JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Cell and Molecular Biology

Research Article

G-Protein Coupled Receptors (Rhodopsin-Like Receptors-Visual Signal)

Bekesho Geleta[*1]

[1]*Directorate of Traditional and Modern Medicine Research, Ethiopian Public Health Institute, P.O Box 1242, Addis Ababa, Ethiopia*

**Corresponding author: Dr. Bekesho Geleta, Directorate of Traditional and Modern, Medicine Research, Ethiopian Public Health Institute, P.O Box 1242, Addis Ababa, Ethiopia, Tel: +251911091969; Email: bekeshog@gmail.com*

Received: 10-03-2016

Accepted: 11-02-2016

Published: 10-06-2017

Abstract

G-protein coupled receptor is a multi-functional receptor that has numerous clinical implications. It is physiologically important in maintaining homeostasis, in particular via their ability to mediate responses to circulating hormones and neurotransmitter input in the central, peripheral and autonomic nervous systems. The cloning and characterization of GPCR and of components involved in mediating receptor responses and in regulating receptor expression has provided a number of new insights, because the ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis. Nevertheless, significant progress has been made in the last few years by many investigators. Further clarification of the precise role of GPCR in different biological context will likely lead to new and novel therapeutic strategies for various diseases such as cancer, autoimmunity, and diabetes. In addition, further insights into GPCR biology may reveal novel, unexpected therapeutic targets that influence the GPCR life cycle or "ligand directed signaling". The existence of a large number of orphan GPCRs provides a treasure trove of possibilities. The rhodopsin is the major family of the GPCRs. Unlike others it is activated by light not by the binding of the ligand to the receptor. The light activated transducin is a reason for the scarcity of the cGMP which is a reason for the close of Na^+ channel and hyperpolarization of the membrane.

Keywords: GPCR; Rhodopsin; Transducin

Abbreviations

GPCR: G-Protein Coupled Receptor;
GPLR: G-Protein Linked Receptor;
cGMP: Cyclic Guanine Monophosphate;
TM: Transmembrane;
GTP: Guanine Triphosphate;
cAMP: Cyclic Adenosine Monophosphate;
MCH: Melanin-Concentrating Hormone;
SWS: Slow Wave Sleep;
REM: Rapid Eye Movement

Introduction

Transmembrane signaling plays a critical role in biology, allowing cells to sense and respond to the surrounding environment. In humans, one of the largest classes of transmembrane signaling proteins is the G protein-coupled receptors family, which comprises approximately 800 members [1]. GPCRs, also known as 7TM domain receptors, heptahelical receptors, serpentine receptor, and GPLRs [2].

GPCRs are a large class of integral membrane proteins involved in regulating virtually every aspect of human physiology and are among the most important targets in the treatment of disease. Owing to the profound biomedical importance of GPCRs, a high priority has long been placed on elucidation of the GPCR structure and its relationship to receptor function. However, until recently high resolution structural information was available for only a single GPCR, bovine rhodopsin. In addition to the increasing diversity of GPCRs for which structures have been determined, two of them, rhodopsin and the β2 adrenergic receptor [1].

GPLRs act indirectly to regulate the activity of a separate plasma-membrane-bound target protein, which can be either an enzyme or an ion channel. The interaction between the receptor and this target protein is mediated by a third protein, called a trimeric GTP-binding protein (G protein). The activation of the target protein can change the concentration of one or more intracellular mediators (if the target protein is an enzyme), or it can change the ion permeability of the plasma membrane (if the target protein is an ion channel). The intracellular mediators affected act in turn to alter the behavior of yet other signaling proteins in the cell. All of the GPLRs belong to a large family of homologous, seven-pass TM proteins [3].

GPCRs constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. They are among the largest and most diverse protein families in mammalian genomes. GPCRs transducer extracellular stimuli to give intracellular signals through interaction of their intracellular domains with heterotrimeric G proteins. The diversity of GPCRs is dictated both by the multiplicity of stimuli to which they respond, as well as by the variety of intracellular signaling pathways they activate. These include light, neurotransmitters, odorants, biogenic amines, lipids, proteins, amino acids, hormones, nucleotides, chemokines and, undoubtedly, many others. In addition, there are at least 18 different human Gα proteins to which GPCRs can be coupled. These Gα proteins form heterotrimeric complexes with Gβ subunits, of which there are at least 5 types, and Gγ subunits, of which there are at least 11 types [4].

GPCRs are found only in eukaryotes, including yeast, plants, choanoflagellates, and animals. GPCRs are involved in many diseases, and are also the target of almost half of all modern medicinal drugs [2].

Classification

Estimates of the number of GPCRs in the human genome vary widely. GPCRs are grouped into 6 classes based on sequence homology and functional similarity.

