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Structural and Functional Analysis of Rhodopsin- A G- Protein Coupled Receptor

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Review Article

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ABSTRACT

Cell signaling and signal transduction are very important for a cell so that it can govern basic and advanced cellular activities in a well-coordinated manner. The activities of cells include anabolic and catabolic activities, response to their microenvironment and external environment, response to various ligands either beneficial for cell or harmful for cell and other physiological activities (sight, touch, taste, pain, anxiety, anger etc). Transduction involves the binding of extracellular signaling molecules and ligands to cell-surface receptors that trigger events inside the cell.

Among the various types of receptors studied GPCRs are considered to be of utmost importance. The members of this large family of proteins are activated by a spectrum of structurally diverse ligands, and have been shown to modulate the activity of different signaling pathways in a ligand specific manner. They play a key role in regulating cell activity and physiological function. GPCR malfunction is responsible for a wide range of diseases including nephrogenic diabetes insipidus, and hyperthyroidism and a large proportion of drugs on the market target these receptors.

The knowledge of GPCRs structure and function is very important for elucidating the molecular mechanisms underlying effective signal transduction and diseases and for performing structure-based drug design. Since structural data are restricted to only a handful of GPCRs, a major population of researchers is engaged in deducing the structures of various GPCRs.

The main aim of this text is to introduce the fundamentals of GPCRs with emphasis on structure and function of rhodopsin, a well studied GPCR belonging to the rhodopsin family of GPCR and responsible for vision in vertebrates.

INTRODUCTION

The capacity of cells to communicate with one another to administer fundamental cell activities and coordinates cell activities is known as cell signaling. Biological processes require various cells to work together in a well-coordinated manner. To make this possible, cells communicate with each other, by cell signaling. Cell signaling makes it possible for cells to respond in an appropriate manner to a specific environmental stimulus.

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Types of cell signals

1. ***Autocrine signaling***- cell that is giving the signal by producing the messenger molecule also expresses receptors on its surface that can respond to that messenger. The start and end of the signal is the same cell.

2. ***Paracrine signaling***- messenger molecules travel only short distances through the extracellular space to cells that are in close proximity to the cell that is generating the message. Paracrine messenger molecules travel only short distances because they are unstable and can be degraded by enzymes.

3. ***Endocrine signaling***- messenger molecules reach their target cells via passage through the bloodstream. The start of the signal is basically a gland and the end of the signal is a distantly located cell.

4. ***Juxtacrine signaling***(contact-dependent signaling)- Two adjacent cells must make physical contact in order to communicate. This requirement for direct contact allows for very precise control of cell differentiation during embryonic development.

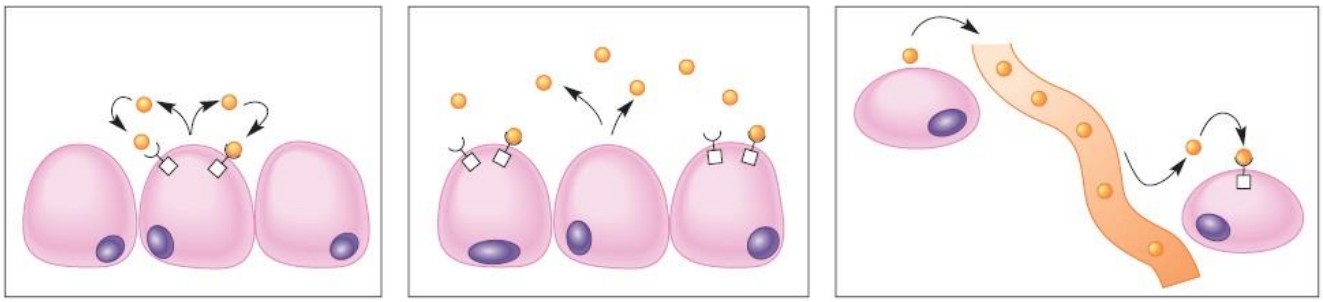

Figure 1: (a) Autocrine (b) paracrine (c) endocrine

Signal transduction occurs when an extracellular signaling molecule activates a cell surface receptor and this receptor then alters the intracellular molecules creating a response. The signal can also be amplified depending on the need of the tissue, organ or body. There are two stages in this process-

1. A signaling molecule activates a specific receptor protein on the cell membrane.
2. A second messenger transmits the signal into the cell, producing a physiological response.

Cell signaling is initiated with the release of a messenger molecule/ligand by a cell that sends messages to other cells in the body. Other cells can only respond to an extracellular message if they express receptors that specifically recognize and bind that particular messenger molecule [1]. The messenger molecule/ligand binds to a receptor at the extracellular surface of the responding cell. This interaction causes a signal to be relayed across the membrane to the receptor's cytoplasmic domain [2]. A route taken to elicit a particular response depends on the type of receptor that is activated. There are 2 major routes of signal transduction-

1. Receptor transmits a signal from its cytoplasmic domain to a nearby enzyme, which generates a second messenger. Because it brings about the cellular response by generating a second messenger, the enzyme responsible is referred to as an effector [3]. Second messengers are small substances that

typically activate (or inactivate) specific proteins. Depending on its chemical structure, a second messenger may diffuse through the cytosol or remain embedded in the lipid bilayer of a membrane [4].

2. Another type of receptor transmits a signal by transforming its cytoplasmic domain into a recruiting station for cellular signaling proteins. Proteins interact with one another, or with components of a cellular membrane, by means of specific types of interaction domains [5].

