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Regulation of cellular signals by G-proteins

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Abstract. Extracellular signals are transduced across the cell by the cell surface receptors, with the aid of G-proteins, which act at a critical point of signal transduction and cellular regulation. Structurally, G-proteins are heterotrimeric consisting α, β and γ subunits but in functionally active state they dissociate into α subunit coupled to GTP and as βγ dimer. G-proteins can be broadly divided into two classes based on their sensitivity to pertussis toxin and cholera toxin. Existence of various forms of each of the subunit allows molecular diversity in the subunit species of G-proteins. These subunits interact with a wide range of receptors and effectors, facilitated by post translational modification of their subunits. Different types of G-proteins mediate several signalling events in different parts of the body. This review summarizes the features of (i) structural and functional heterogenity among different subunits of G-proteins, (ii) interaction of G-proteins and their subunits with effectors with specific cases of G-protein mediated signalling in olfaction, phototransduction in the retina, *ras* and *ras* related transduction and (iii) disease conditions associated with malfunctioning of G-proteins.

Keywords. G-proteins; G-protein subtypes; receptors; effectors; diseases.

1. Introduction

Of the numerous physical and chemical signals which constantly reach the cell surface, some signals bind to the receptors on the cell membrane and initiate a cascade of events that transduces to the cell interior. This process of signal transduction across the cell membrane is facilitated by a group of intracellular/transmembrane coupling proteins having GTP binding nature, called as G-proteins. G-proteins achieve cellular responses by bringing about a change in the activity of the target ion channels or enzymes, which modulates ionic composition or second messenger levels. Receptors transmitting signals through G-proteins have characteristic structural features with seven transmembrane helices (Dohlman *et al* 1991). Every eukaryotic cell has receptors for many kinds of signals, different types of G-proteins and effectors. A steady increase in the heterotrimeric subunit diversity of G-proteins has been recognized which eventually leads to a major challenge of defining the specific interactions in the receptor-G-protein-effector coupling, besides understanding their function. Excellent reviews have appeared in terms of G-protein characteristics and topology (Dohlman *et al* 1991), receptor effector coupling (Spiegel *et al* 1992) various aspects of receptor-G-protein action (Savarese and Fraser 1992; Spiegel *et al* 1992), role of G-protein *βγ* dimer in transmembrane signalling (Clapham and Neer 1993) and G-proteins as regulators of transmembrane signals (Neer 1995). This review attempts to summarize some of the structural and functional features of G-proteins; how these can help in an orchestrated cellular signal transduction, discusses the interactions of G-proteins with effectors, and their relevance in certain disease conditions where the intricate balance

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rendered by G-proteins in signal transduction is affected. This review is timely, given the rapidity of progress in this filed particularly with the number of G-protein subunit species being identified, crystal structure deciphered and the defective expression of G-proteins in different disease states.

2. Structural and functional heterogenity of G-proteins

2.1 *Structural features and the mechanism of action of G-proteins*

G-proteins are made up of three polypeptides, α, *β* and *γ* subunit. All three peptide chains are encoded by specific genes (Cali *et al* 1992; Simon *et al* 1991). Combination of the different peptide chains allows the generation of different G-proteins. Upon receptor stimulation by the ligand, the α subunit dissociates from the *βγ* subunit and binds to GTP converting G-protein into its active form. This form initiates the formation of a second messenger, cAMP. In the activated state, α subunit loses its affinity for GDP and binds to GTP since the concentration of GTP is more than that of GDP in the cells. The activated state lasts until the GTP is hydrolyzed to GDP by the intrinsic GTPase activity of α subunit. Once GTP is cleaved to GDP, the α and *βγ* subunits reassociate, become an inactive heterotrimer and return to the receptor (figure 1). Recent findings have shown that an effector can modulate the GTPase activity of the α subunit. This means that an effector can influence the duration of its own activation. For e.g., two effectors, phospholipase C and cGMP phosphodiesterase have been shown to enhance the GTPase activity of the α subunit that regulates them (Arshavsky and Bownds 1992; Bernstein *et al* 1992; Neer 1995).

2.2 *Structure of* α *subunit*

The α subunits have molecular weight ranging from 39 to 52 kDa. There are over 20 types of α subunits of G-proteins of which 16 of them are direct gene products and

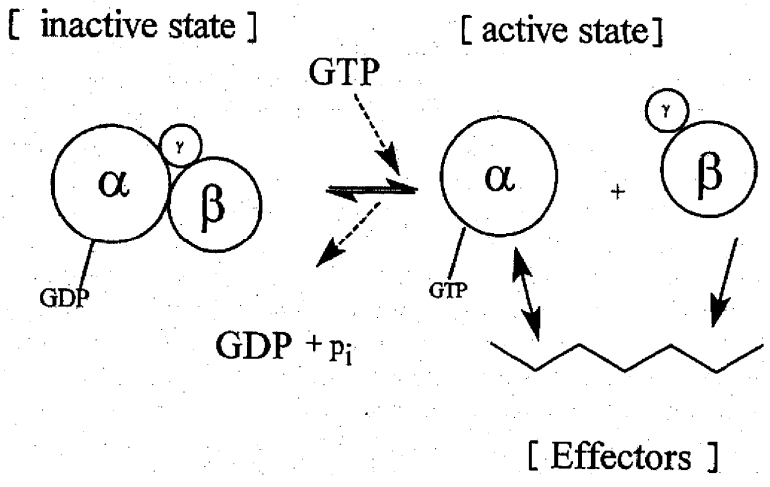

Figure 1. Regulatory cycle of heterotrimeric G-proteins (figure adopted and modified from Neer 1995).