Class 1 (or A) (Rhodopsin-like)
Class 2 (or B) (Secretin receptor family)
Class 3 (or C) (Metabotropic glutamate/pheromone)
Class 4 (or D) (Fungal mating pheromone receptors)
Class 5 (or E) (cAMP receptors)
Class 6 (or F) (Frizzled/Smoothened)

In a recent analysis of the GPCRs in the human genome, more than 800 GPCRs were listed. Of this total, 701 were in the rhodopsin family (type A) and, of these, 241 were non-olfactory [4].

The very large rhodopsin A, group is further sub divided into 19 subgroups (A1-A19). An alternative classification system has been proposed recently called GRAFS (Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, and Secretin) [2].

GPCR Ligands

These are integral membrane proteins that respond to an extracellular signal and couple that signal with biochemical activities within cells, most often through heterotrimeric G proteins. For most GPCRs, the external signal is a small molecule that binds to and causes a conformational change in the protein spanning the membrane. This results in binding and activation of G-proteins on the intracellular surface of the receptor [5].

The three types of ligands are agonists are ligands which shift the equilibrium in favor of the active state; inverse agonists are ligands which shift the equilibrium in favor of inactive states and neutral antagonists are ligands which do not affect the equilibrium [6].

The first step in signal transduction is ligand binding. The nature of GPCR ligand-binding sites is best studied by a combination of site-directed mutagenesis, molecular modelling of the receptors and screening of large numbers of potential ligands [4]. The ligands that bind and activate these receptors include light-sensitive compounds, pheromones, neurotransmitters, odors, and hormones, and these vary in size from small molecules to peptides to large proteins [2].

GPCR is activated by an external signal in the form of a ligand or other signal mediator. This creates a conformational change in the receptor, causing activation of a G protein. Further effect depends on the type of G protein.

Signal Transduction

Signal transduction refers to any process by which a cell converts one kind of signal or stimulus into another, most often involving ordered sequences of biochemical reactions inside the cell, that are carried out by enzymes and linked through second messengers resulting in what is thought of as a "second messenger pathway".

Signal Transduction by GCPR: Step-1: G-Protein Activation

(A) In the unstimulated state, the receptor and the G protein are both inactive. Although they are shown here as separate entities in the plasma membrane, in some cases, at least, they are associated in a preformed complex.

(B) Binding of an extracellular signal to the receptor changes the conformation of the receptor, which in turn alters the conformation of the G protein that is bound to the receptor.

(C) The alteration of the α subunit of the G protein allows it to exchange its GDP for GTP. This causes the G protein to break up into two active components an α subunit and a βγ complex, both of which can regulate the activity of target proteins in the plasma membrane. The receptor stays active while the external signal molecule is bound to it, and it can therefore catalyze the activation of many molecules of G protein.

Signal Transduction by GCPR: Step-2: Receptor Activation

The activated G protein subunits detach from the receptor and initiate signaling from many downstream effector proteins, including phosphodiesterases and adenylyl cyclases, phospholipases, and ion channels that permit the release of second messenger molecules such as cAMP, cGMP, IP3, diacylglycerol (DAG), and Ca^{2+}.

Signal Transduction by GCPR: Step-3: Second Messengers

Intracellular signal transduction is largely carried out by second messenger molecules like cAMP. Second messenger molecules initiates other pathways like gene regulation, metabolism etc (Table 1) [3].

Physiological Roles of GPCRs and its Impact in Clinical Medicine

GPCRs are involved in a wide variety of physiological processes. Some examples of their physiological roles include:

Physiological Roles of GPCRs

Regulation of immune system activity and inflammation: chemokine receptors bind ligands that mediate intercellular communication between cells of the immune system; receptors such as histamine receptors bind inflammatory mediators and engage target cell types in the inflammatory response.

Family	Some Family Members	Action Mediated by	Function
I	G_s	α	activates adenylyl cyclase;
			activates Ca2+ channels
	G_{olf}	α	activates adenylyl cyclase in olfactory
			sensory neurons
II	G_i	α	inhibits adenylyl cyclase
		βγ	activates K+ channels
	G_o	βγ	activates K+ channels;
			inactivates Ca2+ channels
		α and βγ	activates phospholipase C-β
	G_t	α	activates cyclic GMP phosphodiesterase in vertebrate rod photoreceptors
III	G_q	α	activates phospholipase C-β

Table 1. Three Major Families of Trimeric G Proteins

Cell density sensing: A novel GPCR role in regulating cell density sensing.

The sense of smell: receptors of the olfactory epithelium bind odorants (olfactory receptors) and pheromones (vomeronasal receptors).

The visual sense: the opsins use a photo isomerization reaction to translate electromagnetic radiation into cellular signals. Rhodopsin, for example, uses the conversion of 11-cis-retinal to all-trans-retinal for this purpose.