- These receptors are included in every physiological movement, including sight, taste, and hormone regulation [6]. They work as sign transmitters that can sense an immense assortment of signs and increase them inside the cells, prompting diverse cell reactions, and are included in numerous human diseases.
- Among the signals transduced are light (phototransduction), extracellular calcium ions, tastants (gustatory), odorants (smell), pheromones (mating signals), warnings (pain), immunological (chemokines), endocrine (hormones), and neural (neuromodulators and neurotransmitters) [7]. They are the richest source of targets for the pharmaceutical industry as ligands directed at GPCRs represent the largest family of pharmacological agents, accounting for nearly 30% of current clinical pharmaceutical agents available [8].
- Our understanding of GPCR structure is based largely on the high-resolution structures of the inactive state of the human β2 adrenergic receptor (β2AR), the avian β1AR, the human A2A adenosine receptor and rhodopsin [9]. Rhodopsin is better suited for structural studies than most other GPCRs because it is possible to obtain large quantities of highly enriched protein from bovine retina. Rhodopsin is a remarkably stable GPCR, retaining function under conditions that denature many other GPCRs [10] .
- Great progress has been made over the past three decades in understanding diverse GPCRs, from pharmacology to functional characterization in vivo [11]. Cryoelectron microscopy, X ray crystallography and other advanced techniques have provided insights into the molecular mechanisms of GPCR structure, activation and function [12].

Basic Structure of GPCR

G protein coupled receptors (GPCRs) represent the largest family of membrane proteins or cell- surface receptors in the human genome (approx 2%). All GPCRs are located within the plasma membrane [13] . Research using Co-immunoprecipitation and resonance energy-transfer techniques has successfully demonstrated that GPCR structures are present at the plasma membrane [14].

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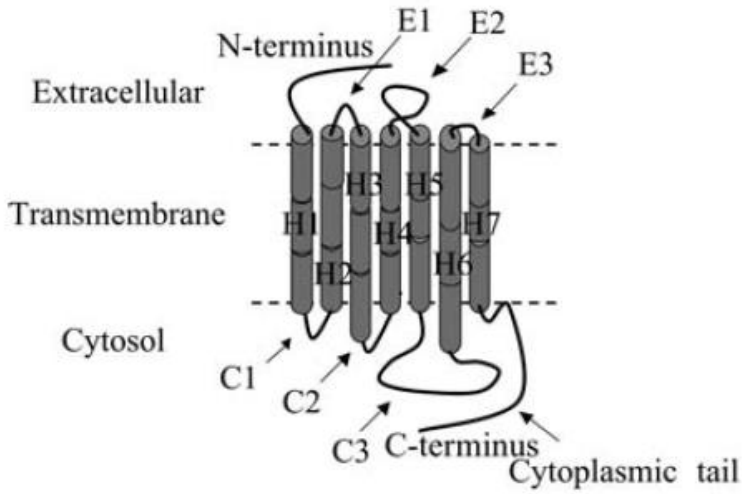

Figure 2: Structure of a GPCR.

GPCRs are cell- surface receptors capable of coupling to specific guanine binding proteins (G proteins), thereby transducing an extracellular signal to an intracellular effector. As a family of proteins, GPCRs share a common structural signature of seven hydrophobic transmembrane (TM) α-helical segments, with an extracellular amino terminus and an intracellular carboxyl terminus [15]. The helices are connected to one another by three extracellular loops E1, E2, E3 and three cytoplasmic loops C1, C2, C3. GPCRs share the greatest homology within the TM segments [16]. The variable structures among the family of GPCRs are the carboxyl terminus, the intracellular loop spanning TM5 and TM6, and the amino terminus. The greatest diversity is observed in the amino terminus. This sequence is relatively short (10–50 amino acids) for monoamine and peptide receptors, and much larger (350–600 amino acids) for glycoprotein hormone receptors, and the glutamate family receptors [17]. The largest amino terminal domains are observed in the adhesion family receptors. The extracellular loop E2 and the N-terminus segment are especially well situated for ligand binding. These structures vary in size from one class of GPCR to another [18]. The intracellular loops plus the cytoplasmic ends of the trans membrane helices participate in protein binding and activation. The cytoplasmic tail and third intracellular loop (C3), in particular, provide multiple sites for protein docking and interactions [19,20].

Examples of GPCRs with well-ascribed roles in cardiovascular biology include the β1- and β2- adrenergic receptors (ARs), the α1- and α2- ARs, the M2- and M3-muscarinic acetylcholine receptors, the angiotensin II (Ang II) receptors, the endothelin receptors, the adenosine receptor, the thrombin receptor, and the vasopressin receptor [21–25].