Table 1. Classes of Gα subunits

Class	Sub types/isoforms	Some functions
α_s	α_s, α_{olf}	Stimulate adenylyl cyclase Regulate Ca^{2+} channels
α_i	α_{i1}, α_{i2}, α_{i3}, α_0, α_{t1}, α_{t2}, α_{gust}, α_z	Inhibit AC; Regulate K^+ and Ca^{2+} channels Activate cGMP phosphodiesterase
α_q	α_q, α_{11}, α_{14}, α_{15}, α_{16}	Activate phospholipase C
α_{12}	α_{12}, α_{13}	Regulate Na^+/K^+ exchange

others are alternatively spliced isoforms (Kaziro *et al* 1991; Simon *et al* 1991). There are four major classes of α subunits defined by amino acid similarity (table 1). The α_s class was first recognized by its ability to activate adenylyl cyclase and includes the ubiquitous α_s and α_{olf} (an α subunit from olfactory epithelium). The α_i class is so called because of its ability to inhibit adenylyl cyclase and includes α_{il}, α_{i2}, α_{i3}, α_o (a predominately neural α subunit) α_{tl}, α_{t2} (the retinal α subunits) and α_z members of the α_q class including α_q, α_{11}, α_{14}, α_{15}, α_{16}, activate phospholipase C. The fourth class is the α_{12} (includes α_{12}, α_{13}) class whose main function is the regulation of Na^+/K^+ exchange. Except for some α subunits like α_t found only in the retina and α_{olf} found only in olfactory neuroepithelium, most others are widely expressed with many subtypes in individual cells (Kim *et al* 1988). Structural analysis of the α subunit shows five different regions (Pennington 1994). They are classified as P or Gl; E or G-2 region; G-3 region; G-4 region and G-5 region.

2.2a *P or G-1 region:* This region shows a consensus sequence GXXXXGK(S/T). This region interacts with GTP, GDP and guanine nucleotides.

2.2b *E or G-2 region:* This region has a consensus sequence D–$(X)_n$ — T. It has a conserved threonine residue (corresponding to Thr-35 in $p21^{ras}$) co-ordinated to Mg^{2+}. Mutation of this region in $p21^{ras}$ leads to loss of the transforming potential of the protein without affecting its ability to bind GTP. It is therefore suggested that this region (amino acids in 32-42 in $p21_{ras}$) is responsible for interaction with the effector.

2.2c *G-3 region:* This region has a consensus sequence DXXGG. Amino acids in this highly conserved region contribute to the GTPase activity.

2.2d *G-4 region:* This region has a consensus sequence(N/T)(K/Q)XD. Amino acids of this region interact with the guanine ring and stabilize the guanine nucleotide binding site.

2.2e *G-5 region:* This region is not well conserved between the members of the GTPase family. In $p21^{ras}$ this region interacts indirectly with guanine nucleotides.

2,3 *Diversity of α subunits of G proteins*

When the deduced amino acid sequences of all the α subunits that have been cloned were aligned, approximately 20% of the amino acids were found to be invariably

Table 2. Conservation of G-protein α subunit sequences in different mammalian species.

Species	Amino acid sequence	Nucleotide sequence
rGsα vs hGsα	393/394 (99·7%)	1128/1182 (95·4%)
bGi1α vs hGi1α	354/354 (100%)	998/1062 (94·0%)
rGi2α vs hGi2α	350/355 (98·6%)	985/1056 (92·4%)
rGi3α vs hGi3α	349/354 (98·6%)	981/1062 (92·4%)
rGoα vs hGoα	348/354 (98·3%)	992/1062 (93·4%)
rGxα vs hGxα	349/355 (98·3%)	977/1065 (91·7%)

r, Rat; h, human; b, bovine.
Table taken from Kaziro Y p-56 of G-Proteins as mediators of cellular signalling processes (eds) M D Houslay and G Milligan, Wiley publishers, 1990.

conserved. Amino acid sequence similarity provides a measure of the relatedness of different α subunits (table 2). The amino acid sequence of $G_{s\alpha}$ between human and rat is strongly conserved with only one out of 354 amino acids being different. Over 98% identity of amino acids sequences is maintained for $G_{i2\alpha}$, $G_{i3\alpha}$, $G_{x\alpha}$ and $G_{o\alpha}$ among different mammalian species. The strong conservation of amino acid sequence between each G-protein α subunit, from distant mammalian species is indicative of an evolutionary pressure to maintain the specific physiological function of each G-protein gene product (Itoh *et al* 1988).