Autonomic nervous system transmission: both the sympathetic and parasympathetic nervous systems are regulated by GPCR pathways, responsible for control of many automatic functions of the body such as blood pressure, heart rate, and digestive processes.

Behavioral and mood regulation: receptors in the mammalian brain bind several different neurotransmitters, including serotonin, dopamine, GABA (Gamma amino butyric acid).

Impact of GPCRs in Clinical Medicine: Genetic Variants and Drug Targets

Obesity, Cardiovascular Disease, Inflammation, Cancer, Diabetes and Alzheimer's disease are the diseases which have association with GPCR (Table 2) [7].

Diseases – GPCRs

Disease	Overactive known GPCRs
Schizophrenia	5HT2A, D4
Depression	5HT2A
Hyperthyroidism	Thyrotropin
Hypertension	Angiotensin AT1A
Asthma	Adenosine A1
Melanoma	MC-1
Retinitis Pigmentosa	Rhodopsin

Table 2. The diseases associated with the GPCRs

Widespread distribution and important roles in cell physiology and biochemistry, GPCR play multiple important roles in clinical medicine. Most important are:

• Monogenic diseases of GPCR

• Genetic variants of GPCR

• Clinically useful pharmacological agonists and antagonists of GPCR.

Diseases involving mutations of GPCR are rare, occurring in <1/1000 people, but disorders in which antibodies are directed against GPCR is more common. Genetic variants, especially single nucleotide polymorphisms (SNP), show substantial heterogeneity in frequency among different GPCRs but have not been evaluated for many therapeutic agonists and antagonists target GPCR and show inter-subject variability in terms of efficacy and toxicity.

Monogenic Diseases of GPCR

Monogenic diseases and genetic variants associated with those diseases are generally quite rare, occurring in <1% of the population and often variably among subjects of different ethnicities.

Since GPCR comprise ~3% of the human genome, it is perhaps not surprising that nonlethal mutations can occur in GPCR, especially those that are expressed in sensory and hormonal systems, where they serve as mediators of information transfer from the extracellular environment to the cell interior. One such critical action is in the visual system where rhodopsin in photoreceptor-capturing neurons, retinal rods and color (red, blue and green) opsins in retinal cones, transduce the input from photons of light into electrical impulses that then travel to the brain and are decoded. A large number of monogenic mutations have been identified in rhodopsin, in particular in patients that have the disease retinitis pigmentosa; in addition, a number of hormonally responsive GPCR have been identified as pathologic entities in a variety of endocrine disorders (Table 3) [2].

Receptor/Gene Name	Mutation	Disease
Calcium-Sensing (CaS)/CaSR	Multiple (e.g. Arg185Gln)	Autosomal Dominant Hypocalcemia (ADH) Sporadic Hypoparathyroidism Familial Hypoparathyroidism
CXCR4	Multiple (e.g. Ser338X)	WHIM syndrome
Endothelin receptor B (ETB)/EDNRB	Multiple (e.g. Trp276Cys)	Hirschsprung's disease
Follicle-stimulating hormone (FSH)/FSHR	Multiple (e.g. Ala189Val)	Female infertility
N-formyl-peptide (FPR)/FPR1	Phe110Ser, Cys126Trp	Juvenile periodontitis
Frizzled (FZD4)/FZD4	Multiple (e.g. Arg417Gln)	Familial exudative vitreoretinopathy(FEVR)
Gonadotropin-releasing Hormone (GnRH)/GNRHR	Multiple (e.g. Arg262Gln)	Hypogonadotropic hypogonadism (HH)
GPR54/GPR54	Multiple (e.g. Cys223Arg)	Hypogonadotropic hypogonadism (HH)
GPR56/GPR56	Multiple (e.g. Cys223Arg)	Bilateral frontoparietal polymicrogyria (BFPP)
vGPCR/KSHV-GPCR	(constitutively active)	Kaposi's sarcoma (KS)
Relaxin family peptide receptor 2 (RXFP2)/LGR8	Multiple (e.g. Thr222Pro)	Cryptorchidism
MASS1 (also called VLGR1, USH2C)/MASS1	Multiple (e.g. Ser2652X))	Usher syndrome Febrile seizures (FS)
Melanocortin (MC4)/MC4R	Multiple (e.g. Pro78Leu)	Dominant and recessive obesity
Rhodopsin/RHO	Multiple (e.g. Pro23His)	Retinitis pigmentosa (RP)
Vasopressin receptor (V2)/AVPR2	Multiple (e.g. Arg113Trp)	Nephrogenic diabetes insipidus (NDI)

Table 3. Examples of Rare Mutants of GPCR that Cause Human Diseases

Genetic Variants of GPCR

Genetic variants/polymorphisms identified in GPCRs can influence receptor expression, targeting, function, and receptor turnover; as well as the ability of receptors to recognize and respond to pharmacologic agents. Below we describe selected GPCRs with polymorphisms involved in human diseases, in addition to elucidating their potential for serving as future therapeutic targets (Table 4) [2].