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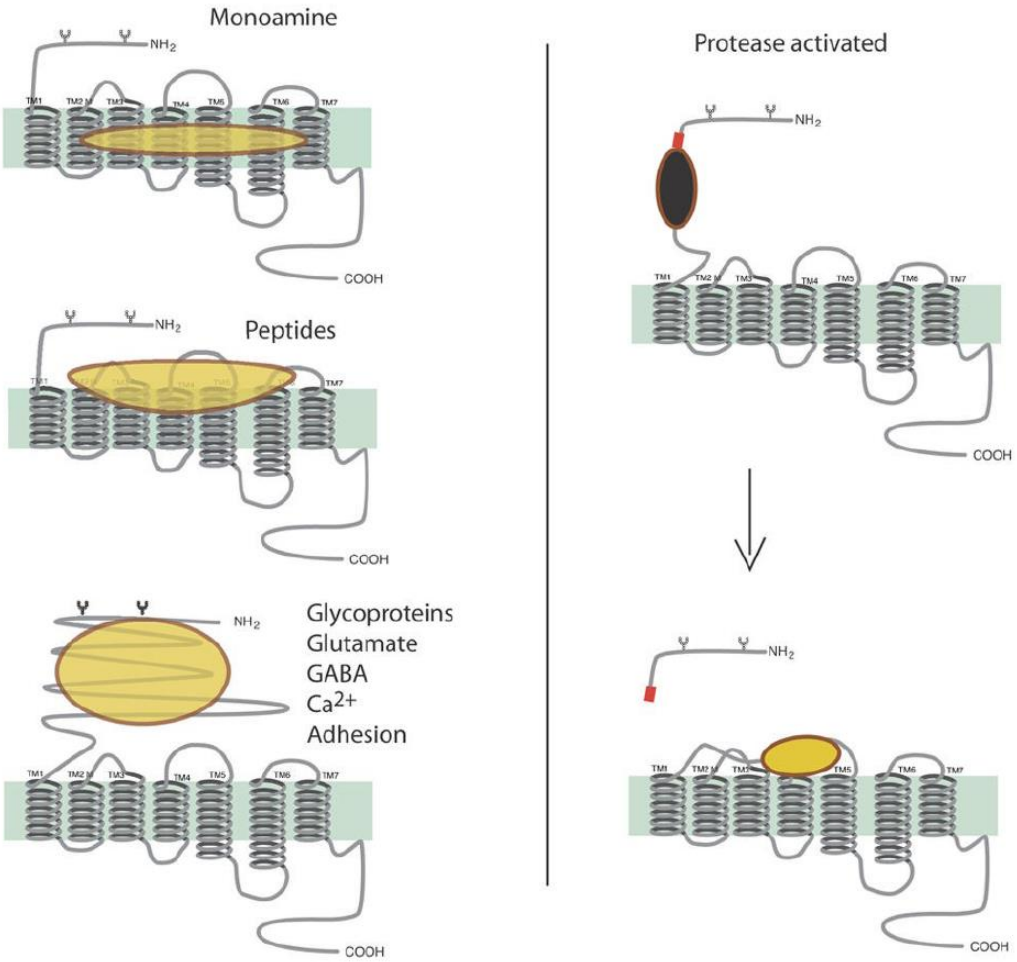

Figure3: Secondary Structure and Location of Agonist Binding Sites for Different GPCRs.

A recent and detailed analysis of the human genome reveals over 800 unique GPCRs (Yona, 2010). Based on sequence similarity within the 7 TM segments, these receptors in vertebrates can be clustered into 5 families-

1. The rhodopsin family (family A, 701 members)
2. The secretin family (family B, 15 members)
3. The glutamate family (family C, 15 members)
4. The adhesion family (24 members)
5. The frizzled/ taste family (24 members) .

The physiologic function of a large fraction of these 800 GPCRs is unknown; these receptors are referred to as orphan GPCRs [26].

The rhodopsin family is the largest and most diverse, and members are characterized by conserved sequence motifs that imply shared structural features and activation mechanisms. Receptors for a diverse spectrum of ligands including most of the amide and peptide hormones and neuromodulators belong to this family [27–29]. Class A receptors bind a number of glycoprotein hormones such as LH, TSH, and MSH using a large extracellular domain. The glycoprotein-binding receptors differ from the GPCRs for the smaller ligands. The latter receptors rely on extracellular loops or utilize a binding pocket formed by the H3 to H6 helices [30].

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Ligands for Class B GPCRs, such as glucagon, secretin, and VIP/PACAP (vasoactive intestinal peptide/pituitary adenylate cyclases activating polypeptide) are fairly large. Receptors in this family utilize an extracellular domain plus nearby extracellular loops, for ligand binding [31–33].

There are no orphans among the Secretin family and the Frizzled family, but two thirds of the glutamate remain as orphans. Only seven of glutamate has been identified as drug targets. An extensive extracellular domain and a large intracellular domain characterize this group [34,35].

The adhesion family has a much extended N terminus thought to be involved in cell-cell contact. There are, as yet, no drugs targeting them, and the majority of them remain orphan receptors [36]. But knockout studies in Caenorhabditis elegans and in rodents showed that they are crucial in the early development of the embryo and also appear to have a number of physiological roles [37].

Synthesis and Maturation

Both during and after the synthesis, GPCRs undergo a continual process of maturation before reaching at the plasma membrane. They achieve proper folding while residing in the endoplasmic reticulum, traverse from the cis- to the trans- Golgi while undergoing modification, and finally targeted to the plasma membrane where they are properly inserted into the membrane [38–40].

Strict quality-control systems inside of cells guarantee that despicably or deficiently collapsed proteins are focused for degradation, ordinarily by means of the proteasome pathway [41]. Folding of many GPCRs into a proper/functional conformation requires the presence of endogenous accessory chaperone proteins. For example, the human DnaJ protein HSJ1b, a member of the heat shock protein (HSP) family of cytoplasmic cochaperones, regulates trafficking of rhodopsin from the endoplasmic reticulum (ER) to the cell surface. Alternatively, single-membrane–spanning chaperone proteins can facilitate GPCR exit from the ER [42–45].