2.4 *Molecular biology of the G_α subunits*

The presence of multiple $G_{i\alpha}$ subtypes have been suggested from studies on the molecular heterogenity, immunological distinction and functional differences of the pertussis toxin (PTX) substrates in mammalian cells (figure 2). Transducin G_t has two subtypes $G_{tl\alpha}$ and $G_{t2\alpha}$ expressed in rods and cones respectively. $G_{i\alpha}$ subunit has three subtypes $G_{i1\alpha}$ $G_{i2\alpha}$, $G_{i3\alpha}$. Four different $G_{s\alpha}$ cDNAs have been generated from a $G_{s\alpha}$ (also known as $G_{sl\alpha}$) gene by alternative splicing. Another subtype of $G_{s\alpha}$ coded by a distinct gene $G_{olf\alpha}$ or $G_{s2\alpha}$ expressed specifically in olfactory cells has been reported (Jones and Reed 1989). Further, a new G_α clone, designated as $G_{x\alpha}$ insensitive to PTX has been isolated (Matsuoka *et al* 1988). The cys residue at the fourth position from the C-terminus commonly seen in all PTX sensitive $G_{s\alpha}$ is replaced by Ile in $G_{x\alpha}$. Human $G_{z\alpha}$ cDNA isolated from retina may be the counterpart of $G_{x\alpha}$. The human Gsα gene has 13 exons and 12 introns and spans about 20 kb of genomic DNA. The coding region of human $G_{i2\alpha}$ and $G_{i3\alpha}$ genes is split into eight exons and seven introns (Kozasa *et al* 1988). There is an additional exon (exon 9) in the 3' non coding region too. For human $G_{il\alpha}$, only sequences of exon 1 to exon 3 are known at present.

The exon intron organization of the $G_{s\alpha}$, $G_{i2\alpha}$,$G_{i3\alpha}$,and $G_{o\alpha}$ when compared with the predicted functional domain structure of protein revealed that the NH_2 terminal domain encoded by exon 1 is hydrophilic and contains the site for limited tryptic digestions. This region may be involved in interaction with $\beta\gamma$ subunits but its precise function has not yet been shown. Exon 2 encodes a short length region and is the most conserved among all G_α proteins and responsible for GTP hydrolysis.

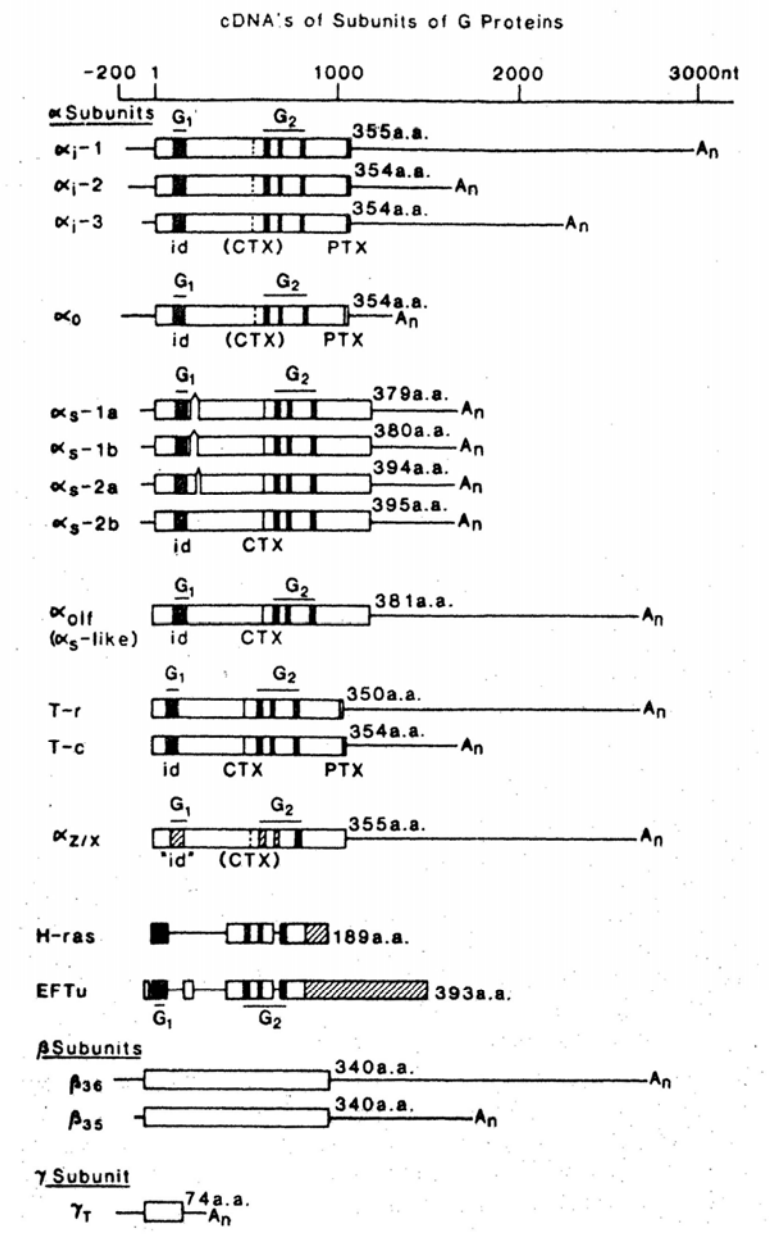

Figure 2. Organization of vertebrate α subunit mRNA molecules based on cDNA cloning. Open boxes represent open reading frames (ORFs) and lines represents' and 3' untranslated sequences. Black boxes within ORFs denote sequences highly homologous to bacterial elongation factor Tu, and to H-*ras,* which are involved in GTP binding and hydrolysis. The mRNA molecules encoding β_{36}, β_{35} and γ_{τ} are shown for comparison. The position of amino acids ADP ribosylated by cholera and/or pertussis toxin (CTX and PTX) are indicated, id, Location of the identity box. The scale is in nucleotides, (Figure taken from Birnbaumer *et al* CRC reviews in Biochem, and Mol. Biol. 25:225-244,1990 CRC press, with permission).