Receptor	Polymorphisms	Examples of Disease Associations
β1 Adrenergic receptor	Arg389Gly	Heart failure
β2 Adrenergic receptor	Multiple	Hypertension, Asthma
β3 Adrenergic receptor	Trp64Arg	Obesity
CC chemokine receptor 2 (CCR2)	Val64Ile	Delayed progression of AIDS
CC chemokine receptor 5 (CCR5)	Multiple	Associated with progression of AIDS
Dopamine receptor 2 (D2)	3'UTR52A/G	Associated with depression and Anxiety
Dopamine receptor 3 (D3)	Ser9Gly, Promoter SNPs	Haplotype associated with Schizophrenia
Muscarinic receptor subtype 3(M3)	Promoter haplotype	Possible association with asthma and atopy
Neuropeptide S receptor (NPSR; also called GPR154,GPRA)	Haplotypes H1, H5 Asn107Ile, rs324981	Asthma susceptibility
P2Y12	CA deletion at Codon 240	Associated with bleeding diathesis

Table 4. Examples of Polymorphisms of GPCR Associated with Human Diseases.

Clinically Useful Pharmacological Agonists and Antagonists of GPCR

GPCR polymorphisms can affect functional responses to some but not other ligands, e.g. with β3- adrenergic receptors. Such concepts may apply not only to acutely measured GPCR responses but also to receptor regulation, especially because certain GPCR polymorphisms alter such regulation. In the following we illustrate these principles based upon examples from several drug classes acting on GPCR that are frequently used in clinical medicine (Table 5) [2].

Rhodopsin, a G protein-Coupled Receptor

Rhodopsin is the visual pigment mediating vision at night and/or dim-light. It consists of five exons and four introns. Its protein-coding sequence is composed of approximately 1044 nucleotides, hence, 348 amino acids. There are seven TM helices (TM), which are embedded in the membrane and encompass 194 amino acids in total [8]. The human retina contains two types of photoreceptors, rods and cones, which are the primary recipients of visual stimulation. Cones are involved in color vision, while rods are stimulated by weak light like moonlight over a range of wavelengths. Rhodopsin, a GPCR that is activated by light, is localized to the thousand or so flattened membrane disks that make up the outer segment of rod cells [9].

Packed in the crystal lattice to form an array of helical tubes, the extracellular surface domain comprises an amino-terminal tail and three interhelical loops; the cytoplasmic domain comprises a carboxyl-terminal tail and three cytoplasmic loops. Rhodopsin is temperature-sensitive. With an isoelectric point at pH 5.43, rhodopsin is an acidic protein; it has more glutamic and aspartic acid than basic lysine and arginine residues. It is ascertained that the chromophore is covalently bound to the opsin at the highly conserved Lys296, but which residues participate in holding the 11-cis retinal inside the binding pocket before photoisomerization is still debated [8].

Rhodopsin Family

The rhodopsin-like family encompasses receptors for a large variety of stimuli, such as biogenic amine neurotransmitters, neuropeptides, peptide hormones, light, nucleotides, pros

Receptor	Drugs and Some Key Indications	Polymorphism	Relevance
AT1 angiotensin II receptor	Antagonists (e.g. losartan) in the treatment of essential hypertension or congestive heart failure	A1166C SNP in untranslated part of exon 5	Inconclusive data for drug responses where multiple studies have been done
α1 Aadrenergic receptor	Antagonists (e.g. tamsulosin) to treat micturition (bladder emptying) disorders associated with enlarged prostate glands	C1475T	Short- and long-term antagonist effects apparently not affected
β1- adrenergic receptor	Antagonists (e.g. propranolol, atenolol, metoprolol, carvedilol) to treat essential hypertension or congestive heart failure	Ser49Gly Arg389Gly	Arg389 linked to increased antagonist effect
β2- adrenergic receptor	Agonists (e.g. terbutaline, salbutamol,formoterol,salmeterol) for treatment of obstructive airway disease or premature labor	Arg16Gly Gln27Glu Thr164Ile	Possibly reduced responses with Ile164 otherwise no consistent association with drug responsiveness
D2 dopamine receptor	Antagonists (e.g. haloperidol and clozapine) to treat schizophrenia Agonists (e.g. levodopa) for the treatment of Parkinson's disease	-141C Ins/Del Taq1A	Reduced antagonist response with Del or homozygous A2 allele No consistent associations with therapeutic response or side effects of agonists
D3 dopamine Receptor	Antagonists (e.g. haloperidol) in the treatment of schizophrenia	Ser9Gly	Increased risk of tardive dyskinesia with Gly allele
5-HT2A Receptor	Antagonists (e.g. clozapine) to treat schizophrenia Indirect agonists (e.g.fluvoxamine) for the treatment of depression	T102C	Reduced response to clozapine with Callele Possibly reduced response to agonists with homozygous T allele