Oligomerization of GPCRs plays in the biosynthesis and trafficking of nascent GPCRs to the cellular surface. Multiple GPCRs including the β2-AR and vasopressin receptors undergo constitutive homodimerization early in the biosynthetic pathway, occurring in the ER. Early studies using rhodopsin, muscarinic, and β-adrenergic receptors as model GPCRs suggested that GPCRs exist primarily as monomers, although modification of the detergent extraction systems used for protein purification also suggest that a varying fraction of GPCRs are present in oligomeric form.

In order for a GPCR to transduce an extracellular signal, it must both traffic correctly to and be retained at the cellular surface to allow for receptor/ligand interaction. Multiple proteins not directly involved in the signal transduction cascade have been identified which stabilize receptor surface expression. These include spinophilin, Homer, actin-binding protein 280/filamin A, protein 4.1N, muskelin, and postsynaptic density-95 (PSD-95) [46-48].

Ligands

The effect of a ligand on the structure and biophysical properties of a receptor, and hence on the biological response, is known as the ligand efficacy [49]. Natural and synthetic ligands can be grouped into different efficacy classes as (a) full agonists are capable of maximal receptor stimulation(b)partial agonists are unable to elicit full activity even at saturating concentrations (c)neutral antagonists have no effect on signaling activity but can prevent other ligands from binding to the receptor(d)Inverse agonists reduce the level of basal or constitutive activity below that of the unliganded receptor [50].

Ligands act as either agonists or as antagonists. An agonist induces the same response in the receptor as that triggered by the natural ligand. An antagonist binds to the receptor but the receptor does not transmit a signal in

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response to the binding event [51]. By binding the receptor the antagonist blocks access of the natural ligand to the receptor and thus prevents transmission of a signal. Drugs that bind in an antagonistic fashion are known as blockers [52]. Prominent examples are beta blockers, which antagonize the beta-adrenergic receptor, and antihistamines, which inhibit the histamine H1 receptor.

The ligands range from subatomic particles (a photon), to ions (H+ and Ca++), odorants, fatty acids, neurotransmitters, amino acids, peptides, proteins and even proteolytic enzymes . The location of the ligand binding domains for many GPCRs has been determined [53]. While many small organic agonists bind within the TM segments, peptide hormones and proteins often bind to the amino terminus and extracellular sequences joining the TM domains.

Heterotrimeric G Proteins and Subunits

GPCRs transmit messages into the cell by activating heterotrimeric G proteins. In the absence of GPCR ligand binding, heterotrimeric G proteins remain in close association with the GPCRs. Ligand binding leads to G protein dissociation and signaling through second messengers that target protein kinases/phosphatases and ion channels. Heterotrimeric G proteins are assembled from three distinct subunits. These subunits are designated as Gα, Gβ, and Gγ. There are 20 different Gα subunits, 6 known Gβ subunits and 12 distinct Gγ subunits.

The Gα subunits function as GTPases. The signaling activity of a Gα subunit is turned off when it is GDP-bound and switched on when it is GTP-bound. The GPCR provides the activation signal and also serves as the GEF (guanine nucleotide exchange factor), catalyzing the dissociation of bound GDP from Gα. A family of proteins called regulators of G protein signaling (RGS proteins) function as GAPs (GTPase Activating Protein) for the Gα subunits. They catalyze the hydrolysis of GTP by the Gα subunits, thereby rapidly switching off Gα signaling [54].

Once activated the Gα and Gβγ subunits are free to diffuse laterally along the cytoplasmic surface of the plasma membrane, and bind nearby signaling targets, or effectors. The cycle of activation and signaling is completed with hydrolysis and reassociation of the Gα and Gβγ subunits. Upon binding Gα, the Gβγ induces substantial conformational changes and increases the affinity of Gα for GDP. When bound to Gα, the Gβγ subunit cannot bind its effectors and signal because the binding sites on Gβγ for its effectors overlap that for Gα.

Families of Gα subunits

There are four kinds of Gα subunits. For most GPCRs one type of GPCR couples to and activates only one of the four kinds of alpha subunits. However, in some cases, the coupling is richer and the GPCR can switch from one kind of subunit to another [55].

Several portions of the GPCR contribute to the G protein-specific binding. Regions at the ends of helices H3, H5, and H6, loops C2 and especially C3, and the short helix (H8) located in the C-terminal region just after helix H7 are all involved in coupling and activating the various members of the heterotrimeric G protein family.

1. Gs subunits- bind to and stimulate adenylyl cyclases
2. Gi subunits- inhibit adenylyl cyclases
3. Gq subunits- stimulate phospholipase C
4. G12 subunits- effectors are not identified yet

Each Gα family contains several variants. Some variants and groups of variants are found in certain tissues while others are more broadly distributed. For example- G0 subunits are brain-specific.

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Gβγ subunits target many of the same second messenger generators as the Gα subunits. Like Gα subunits, some Gβγ subunits stimulate adenylylcyclases while others inhibit them. Still other Gβγ subunits stimulate phosopholipase C. Thus, Gα and Gβγ subunits jointly determine the overall
action of a G protein on second messenger generators [56] .

