization of receptors other than the agonist-activated receptor, could occur via several kinases, including cAMP-dependent protein kinase A or diacylglycerol-activated protein kinase C (51). Point mutation of Ser and Thr residues in loop i3 of the beta-adrenergic receptor, having appropriate phosphorylation consensus sequences, suppresses kinase A-mediated desensitization (37). The complex interplay among these different signaling pathways has been shown to depend upon the receptor types involved and, equally important, on the tissue characteristics. Stimulating cAMP or PI turnover may enhance or inhibit the other pathway, a notable example being the dramatic increase in cAMP levels by beta-2 receptor stimulation with simultaneous alpha-1 activation (PI turnover) in pineal gland tissue (21). Conversely, muscarinic receptors are desensitized following kinase C activation by phorbol esters (51).

Rapid receptor internalization and down-regulation are less understood. The beta-2 adrenergic receptor mutants lacking S and T residues still internalize at similar rates to the wild-type receptor, although desensitization is deficient (37), indicating that the two processes are distinct. Recently, tyrosine residues in the C terminal of human beta-2 receptor were shown to be essential for its slow down-regulation (56). For the muscarinic cholinergic receptors which undergo rapid internalization, receptor phosphorylation by kinase C has been proposed to initiate internalization (51). The muscarinic receptors contain a much larger i3 loop and a shorter C terminus than the adrenergic receptors, and both processes may be mediated at least in part by receptor domains in i3. Although the deletion of a large portion of i3 of muscarinic receptor Hm1 did not affect receptor internalization in a recent study (57), we have found that even greater deletion to within 11 amino acids of the N-terminal junction of i3 yielded a mutant Hm1 receptor which still activated PI turnover when transfected into human embryonic kidney cells U293 but failed to internalize rapidly (58). These results suggest that a receptor domain near the C-terminal junction of i3 participates in receptor internalization.

At this point it is not known what cellular pathway is responsible for GPC-R internalization. Receptor-mediated endocytosis requires an interaction of receptor domains with the proteins associated with coated pits, e.g., the clathrins and assembly polypeptides; however, detailed studies on the mechanism of GPC-R internalization are lacking.

DRUG DEVELOPMENT

The ability to clone the individual receptor subtypes and express them in mammalian target tissue will prove a boon to the development of more selective drugs, as agonists or antagonists, that fit into the ligand binding pocket. However, we now recognize many functional domains of the GPCRs. The recent finding that G proteins can be activated by short peptides derived from the GAP-43 protein of the neuronal growth cone (49) suggests a novel class of agents that mimic the receptor (receptomimetics), rather than stimulate it. Similarly, the bee venom, mastoparan, is capable of activating G proteins directly, possibly by mimicking a critical receptor-G protein contact site (59). Further, Takemoto *et al.* (60) found that synthetic peptides from the C terminal of rhodopsin inhibit the GTPase activity of transducin, while allosteric nonpeptide inhibitors of R-G interactions or G protein function were described by Huang *et al.* (61) and Ramkumar and Stiles (62). Although structural models for the G proteins have been proposed, the exact nature of the receptor–G protein interaction remains unknown, and more work is needed to exploit receptomimetics as drugs.

Another potential drug target is receptor desensitization or internalization. As many of the GPCRs rapidly desensitize or internalize upon agonist activation, blockers of these processes could greatly potentiate or prolong agonist action. Such strategies may be crucial to the successful treatment of chronic mental disorders, such as Parkinsonism and Alzheimer's disease. However, targeting the interaction among receptors and the many proteins that regulate receptor function requires a greater knowledge of the mechanism of these processes at the molecular level.

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