Table 5. GPCR as Drug Targets: Some Examples of the Impact of Receptor Polymorphisms.

taglandins, leukotrienes, chemotactic peptides, and chemokines. Although their ligands vary considerably in structure, the rhodopsin-like GPCRs show sequence conservation within their seven putative TM domains [10]. The Rhodopsin family is made up of the largest number of receptors with about 278 non-olfactory receptors. In addition there are at least 347 olfactory receptors and another 11 receptors currently placed in the "Other" group that are likely to be of Rhodopsin type and this adds up to 636 known Rhodopsin GPCRs. The crystal structure of bovine rhodopsin has been revealed and this is the only animal GPCR that has had its exact structure determined. Therefore bovine rhodopsin has frequently been used as a template for modeling the structure of other GPCRs from the rhodopsin family. It should be noted that bacterio-rhodopsin, which has also had its three-dimensional structure determined, has no sequence similarity with the GPCRs in the human genome. The ligands for most of the rhodopsin receptors bind within a cavity between the TM regions. There are however important exceptions to this, in particular for the glycoprotein binding receptors (luteinizing hormone, follicle stimulating hormone, thyroid stimulating hormone), where the ligand-binding domain is in the N-terminal [11]. The importance of GPCRs in biological processes and their roles as drug targets have promoted interest in the rhodopsin structure as a model for understanding ligand binding and signaling processes in this class of receptors [12]. Rhodopsin is a typical GPCR, except that its initial signal is not connected with binding of a small molecule ligand. In other GPCRs, the photo-induced conformational change in retinal is replaced by a ligand binding event. For many GPCRs, the ligand is believed to bind in a site similar to that of retinal in rhodopsin. Binding of a small molecule to its specific GPCR initiates the conformational change in the protein that is then coupled to its G-protein [5].

The Rhodopsin alpha-Group

This is the largest of the four main groups in the Rhodopsin family with 101 members in total.

It includes:

- The Prostaglandin Receptor Cluster
- The Amine Receptor Cluster
- The Opsin Receptor Cluster

The Melatonin Receptor Cluster

- The MECA Receptor Cluster
- Other Rhodopsin alpha-Receptors

The Rhodopsin beta-Group

The beta-group in the Rhodopsin family is not subdivided further into named sub branches. All the known ligands to the receptors in this cluster are peptides. The group includes the branch containing orexin-, neuropeptide FF-, tachykinin-, cholecystokinin-, prolactin-releasing hormone receptor and the neuropeptide Y receptors.

The Rhodopsin gamma-Group

This group comprises three main clusters termed the super family of GPCR, melanin concentrating hormone receptor, and Chemokine receptor clusters.

Post-translational prenylation of the carboxyl-terminal cysteine is a characteristic feature of the guanine nucleotide-binding protein (G protein) gamma subunits. Recent findings show that the farnesylated COOH-terminal tail of the gamma 1 subunit is a specific determinant of rhodopsin-transducin coupling. We show here that when synthetic peptides specific to the COOH-terminal tail of gamma 1 are chemically modified with geranyl, farnesyl, or geranylgeranyl groups and tested for their ability to interact with light activated rhodopsin, the farnesylated peptide is significantly more effective. These results show that an appropriate isoprenoid on the G protein gamma subunit serves not only a membrane anchoring function but in combination with the COOH-terminal domain specifies receptor-G protein coupling [13].

The Super-Family of GPCR Cluster

This cluster of receptors contains the somatostatin, opioid, galanin, and neuropeptide receptors