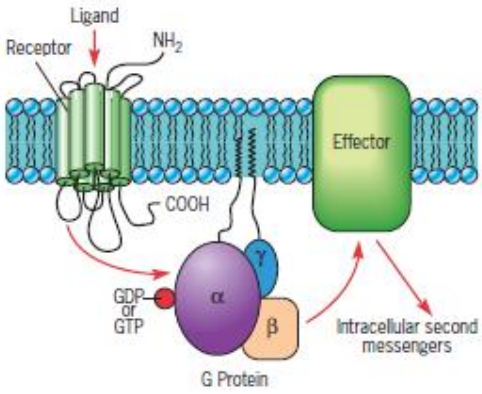

Figure 4: Signal transduction by means of a seven transmembrane receptor and a heterotrimeric G protein.

Structure of Rhodopsin: A G Protein-Coupled Receptor

Rhodopsin, also known as visual purple or visual pigment, is a biological pigment in photoreceptor cells of the retina that is responsible for the first events in the perception of light. Rhodopsins belong to the G-protein-coupled receptor family and are extremely sensitive to light, enabling vision in low-light conditions.

Rhodopsins are a member of the largest GPCR family i.e. the rhodopsin family. These are activated by light and turn on the signaling pathway that leads to vision. Rhodopsin is an integral membrane protein with about 50% of its mass in the phospholipid bilayer [57].

Rhodopsin is composed of the protein opsin (Bovine opsin consists of 348 amino acids ([9]), 40 kD) covalently linked via a protonated positively charged Schiff base linkage to 11-cis-retinal through Lys- 296. Glu-113 serves as the counterion for the Schiff base attraction of the chromophore to Lys-296. Lys residue is conserved within H7 in all pigments, and Glu residue that serves as the counterion to the Schiff base is conserved within H3.

The 11-cis-retinal chromophore is a derivative of vitamin A1, with a total of 20 carbon atoms. The binding site of the chromophore lies within the membrane-embedded domain of the receptor. All seven transmembrane segments and part of the extracellular domain contribute interactions with the bound chromophore. The chromophore is located closer to the extracellular side of the transmembrane domain of the receptor than to the cytoplasmic side. In all, at least 16 amino acid residues are within 4.5 Å of the chromophore: Glu-113, Ala- 117, Thr-118, Gly-121, Glu-122, Glu-181, Ser-186, Tyr-191, Met-207, His-211, Phe-212, Phe-261, Trp-265, Tyr-268, Ala-269, and Ala-292.

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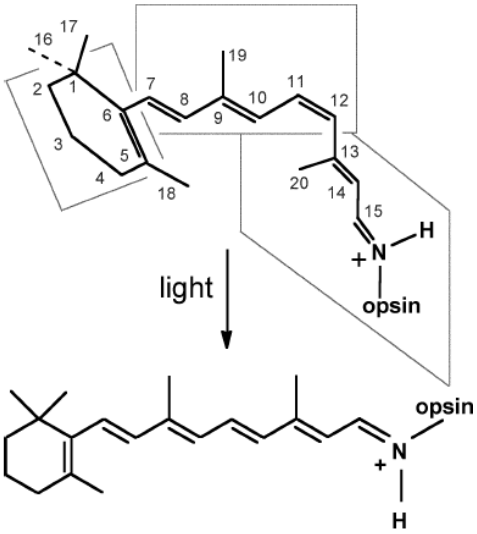


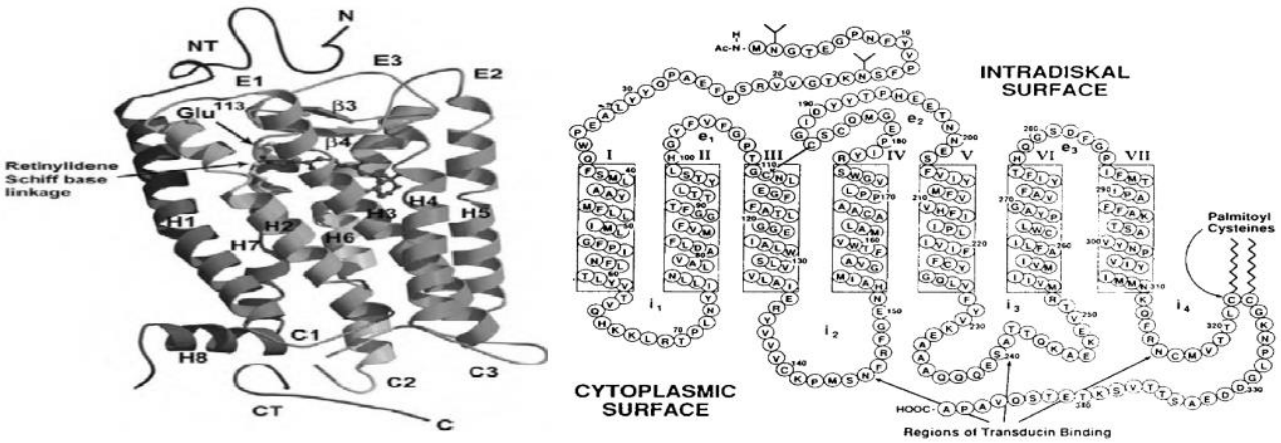
Figure 5- photoisomerization of 11-cis-retinal chromophore.

Two Dimensional Crystals

The first structure of rhodopsin came from cryoelectron microsopy and X- ray crystallography of bovine rhodopsin from Gebhard Schertler's group. While the resolution of these structures was limited (ranging from 5 to 9Å), they provided the first picture of the orientation of the TM segments in a lipid environment.