Comparision of the exon organization of Giα subfamily and $G_{o\alpha}$ with $G_{s\alpha}$ indicates that some exon functions are conserved between Giα subfamily and $G_{s\alpha}$. Thus three out of twelve splice sites of the human $G_{s\alpha}$ gene are shared with human Giα genes and exons. 1, 7 and 8 of $G_{s\alpha}$ correspond to exon 1 and exon 5 of Giα respectively. It has been shown that $G_{tl\alpha}$ and $G_{t2\alpha}$ gene posses the same organization as human $G_{i\alpha}/G_{o\alpha}$ genes, while human gene for $G_{x\alpha}$ has only two coding exons (Raport *et al* 1989).

2.5 *Crystal structure of G protein subunits*

The three-dimensional crystal structure of activated α subunit of the heterodimeric G-protein was resolved using transducin-a complexed with GTP analogue GTPγS. In a crystal structure of activated rod transducin, the $G_{t\alpha}$ GTPγS molecule is occluded, deep in a cleft between a domain structurally homologous to small GTPases and a helical domain unique to heterotrimeric G-proteins. This X-ray crystal structure data when taken along with the data from biochemical and genetic studies explain the mechanism for GTP induced changes in effector and receptor binding surface (Noel *et al* 1993). Similarly crystal structure of transducin's *β*γ subunits complexed with phosducin was resolved at a resolution of 2·4Å. Phosducin's Ser-73 when phosphorylated, points away from G_iSγ and inhibits phosducin's function indirectly through an induced conformational change and thus regulate G*β*γ interactions with Gα and with G*β*γ effector molecules (Gaudet *et al* 1996).

2.6 *Structure of β and y subunits*

The four known mammalian *β* subunits are identical to each other by 83 –9 0 % (Simon *et* al 1991; Watson *et al* 1994). In contrast, the six y subunits differ greatly from each other, than the *β* or *α* subunits (Cali *et al* 1992). Four different *β* subunits and at least six *γ* subunits could produce many different combinations. The *β, y* subunits are known to interact directly with seven kinds of proteins (a subunits, receptors, adenylyl cyclase, phospholipase *C-β, β*-adrenergic receptor kinase, calmodulin and phosducin) and probably also interacts directly with phospholipase A_2, K^+ channels and inositol triphosphate kinase (Clapham and Neer 1993). These proteins have no obvious common *β,* γ-binding sequence motif. The *β* and γ subunits bind very tightly to each other and can be separated by denaturants. The *β* subunit is predicted to contain two types of structures, an amphipathic α-helix followed by seven repeating units of approximately 43 amino acids each (Simon *et al* 1991). These repeating sequences called WD repeats, consist of a conserved core of 23–41 residues usually bounded by Gly-His and Trp-Asp (Neer *et al* 1994). The conserved core sequences are separated by short regions of about 7–11 amino acids which probably form loops. The N-terminal portion of *β* must lie close to γ because it contains a cysteine that can be cross-linked to a cysteine in γ subunit. However, selectivity is determined by multiple sites in the WD repeat region especially residues 215–255 in repeat 5 (Pronin and Gautam 1992).

The γ subunit has been predicted to be largely α-helical and is shown to be extending along the repeat units of *β* subunit (figure 3) and is held in place by the N-terminal α-helix of the *β* subunit (Lupas *et al* 1992). The prenyl group at the C-terminus shown by a zig-zag line, is probably essential for membrane attachment. Selectivity of the γ subunits for different *β* subunits is determined by about 14 amino acids in the middle

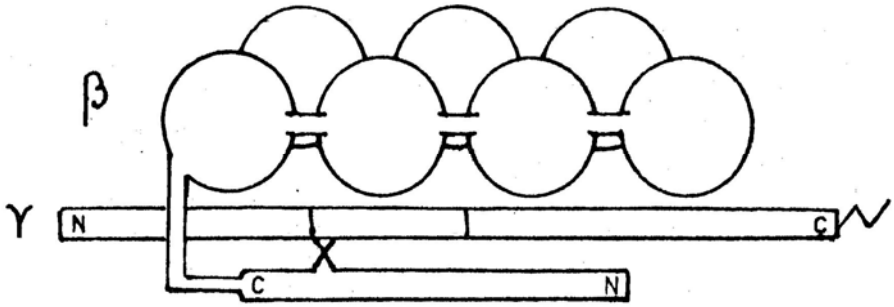

Figure 3. The cores of the WD repeats are represented by circles connected by the variable regions. Each core is predicted to be a structure made up of a *β* strand-turn-*β*. The putative α-helical region in the N-terminus of *β* is shown as a rectangle. The X represents the site of the cross-link introduced by Bubis and Khorana (1990). The C-terminal prenyl group of γ is indicated by a zig-zag line (figure adopted and taken from Neer 1995 with permission).

of the *γ* subunit which contains a cysteine cross-linked to another cysteine in the *β* subunit (Bubis and Khorana 1990).

2.7 *Diversity of $G_{\beta\gamma}$ subunits*

Several $G_{\beta\gamma}$ dimers are formed due to the combination of different *β* and γ subunits. G_{γ} subunits are more heterogenous than G_{β} subunits. G_{γ} subunits also differ in their modification by prenyl groups. G_{yl} is farnesylated whereas $G_{\gamma 2}$ is geranyl geranylated (Spiegel *et al* 1991). Prenylation is necessary for membrane attachment (Simonds *et al* 1991). $G_{\beta\gamma}$ dimers having the same *β* subunit but different γ subunits have different functions in different regions of the body. G_{γ} subunit is essential for determining the functioning the $G_{\beta\gamma}$ subunits (Clapham and Neer 1993).