The Melanocyte Concentrating Hormone Receptor Cluster

MCH, a neuropeptide expressed in central and peripheral nervous systems, plays an important role in the control of feeding behaviors and energy metabolism. It regulates feeding and complex behaviors in mammals and fish. MCH is a key regulator of food intake and homeostasis [14-17]. Neurons containing the neuropeptide MCH are mainly located in the lateral hypothalamus and the incerto-hypothalamic area, and have widespread projections throughout the brain. While the biological functions of this neuropeptide are exerted in humans through two metabotropic receptors, the MCHR1 and MCHR2, only the MCHR1 is present in rodents. Recently, it has been shown that the MCHergic system is involved in the control of sleep. Different experimental findings summarized the major roles of MCH as follows: (1) The areas related to the control of sleep and wakefulness have a high density of MCHergic fibers and receptors. (2) MCHergic neurons are active during sleep, especially during REM sleep. (3) MCH knockout mice have less REM sleep, notably under conditions of negative energy balance. Animals with genetically inactivated MCHR1 also exhibit altered vigilance state architecture and sleep homeostasis. (4) Systemically administered MCHR1 antagonists reduce sleep. (5) Intraventricular microinjection of MCH increases both SWS and REM sleep; however, the increment in REM sleep is more pronounced. (6) Microinjection of MCH into the dorsal raphe nucleus increases REM sleep time. REM seep is inhibited by immunoneutralization of MCH within this nucleus. (7) Microinjection of MCH in the nucleus pontis oralis of the cat enhances REM sleep time and reduces REM sleep latency. All these data strongly suggest that MCH has a potent role in the promotion of sleep. Although both SWS and REM sleep are facilitated by MCH, REM sleep seems to be more sensitive to MCH modulation [16]. An orphan G protein-coupled receptor (SLC-1/GPR24) has recently been identified as a receptor for MCH (MCHR1). Full understanding of MCH biology is important for the development of potential therapeutics for diseases involving MCH, including obesity [14].

The Chemokine Receptor Cluster

This cluster is by far the largest in the Rhodopsin gamma-group.

Other Rhodopsin gamma-Receptors

Post-translational prenylation of the carboxyl-terminal cysteine is a characteristic feature of the guanine nucleotide-binding protein (G protein) gamma subunits. Previous findings show that the farnesylated COOH-terminal tail of the gamma 1 subunit is a specific determinant of rhodopsin-transducin coupling. Other previous findings show that when synthetic peptides specific to the COOH-terminal tail of gamma 1 are chemically modified with geranyl, farnesyl, or geranylgeranyl groups and tested for their ability to interact with light activated rhodopsin, the farnesylated peptide is significantly more effective. These results show that an appropriate isoprenoid on the G protein gamma subunit serves not only a membrane anchoring function but in combination with the COOH-terminal domain specifies receptor-G protein coupling [12].

The Rhodopsin σ-Group

This group has five main branches. These are:

- The MAS-related Receptor Cluster
- The Glycoprotein Receptor Cluster
- The Coagulation Factor Receptor Cluster
- The Purinergic Receptor Cluster
- The Olfactory Receptor Cluster
- Other Rhodopsin alpha-Receptors [10].

Mechanism of Photo-Transduction by Rhodopsin

There are three mechanisms:

• *hv* discrimination & absorbance

• Receptor activation

• G-protein activation

1 photon

↓

1 rhodopsin activated

↓

500 transducin molecules activated

↓

1 cGMP phosphodiesterase activated per transducin

↓

1 cGMP phosphodiesterase activated per transducin

↓

1000 cGMP/s cleaved per phosphodiesterase [18].

In the vision system, rhodopsin and the cone opsins respond to light. The receptors are located in the outer segments of the cone and rod cells in the retina. Rhodopsin resides within internal membrane structures called disc membranes that are found in the rod outer segments of the retinal photoreceptors [5]. The trimeric G protein coupled to rhodopsin, often called transducin (Gt), is found only in rod cells [9]. The activating extracellular signal, however, is not a molecule but a photon of light. Each rhodopsin molecule contains a covalently attached chromophore, 11-*cis* retinal, which isomerizes almost instantaneously to all-*trans* retinal when it absorbs a single photon. The isomerization alters the shape of the retinal, forcing a conformational change in the protein (opsin) [3]. The resulting form in which opsin is covalently bound to all-trans-retinal is called metarhodopsin II, or activated opsin [9]. This alters the interactions between the retinal and the polypeptide. These changes cause a re-packing of the TM helices and result in changes on the surface of the protein facing the cytoplasm of the cell. This cytoplasmic surface is where the rhodopsins and cone opsins interact with the G-protein, transducin, and activate it [5].

In dark-adapted rod cells, a high level of cGMP keeps nucleotide-gated nonselective cation channels open. Light absorption generates activated opsin (Step-1), which binds inactive GDP-bound Gt protein and mediates replacement of guanine diphosphate (GDP) with GTP (Step-2). The free Gt•GTP generated then activates cGMP phosphodiesterase (PDE) by binding to its alpha inhibitory subunits (Step-3) and dissociating them from the catalytic alpha and beta subunits (Step-4). Relieved of their inhibition, the alpha and beta subunits convert cGMP to guanine monophosphate (GMP) (Step-5). The resulting decrease in cytosolic cGMP leads to dissociation of cGMP from the nucleotide-gated channels in the plasma membrane and closing of the channels (Step-6). The membrane then becomes transiently hyperpolarized [9].