The seven alpha helices in rhodopsin are arranged sequentially in a clockwise manner when viewed from the intracellular side. Helices 1, 2, 3, and 6 are tilted. That is, their axes are inclined by about 25° relative to an axis drawn perpendicular to the surface. Helices 4 and 7 are nearly perpendicular to the membrane bilayer. Helix 6 is bent; one part is nearly perpendicular to the plane of the membrane and the other part is inclined by about 25° (helix 5 is also bent, but to a lesser extent) [58]. Palmitoyl acyl chains, covalently bound to Cys 322 and Cys 323, are thought to anchor part of the carboxyl terminal tail to the membrane forming a putative fourth loop consisting of 11 amino acids on the cytoplasmic surface of the rod outer segment disk membrane. An additional posttranslational covalent modification is found in the amino terminal tail at Asn 2 and Asn 15, where N-linked oligosaccharides are added.

A pair of highly conserved Cys residues is found on the extracellular surface of the receptor and forms a disulfide bond. A Glu(Asp)/Arg/Tyr(Trp) tripeptide sequence is found at the cytoplasmic border of H3. This sequence is conserved in family A GPCRs and has been shown to be involved in G-protein interaction [59].



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(a) (b)

Figure 6a and 6b: Structure of rhodopsin determined by means of X-ray crystallography.

Three Dimensional Crystals

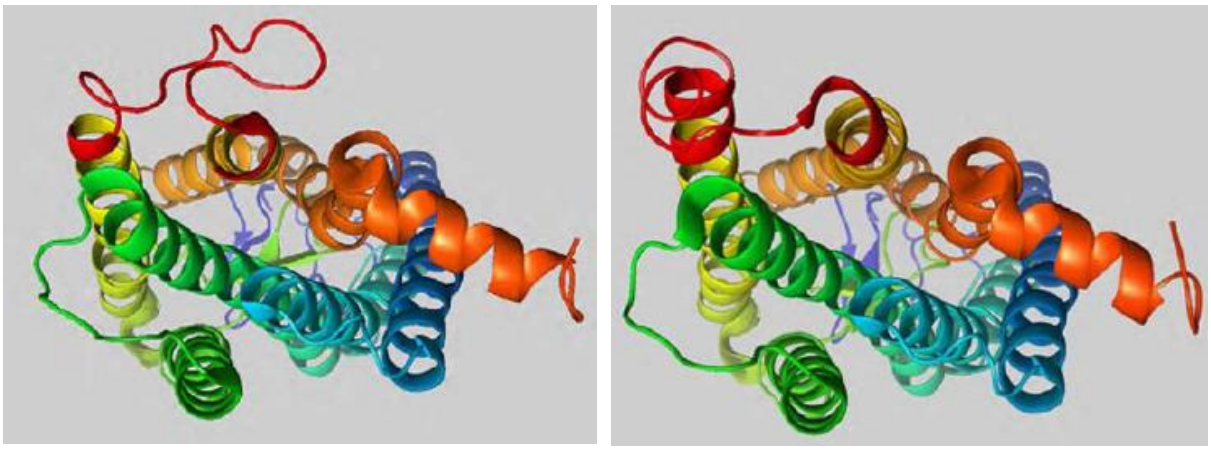
Three dimensional crystals structures of rhodopsin have been obtained by several groups. Three-dimensional crystals of bovine rhodopsin have been grown using at least two different approaches.

The first published crystals of rhodopsin were obtained from rhodopsin selectively solubilized from rod outer segments using a combination of alkyl (thio) glucoside detergents and divalent cations. The procedure used no additional purification steps. The mixture likely contained more rod out segment lipids than would be expected from protein purified by column chromatography, and it was speculated that the presence of these lipids may influence crystal formation [60]. Crystals grown from this preparation of rhodopsin initially diffracted at 2.8 Å and subsequent improvements have led to diffraction at 2.2 Å. All of these crystals have a P41 space group, and crystal contacts form between hydrophilic domains of the receptor.

Three-dimensional crystals have also been obtained from bovine rhodopsin solubilized from rod outer segments using the detergent lauryldimethylamine-oxide (LDAO) and subjected to lectin chromatography followed by detergent exchange into n-octyltetraoxyethylene (C8E4) followed by anion exchange chromatography. This more extensive purification procedure, which would be expected to remove all but tightly bound lipid, resulted in crystals diffracting at 2.6 Å. These crystals have a P31 space group and crystal contacts form primarily within the transmembrane domains and the intracellular and extracellular loop structures point into solvent filled cavities [61]. So the loop structures may assume a more native structure in the P31 crystal form compared to the P41 crystals.

Comparison of P41 and P31 rhodopsin structures

The structures obtained from P41 and P31 crystals are very similar overall, particularly in the transmembrane and extracellular domains. However, there are significant differences in the cytoplasmic loop linking TM5 and TM6, which is known to be involved in G protein coupling. The structure from the P31 crystals is in agreement with structures obtained from electron diffraction of two-dimensional crystals and with electron paramagnetic resonance spectroscopy studies. As discussed above, this loop may assume a more native structure in the P31 crystals because none of the cytoplasmic domains are involved in crystal lattice contacts [62].



(a) P41 rhodopsin crystal (b) P31 rhodopsin crystal

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Figure 7a and 7b**:** A comparison of the rhodopsin structures determined from the P41 and P31crystal forms. The loop connecting TM5 and TM6 (shown in red) is the most divergent sequence.