2.8 *Interaction of α and γ subunits*

Another finding is that the $G_o\alpha$ subunit is rapidly cleaved to a 24 kDa fragment by trypsin (Rahmatullah and Robishaw 1994). However, in the presence of γ_2 subunit, trypsin cleavage is completely prevented. Thus, a direct association between *γ* and α subunits may provide the basis for the selective assembly of these subunits into functionally diverse G-proteins.

2.9 *Specificity of G-,proteins in cellular responses*

How target oriented specific cellular responses are achieved by G-proteins and their interaction with receptors and effectors, still remains unclear. Experiments done on heart have shown that the opposing signals could be kept distinct which needs the mediation of different G-proteins.

The *β* adrenergic receptor in heart is coupled to G_s, thereby increasing cAMP levels when stimulated, while the muscarinic receptor is coupled to G_i class of proteins which modulates several functions like activation of PLC and a *K*+ channel. These pathways

are quite distinct and occur independently without affecting the other. However, using natural systems, in reconstitution experiments, the *ß*-adrenergic receptor preferred to bind to G_s two to three-fold better than G_i (Asano *et al* 1984). Though the concentrations of G_i are much higher than G_s, why *ß*-adrenergic receptor binds to G_s, why it fails to activate K^+ channel still remains to be answered.

Another example of the specificity of G-proteins comes from a different study (Kleus *et al* 1992, 1993). Specific mechanism of action by the G-proteins was revealed by blocking the synthesis of two alternatively spliced forms of G_o α and the *ß,* γ subunits in GH_3 cells by injection of antisense oligonucleotides. Elimination of one isoform of G_o blocked the inhibition of a Ca^{2+} channel by somatostatin receptor, while the same Ca^{2+} channel regulated by muscarinic receptor is inhibited when the other isoform of G_o is eliminated. The C-terminus of G protein α subunit is usually the site of interaction with receptors. Since alternatively spliced isoforms of G_o α differ at the C-terminus end; this could be the mechanism underlying the differential interaction of the two isoforms of $G_{o\ \alpha}$ with different receptors (Strathmann *et al* 1990).

G-protein subunits can also interact with few specific molecules by different mechanisms. Phosducin, a retinal phosphoprotein modulates *ßγ* activity. It complexes with $G_{\beta\gamma}$ and affects its interaction with retinal G_α (Lee *et al* 1992). Calmodulin is another molecule affecting *ßγ* function, Neuromodulin or GAP_{43}, a growth cone associated protein, modulates α subunit activity, enhances GTPγS binding to G_o subunit.

2.10 *Post translational modification of G-proteins*

2.10a *Myristoylation :* The NH_2 terminal region of the G_α subunit is thought to be involved in the interaction with the *ßγ* subunit. This is supported by the fact that proteolysis of the N-terminus end prevents the α subunit from binding to the *ßγ* subunit. N-terminal site is also the site for myristoylation of some G-proteins (where the N-terminal glycine is myristoylated). This is seen in case of α_o, α_i, and α_z, while α_s and α_q are not myristolated. This is necessary for membrane attachment and facilitates binding of *ßγ*. It is an irreversible coValent modification and does not serve a regulatory role (Jones *et al* 1990; Mumby *et al* 1990b).

2.10b *Palmitoylation:* In addition, some α subunits are also palmitoylated at cys-3. Palmitoylation is a reversible modification. Activation of the *ß*-adrenergic receptor leads to rapid depalmitoylation of α_s and depalmitoylated α_s cannot activate adenylyl cyclase. This might be a mechanism to turn off α_s and therefore to desensitize the cell to *ß*-adrenergic stimulation. Control of palmitoylation might also be a mechanism to control the pathway that is activated (Linder *et al* 1993),

It has been suggested that the C-terminus region of the *α* subunit is involved in receptor interactions. Modification of the Gα subunit by PTX blocks its interaction with the receptor. G-protein effector studies using chimeric α subunits suggest that sequences in the C-terminal half of the Gα subunit can determine the effector specificity (Masters *et al* 1988; Gupta *et al* 1990).

2.10c *Prenylation:* Mammalian γ subunits are modified in three ways by sequential post translational modifications. To begin with, a prenylation occurs on a cys-4 residue, from the carboxy terminus end (Spiegel *et al* 1991). Isoprenylation at the

carboxy terminus end may involve addition of a farnesyl or geranylgeranyl moiety depending on the last amino acid of the ã subunit (Yamane *et al* 1990; Sanford *et al* 1991). The $\beta\gamma$ subunits must be prenylated in order to associate with the membrane. A mutation in cys-68 of γ subunit to ser-68 can prevent membrane attachment of $\beta\gamma$ dimer (Muntz *et al* 1992). The second modification is cleavage of the last three amino acids followed by a third modification involving carboxy methylation of the C-terminus (Backlund *et al* 1990; Maltese and Robishaw 1990; Mumby *et al* 1990a).

2.10d *Phosphorylation:* Another type of covalent modification which may affect the specificity of the interactions of G-proteins is phosphorylation. Several types of G-protein subunits (α_{i2} and α_z) can be phosphorylated on *ser* or *thr* residues but to correlate the exact changes due to phosphorylation is yet to be experimented. It is also to be seen whether phosphorylation of a G-protein affects the specificity of its interactions with its receptors and effectors. It probably changes the localization or association of the G-protein with other proteins (Koch *et al* 1991).