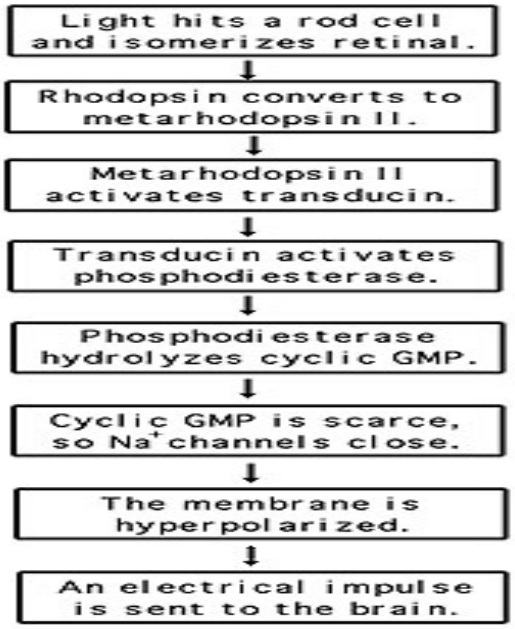

Figure 1. Operational model for rhodopsin-induced closing of cation channels in rod cells [5].

The activated rhodopsin molecule then alters the Gprotein *transducin* (Gt), causing its α subunit to dissociate and activate cyclic GMP phosphodiesterase. The phosphodiesterase then hydrolyzes cGMP, so that cyclic GMP levels in the cytosol fall. This drop in cyclic GMP concentration leads to a decrease in the amount of cyclic GMP bound to the plasma membrane Na+ channels, allowing more of these highly cGMP sensitive channels to close. In this way, the signal quickly passes from the disc membrane to the plasma membrane, and a light signal is converted into an electrical one which forms the bases for vision [3]. The more photons absorbed by rhodopsin, the more channels are closed, the fewer Na ions cross the membrane from the outside, the more negative the membrane potential becomes, and the less neurotransmitter is released. This change is transmitted to the brain where it is perceived as light [9].

Activated opsin is unstable and spontaneously dissociates into its component parts, releasing opsin and all trans-retinal, thereby terminating visual signaling. In the dark, free all-trans-retinal is converted back to 11-cis-retinal, which can then rebind to opsin, re-forming rhodopsin. In the dark, the membrane potential of a rod cell is about 30 mV, considerably less than the resting potential (60 to 90 mV) typical of neurons and other electrically active cells. As a consequence of this depolarization, rod cells in the dark are constantly secreting

neurotransmitters, and the bipolar interneurons with which they synapse are continually being stimulated. The depolarized state of the plasma membrane of resting rod cells is due to the presence of a large number of open nonselective ion channels that admit Na, and Ca, as well as K ions [9].

Activation and Deactivation of Rhodopsin

The key transducing molecule linking activated opsin to the closing of cation channels in the rod-cell plasma membrane is the second messenger cGMP. Rod outer segments contain an unusually high concentration (≈0.07 mM) of cGMP, which is continuously formed from GTP in a reaction catalyzed by guanylyl cyclase that appears to be unaffected by light. However, light absorption by rhodopsin induces activation of a cGMP phosphodiesterase, which hydrolyzes cGMP to 5-GMP. As a result, the cGMP concentration decreases upon illumination. The high level of cGMP present in the dark acts to keep cGMP-gated cation channels open; the light-induced decrease in cGMP leads to channel closing, membrane hyperpolarization, and reduced neurotransmitter release. cGMP phosphodiesterase is the effector protein for Gt. The free Gt•GTP complex that is generated after light absorption by rhodopsin binds to the two inhibitory gamma subunits of cGMP phosphodiesterase, releasing the active catalytic alpha and beta subunits, which then convert cGMP to GMP [9].

A number of mechanisms operate in rods to allow the cells to revert quickly to a resting, dark state in the aftermath of a flash of light a requirement for perceiving the shortness of the flash. A rhodopsin-specific kinase (RK) phosphorylates the cytosolic tail of activated rhodopsin on multiple serines, partially inhibiting the ability of the rhodopsin to activate transducin. An inhibitory protein called arrestin then binds to the phosphorylated rhodopsin, further inhibiting rhodopsin's activity. If the gene encoding RK is inactivated by mutation in mice or humans, the light response of rods is greatly prolonged, and the rods eventually die.

At the same time as rhodopsin is being shut off, regular G-protein signaling (RGS-protein) binds to activated transducin, stimulating the transducin to hydrolyze its bound GTP to GDP, which returns transducin to its inactive state. In addition, the Na+ channels that close in response to light are also permeable to Ca^{2+}, so that when they close, the normal influx of Ca^{2+} is inhibited, causing the Ca^{2+} concentration in the cytosol to fall. The decrease in Ca^{2+} concentration stimulates guanylyl cyclase to replenish the cyclic GMP, rapidly returning its level to where it was before the light was switched on. A specific Ca^{2+} sensitive protein mediates the activation of guanylyl cyclase in response to a fall in Ca^{2+} levels. In contrast to calmodulin, this protein is inactive when Ca^{2+} is bound to it and active when it is Ca^{2+} free. It therefore stimulates the cyclase when Ca^{2+} levels fall following a light response [3].