Function of Rhodopsin

The rhodopsin molecule operates in a switchlike manner to activate the G protein. Two of the helices, H3 and H6, project into the cytoplasm further than the others. Absorption of a photon by the 11-cis-retinal causes its isomerization to all-trans-retinal, leading to a conformational change of the protein moiety H3 and H6, including the cytoplasmic surface (martin, 9). The photolyzed chromophore only transiently activates opsin, before the all trans-retinal is hydrolyzed and dissociated from the opsin [63].

When activated by ligand binding the GPCR acts as a guanine nucleotide exchange factor, or GEF, for its G protein (transducin, Gt). In its active shifted conformation, the rhodopsin GPCR is able to catalyze the release of GDP from the alpha subunit of the G protein leading to its binding GTP. The GDP-GTP exchange triggers the dissociation of Gαβγ into Gα and Gβγ subunits and the subunits migration towards their effectors. The switch is reset by the dissociation of the ligand from the GPCR. The absorption of a single photon results in the activation of hundreds of G-protein molecules with extraordinary reproducibility, whereas the 11-cis-retinal–bound rhodopsin has extremely low activity [64].

The α subunit of Gt, Gtα, activates the effector enzyme, a cGMP phosphodiesterase (PDE), by binding its inhibitor subunit, thus initiating cGMP hydrolysis. The reduction in cGMP concentration results in a hyperpolarization of the ROS plasma membrane, due to the reduced flux of Na+ ions through the cGMP-gated channels, with concomitant change in neurotransmitter release at the synaptic end of the rod cell, which generates the neuronal response to light [65].

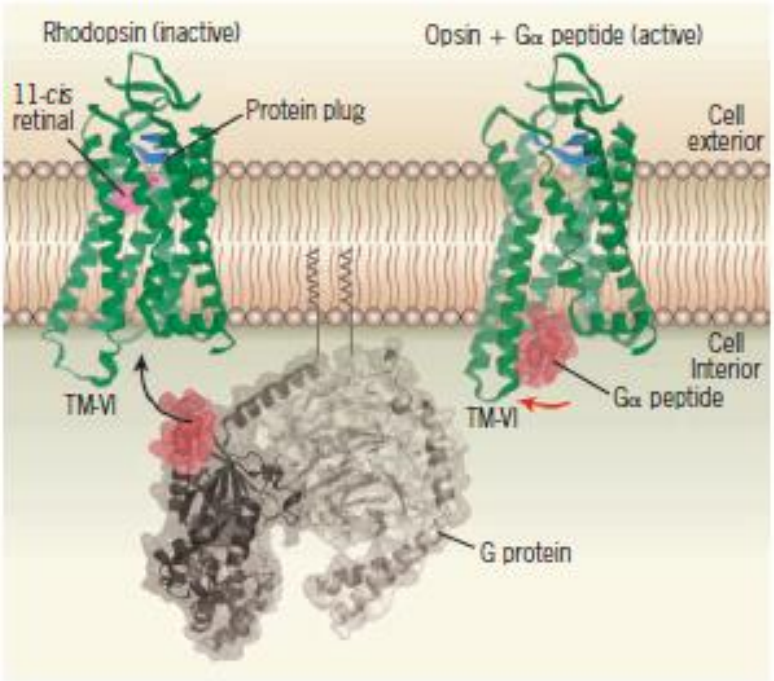

Figure 8: Model depicting the activation of the GPCR rhodopsin based on recent X-ray crystallographic structures. On the left, rhodopsin is shown in its inactive (dark-adapted) conformation together with an unbound heterotrimeric G protein (called transducin). When the retinal cofactor (shown in red on the left rhodopsin molecule) absorbs a photon, it undergoes an isomerization reaction (from a cis to a trans form), which leads to the disruption of an ionic

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linkage between residues on the third and sixth transmembrane helix of the protein. This event in turn leads to a change in conformation of the protein, including the outward movement of the sixth transmembrane helix (red curved arrow), which exposes a binding site for the Gα subunit of the G protein. The rhodopsin molecule on the right is shown in the proposed active conformation with a portion of the Gα subunit (in red) bound to the receptor's cytoplasmic face.