The $\beta\gamma$ subunit has been shown to be phosphorylated on histidine residues in membranes from human leukemia cells (Wieland *et al* 1993) using GTP as phosphate donor. Phosphorylation of histidine on β subunit could probably assign a new regulatory role for $\beta\gamma$, rarely seen in higher eukaryotes.

3. Interaction between receptors, effectors and G-proteins

3.1 *Ion channels (K^+ and calcium channels)*

3.1a K^+ *channel* ($I_{K\text{-}acetyl\ choline}$): The first evidence that $G_{\beta\gamma}$ could regulate effectors came with the report that $G_{\beta\gamma}$ purified from bovine brain activated the cardiac K^+ channel which is normally regulated by the muscarinic acetyl choline (ACh) receptor (Logothetis *et al* 1987). Using patch clamp techniques it was shown that purified $G_{\beta\gamma}$ or G_α subunits greatly enhanced the frequency with which the K^+ channels open. Either G_α or $G_{\beta\gamma}$ can activate the channel (Logothetis *et al* 1988). Experiments by Itoh *et al* (1992), show that $G_{\beta\gamma}$ activates $I_{K.Ach}$ more effectively than G_α where as G_α alone activates another type of K^+ channel $I_{K\ ATP}$ in the same patch.

Experiments conducted with purified $I_{K.ACh}$ channel protein may prove whether G_α or $G_{\beta\gamma}$ activates the channel directly or via intermediates. Kim *et al* (1989) suggested that $G_{\beta\gamma}$ activated $I_{K.ACh}$ via phospholipase (PLA_2) because an antibody shown to block PLA_2 activity in other systems blocked $G_{\beta\gamma}$ activation of $I_{K.ACh}$. The current hypothesis is that its action is direct and arachidonic acid modulates channel activity.

3.2 *Calcium channels*

Nuclear microinjection of antisense oligonucleotides to selective base sequences of each α subunit into GH3 cells showed that somatostatin receptors inhibit a Ca^{2+} channel via $G\alpha_{o2}\beta_1\gamma_3$ where as the M_4 muscarinic receptor inhibits the same channel by $G\alpha_{0}\beta_3\ \gamma_4$. Elimination of either of the appropriate G_α or $G_{\beta\gamma}$ completely blocked the Ca^{2+} channel inhibition suggesting that the two subunits independently regulate the channel (Kleus *et al* 1992,1993). Different $G_{\beta\gamma}$ subunits may specify which receptor activates which $G_{\alpha 0}$ subtype or each $G_{\alpha o}$ may have a strict preference for a particular $G_{\beta\gamma}$.

3.3 *Phospholipase A_2*

$G_{\beta\gamma}$ subunits increase arachidonic acid production by PLA_2 in G-protein depleted retinal membranes reconstituted with purified retinal $G_{\beta\gamma}$ (Jelsema and Axelrod 1987). The concentration of $G_{\beta\gamma}$ required is high (0·6 μM) but the $G_{\beta\gamma}$ concentration in rod outer segment s is estimated to approach 500 μM. PLA_2 can also be activated by G_α subunits but not much is known about the efects of G_α and $G_{\beta\gamma}$ on purified PLA_2.

3.4 *Adenylyl cyclase*

This is one of the best studied examples of an effector regulated by a G_α subunit. cAMP production can be activated by GTP-γS bound $G_{\alpha s}$ and this activation is reversed by excess $G_{\beta\gamma}$ (Tang and Gilman 1991) probably by sequestering $G_{\alpha s}$ an inactive heterotrimer. Inactivation of $G_{\alpha s}$ by $G_{\beta\gamma}$ may be one mechanism for hormonal inhibition of adenylyl cyclase though direct Inactivation by other G_α subunits is also possible.

Four isoforms of adenylyl cyclase have been cloned (table 3). Type I is calmodulin sensitive and is present in the central nervous system. It is activated GTP-γs bound $G_{\alpha s}$ and inhibited by $G_{\beta\gamma}$. Type II and IV bear structural homology to type I adenylyl cyclase but are insensitive to calmodulin. They are moderately activated by $G_{\alpha s}$. However, they can be activated synergistically five to six-fold by $G_{\beta\gamma}$. Type III adenylyl cyclase can not be activated or inhibited by $G_{\beta\gamma}$ (Tang and Gilman 1991, 1992).

Table 3. Subtypes of adenylyl cyclase - response to G-protein subunits.

Type of adenylyl cyclase	Tissue location	$G_{s\alpha}$	$G_{\beta\gamma}$
Type I	Brain	+	–
Type II	Brain, lung	+	+
Type III	Olfactory	+	O
Type IV	Brain, other tissues	+	+

+, Stimulation; –, Inhibition; O, No action.

3.5 *Phospholipase C*

Phospholipase C (PLC) exists in several isoforms some of which are regulated by G-proteins. PLC $\beta_{1\text{-}4}$ are regulated by G-proteins while PLCγ is an exception since it is regulated by growth factors rather than by G-proteins (Rhee 1991). PLC $_{\beta 1\text{-}3}$ isoforms are regulated by both G_α subunits of the G_q class ($G_{\alpha q/11}$, $G_{\alpha l6}$) and by $G_{\beta\gamma}$ subunits (Blank *et al* 1992). PLC β_4 is regulated only by G_α. It has been shown that $G_{\beta\gamma}$ stimulates PLC β_3 most and PLC β_1 the least where as $G_{\alpha q/11}$ subunits stimulate PLC β_1 the most and PLC β_3 least. The action of the G_α and $G_{\beta\gamma}$ subunits is independent suggesting that they bind to different sites on the PLC β molecule. $G_{\beta\gamma}$ binds to N-terminal and G_α binds to the C-terminal region.