Summary

G-protein-coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes. These cell surface receptors act like an inbox for messages in the form of light energy, peptides, lipids, sugars, and proteins. Such messages inform cells about the presence or absence of life-sustaining light or nutrients in their environment, or they convey information sent by other cells.

GPCRs play a role in an incredible array of functions in the human body, and increased understanding of these receptors has greatly affected modern medicine. G protein-coupled receptors (GPCRs) constitute a vast protein family that encompasses a wide range of functions, including various autocrine, paracrine and endocrine processes. They show considerable diversity at the sequence level, on the basis of which they can be separated into distinct groups. G-protein coupled receptors are remarkably versatile signaling molecules. The currently known clan members include rhodopsin-like GPCRs (Class A, GPCRA), secretin-like GPCRs (Class B, GPCRB), metabotropic glutamate receptor family (Class C, GPCRC), fungal mating pheromone receptors (Class D, GPCRD), cAMP receptors (Class E, GPCRE) and frizzled/smoothened (Class F, GPCRF). GPCRs are major drug targets, and are consequently the subject of considerable research interest. he rhodopsin-like GPCRs (GPCRA) represent a widespread protein family that includes hormone, neurotransmitter and light receptors, all of which transduce extracellular signals through interaction with guanine nucleotide-binding (G) proteins. Although their activating ligands vary widely in structure and character, the amino acid sequences of the receptors are very similar and are believed to adopt a common structural framework comprising 7 transmembrane (TM) helices.

References

1. Kruse AC, Manglik A, Kobilkaa BK, Weisa WI. Applications of molecular replacement to G protein coupled Receptors. Acta Crystallographica Section D. 2013, D69: 2287-2292.

2. Marasani A, Talla V, Gottemukkala JR, Rudrapati D. Cell signaling: Role of GPCR; Scholars Research Library. Archives of Applied Science Research. 2010, 2(5): 363-377.

3. Alberts, Johnson, Lewis, Raff, Roberts et al. Molecular Biology of The Cell; 4th edition, 2002.

4. Kroeze WK, Sheffler DJ, Roth BL. G-protein-coupled receptors at a glance. Journal of Cell Science. 2003, 116(Pt 24): 4867-4869.

5. Deupi X, Kobilka B. Activation of G-Protein Coupled Receptors. Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California.

6. Latek D, Modzelewska A, Trzaskowski B, Palczewski K, Filipek S. G-protein-coupled receptors-recent advances. Acta Biochimica Polonica. 2012, 59(4): 515-529.

7. Hanson, Stevens. G-protein Coupled Receptors: Graduate Institute of Anatomy and Cell Biology 2008.

8. Bickelmann C. Visual pigment evolution and the paleobiology of early mammals.

9. Lodish, Berk, Matsudaira, Kaiser, Kreiger, et al. Molecular Cell Biology, Fifth Edition, 2003.

10. Brody T, Cravchik A. Analysis Drosophila melanogaster G Protein–coupled Receptors. The Journal of Cell Biology. 2000, 150(2): F83-F88.

11. Fredriksson R and Schiöth HB. G Protein-coupled Receptors in the Human Genome.

12. Stenkamp RE, Teller DC and Palczewski K. Crystal Structure of Rhodopsin: A G-Protein-Coupled Receptor. 2002, 3: 963-967. K

13. Kisselev O, Ermolaeva M, Gautam N. Efficient interaction with a receptor requires a specific type of prenyl group on the G protein γ subunit. Journal of Biological Chemistry. 1995, 270(43): 25356-25358.

14. An S, Cutler G, Zhao JJ, Huang SG, Tian H et al. Identification and characterization of a melanin-concentrating hormone receptor. Proceedings of the National Academy of Sciences. 2001, 98(13): 7576-7581.

15. Berman JR, Skariah G, Maro GS, Mignot E, Mourrain P. Characterization of two melanin-concentrating hormone genes in zebrafish reveals evolutionary and physiological links with the mammalian MCH system. Journal of Comparative Neurology. 2009, 517(5): 695-710.

16. Gibson WT, Pissios P, Trombly DJ, Luan JA, Keogh J et al. Melanin-Concentrating Hormone Receptor Mutations and Human Obesity: Functional Analysis. Obesity research. 2004, 12(5): 743-749.

17. Torterolo P, Lagos P, Monti JM. Melanin-concentrating hormone: a new sleep factor?. Frontiers in neurology. 2011, 2: 14.

18. Rhodopsin, a G-Protein Coupled Receptor.