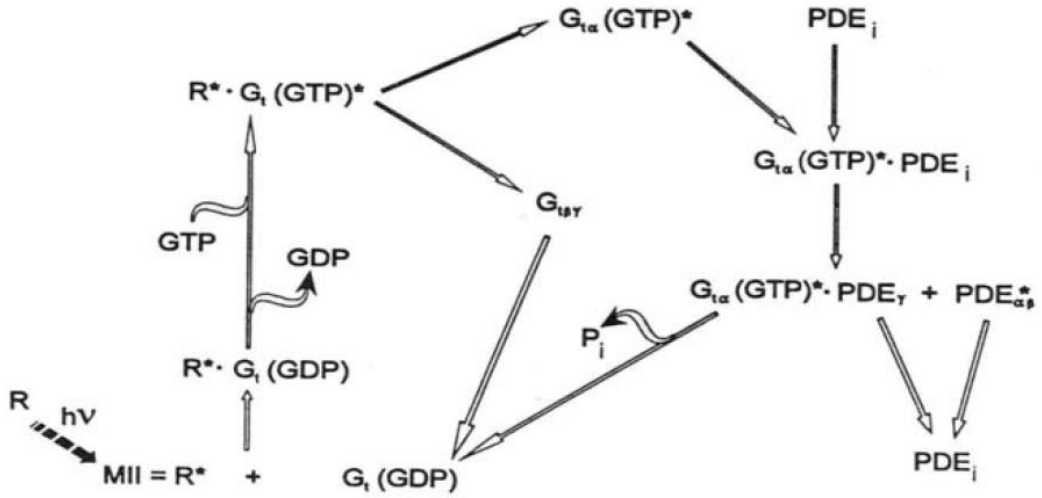

Figure 9: Visual signal transduction in rod cell. Light (hv) converts rhodopsin (R) to an activated form (R*), which binds and activates Gt(GDP) by catalyzing the exchange of bound GDP for GTP. Gt(GTP)* then dissociates and Gtα(GTP) binds to the the hydrolysis of cGMP by PDE* αβ. The lowered concentration of cGMP induces closure of cGMP-gated Na+ channels in the plasma membrane, hyperpolarizing the cell.inactive form of the cGMP-phosphodiesterase (PDEi). This complex dissociates to yield the active subunit complex PDE* αβ and Gtα (GTP)*. PDEγ , where PDEγ is the inhibitory subunit of the PDE, initiating

CONCLUSION AND FUTURE ASPECTS

Much of vertebrate physiology is based on GPCR signal transduction. GPCRs convey the majority (80%) of signal transduction across cell membranes . As the receptors for hormones, neurotransmitters, ions, photons and other stimuli, GPCRs are among the essential modes of communication between the internal and external environments of cells. Since GPCRs mediate most of our physiological responses to hormones, neurotransmitters and environmental stimulants they are the major therapeutic targets for a broad spectrum of diseases.

Although all GPCRs are characterized by the presence of seven membranespanning α-helical segments separated by alternating intracellular and extracellular loop regions but individual GPCRs have unique combinations of signal-transduction activities involving different types of ligands and multiple G-protein subtypes and complex regulatory processes [66].

The wide spectrum of ligand efficacies for individual GPCRs shows that efficient energy transfer between the binding pocket and the site of G-protein interaction is dependent on multiple interactions between receptor and hormone and requires more than simply occupying the binding site. Many GPCRs can stimulate multiple signaling systems, and specific ligands can have different relative efficacies to different pathways [67].

Taking the human β2AR as an example, the binding of adrenaline and noradrenaline to cells in the target tissues leads to the activation of the stimulatory subunit of the heterotrimeric G protein (Gαs), the stimulation of adenylyl cyclase, the accumulation of cyclic AMP (cAMP), the activation of cAMP-dependent protein kinase A (PKA) and the

phosphorylation of proteins involved in muscle-cell contraction. For example, β2AR exhibits significant constitutive activity, which can be blocked by inverse agonists. The β2AR couples to both Gαs and the inhibitory subunit (Gαi) in cardiac myocytes, and can also signal through MAP kinase pathways in a G-protein-independent manner through arrestin. Similarly, the process of GPCR desensitization involves multiple pathways, including receptor phosphorylation events, arrestin-mediated internalization into endosomes, receptor recycling and lysosomal degradation. These activities are further complicated by factors such as GPCR oligomerization, localization to specific membrane compartments and resulting differences in lipid-bilayer composition. Such multifaceted functional behavior has been observed for many different GPCRs [68].

There has been remarkable progress in the field of GPCR biology during the past two decades. Notable milestones include the cloning of the first GPCR genes, and the sequencing of the human genome revealing the size of the GPCR family and the number of orphan GPCRs. Moreover, there is a growing appreciation that GPCR regulation and signaling is much more complex than originally described, and includes signaling through G protein independent pathways.

Ligand-induced selective signaling (LiSS)

The LiSS concept was introduced by Terry Kenakin and is rapidly becoming a generic theme for GPCRs. It has important implications in specific drug development and in minimizing side effects. As we are clear by now that different ligands selectively recruit different intracellular signaling proteins to produce different phenotypic effects in cells. How GPCRs operate is conventionally viewed as the receptor occurring in two states: an inactive state and an active state. In the active state the receptor induces and activates a cascade of events culminating in the physiological function of the cell [69].

What has recently emerged is that receptors can assume many conformations. Each of these conformations can potentially interact with a ligand in a highly selective manner. In turn, this specific receptor conformation interacts with a specific intracellular signaling complex. Nowadays it is becoming popular that the nature of the ligand and the dynamically changing intracellular environment alter the level and path of signaling. Now screening for novel ligands will not simply involve receptor binding but will screen for the appropriate intracellular signal, which reflects the desired phenotypic response of a cell for a disease state or pathophysiology [70].

GPCR signaling independent of G proteins

Another area of research is the GPCR signaling through proteins other than G proteins. It is becoming increasingly apparent that there are many ways in which GPCRs can signal independently of G proteins. The first convincing evidence for the existence of GPCR-independent signaling was given by Lefkowitz and associates. Angiotensin II binds to AT1 receptor and activates both β-arrestin and G proteins [71,72]. When blockers like losartan and valsartan (antagonist) blocks the AT1 receptor, no intracellular signal was propagated. However, another type of antagonist (SII) does not activate the G protein pathway but exclusively recruits β -arrestin and activates ERK.

CONCLUSION

GPCRs are a large family of cell surface receptors that respond to a variety of external signals. Binding of a signaling molecule to a GPCR results in G protein activation, which in turn triggers the production of any number of second messengers. Through this sequence of events, GPCRs help regulate an incredible range of bodily functions, from sensation to growth to hormone responses.

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