3.6 *Receptor kinases*

$G_{\beta\gamma}$ has been known to regulate receptor function directly by enhancing receptor interaction with G_α GDP. $G_{\beta\gamma}$ may also modulate receptor function by controlling the

location of receptor specific protein kinases. Agonist dependent phosphorylation of purified muscarinic and β_2 adrenergic receptors by their respective kinase has been shown to increase 10-fold by $G_{\beta\gamma}$ (Haga and Haga 1992; Pitcher *et al* 1992). In this process $G_{\beta\gamma}$ appears to move β-adrenergic receptor kinase (β-ARK) from the cytosol to the plasma membrane facilitating phosphorylation of agonist bound receptor. However, it is not clear whether the receptor activates the kinase or it is simply a substrate for β-ARK. This finding that the $G_{\beta\gamma}$ can regulate receptors by controlling their phosphorylation and subsequent desensitization gives an insight into the complex feed back mechanisms for the fine tuning of hormone responses.

3.7 G-protein mediated signalling events

3.7a *Photo-transduction in retina:* Photo -transduction in the retina serves an important role in converting light into neural signals. In vertebrate rod photoreceptor cells, this process occurs exclusively in the rod outer segment (ROS) and involves a light activated cGMP enzyme cascade (figure 4). Absorption of a photon by the receptor molecule, causes the photo-isomerization of the 11-cis-retinal chromophore

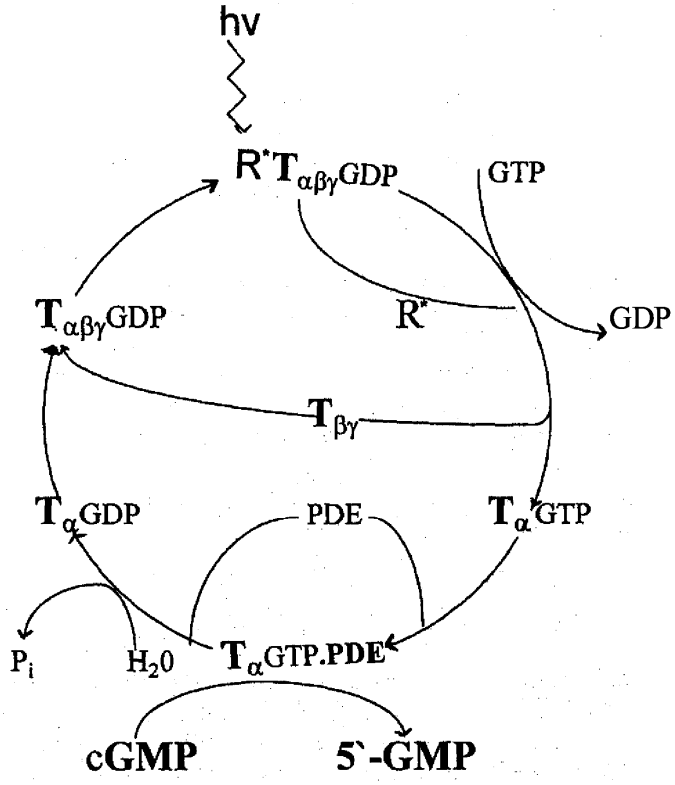

Figure 4. The signal coupling cycle of transducin in the retinal cGMP cascade. T represents transducin, R* photolysed rhodopsin and PDE is the cGMP phosphodiesterase. Figure adopted from Hingorani V N and Ho Y K page 97, G-Proteins as mediators of cellular signalling processes (eds.) M D Houslay and G Milligan, Willey publishers, 1990.

of rhodopsin to all trans-retinal. Photolyzed rhodopsin (R*) triggers a series of biochemical events that lead to the activation of a latent cGMP phosphodiesterase (PDE) which rapidly hydrolyses cytosolic cGMP. The coupling between R* and the latent PDE is mediated by a GTP binding protein called transducin (T) via a GTP binding and hydrolysis cycle. The activation signal is highly amplified, a single R* causes the hydrolysis of 10 cGMP molecules in a fraction of a second. The transient decrease of cGMP concentration causes the closure of the cGMP sensitive cation channels on the plasma membrane of the cell. Reduction of the cations permeability across the plasma membrane results in hyperpolarization of the cell.

The transducin in its latent form, contains a bound GDP (T-GDP). R* binds T-GDP to form a complex with reduced affinity for guanine nucleotides. As a result, bound GDP is exchanged for GTP, converting transducin to an active form (T-GTP), that switches on the PDE. The formation of T-GTP is accompanied by the release from R* which can then interact with another T-GDP. This rapid recycling of R* generates hundreds of active T-GTP and each is capable of activating PDE. Hence, signal amplification is achieved in two stages. In the first stage a gain of 10^2 due to the formation of hundreds of activated PDE and in the second stage a gain of 10^3 due to the rapid hydrolysis of thousands of molecules of cGMP/s by an activated PDE. Deactivation of the T-GTP involves an intrinsic GTPase activity of transducin which hydrolyses the bound GTP and converts the transducin to its latent form of T-GDP.

Transducin has three polypeptides T_α, $T\beta$ and 7_γ . PDE also has three polypeptides, two catalytic subunits P_α and $P\beta$ and an inhibitory subunit P. T_α contains the GTP binding site and is the activator of PDE. The GDP bound form of T_α has a high affinity for $T_{\beta\gamma}$ and rhodopsin a low affinity for PDE. R* catalyses the exchange of GTP for the bound GDP on T_α. The T_α-GTP complex then dissociates from $T_{\beta\gamma}$/R* and acquires a high affinity for the effector enzyme PDE. T_α-GTP interacts with P_γ removing the inhibition exerted by P_γ on the catalytic sites of PDE. T_β and T_γ form a tight complex and play a role in presenting T_α to R*. Individually T_α- GDP or $T\beta\gamma$ does not bind well to R*. However, a complex of T_α -GDP - $T_{\beta\gamma}$ tightly associates with R*. The dissociation of these 3 protein components ensures an irreversible step in the cascade (Houslay and Milligan 1990). Hence, GTP cannot bind to the $T_\alpha$site in the absence of R * and $T_{\beta\gamma}$, and the T_α bound GTP will not be released from the site easily which allows T_α to remain in its active GTP bound state long enough to find its target, a latent PDE. T_α-GTP can only be converted to its GDP bound form by the hydrolysis of the bound GTP which terminates the interaction with PDE.

3.8 *Ras and Ras related guanine nucleotide binding proteins*

In the last few years a novel class of small (21 to 24 kDa) monomeric guanine nucleotide binding proteins have been described ofwhich the prototype is *ras.* These proteins have some similarities with the classical G-proteins. They exist in a resting G D P form and an active GTP form. Though they are capable of hydrolysing GTP, this is not required for activity. Ras genes were first characterized as viral oncogenes carried by two closely related transforming retroviruses, Harvey Murine sarcoma virus (v-Ha-*ras*) and Kirsten Murine sarcoma virus (v-Ki-*ras*). A significant proportion of human malignancies contain a constitutively expressed *ras* oncogene. This leads to the production of an

oncoprotein carrying a single amino acid substitution either at position Glyl2/Glyl3 or Gln61. An understanding of the role of the three *ras* proteins in the control of normal bell growth and of the biochemical consequences of amino acid substitutions that ultimately lead to malignancy has become a valuable tool to explain the mechanisms of malignancy.

Each of three mammalian *ras* protooncogenes encodes a 21 kDa protein, $p21^{ras}$ and in case of protooncogenes N-ras and Ha-*ras,* the protein is 189 amino acids. All three are highly similar with a maximum of 15 differences between any two proteins within the first 164 amino acids. The C-terminal amino acids 186-189 form a conserved CAAX box (C-cysteine, A-aliphatic residue, X- any residue) required for correct post translation processing of $p21^{ras}$. These proteins have been localized to the inner surface of the plasma membrane (Willingham *et al* 1980) and mutational analysis has revealed that cys 186 is essential for a post translational modification step permitting membrane localization (Willumsen *et al* 1984).

Although mutations have been found at the residues 12/13, or 61 in human tumours, alterations at 59, 63,116,117 and 119 have also been implicated in oncogenic activation by *ras* protein (Trahey and McCormick 1987). A complete analysis of the possible substitutions at codon 12 reveals that Gly can be replaced by any other amino acid except proline (Seeburg *et al* 1984) and that Gin at 61 can be replaced by any amino acid except proline and glutamic acid (Der *et al* 1986) to generate a transforming gene. The biochemical basis of *ras* oncogenic activation by a point mutation results in the inability of these mutants to hydrolyze the GTP in the absence or presence of GAP (Vogel *et al* 1988). This leads to a continuous constitutive expression of *ras* oncoproteins favouring malignancy.

The biological phenomenon associated with oncogenic *ras* protein is cellular transformation. Introduction of an oncogenic *ras* gene into rodent fibroblast cell lines leads to cellular transformation. This is further strengthened by the formation of tumours when cells are injected into nude mice. However, caution should be exercised since all cells do not respond similarly to *ras* oncoprotein. In fibroblasts *ras* proteins stimulate cell proliferation, while in PC 12 cell line, *ras* blocks proliferation but induces differentiation to neuron like cells mimicking the action of nerve growth factor (Bar-Sagi and Feramisco 1985). It is assumed that the signal generated by *ras* is same in these two cell types but the target effect is different in different cells.

3.9 *The ras GTPase activating protein*

GTPase activation protein (GAP) predominantly located in cytoplasm of all eukaryotes except yeast, though *ras* is active at the plasma membrane. This 116 kDa GAP protein has been purified and cloned from bovine brain and human placenta (Gibbs *et al* 1988). It is likely that GAP translocated to the membrane to work enzymatically to,convert $p21^{ras}$GTP to $p21^{ras}$GDP (Adari *et al* 1988). It has weak (20%) homology to the regulatory subunit of yeast adenylyl cyclase.

3.10 *ras related proteins*

A growing number of *ras* related proteins have been identified in mammalian cells. The *ras* subfamily consists of Ras (with three isoforms H, K and N-Ras) R-ras, TC21 and

Rap (four isoforms, Rap 1A, 1B and Rap2A and 2B) (Wittinghofer and Nassar 1996). The use of oligonucleotide probes has been very useful in cloning sequences from cDNA libraries to reveal *ral,* the *rap* family *(rap* 1A, 1B and 2) and the *rab* genes (Darchen *et al* 1990; Mollard *et al* 1990; Denhardt 1996). Three *rho* genes A, B and C were identified in mammalian genomes (Denhardt 1996; Yeramian *et al* 1987). The SEC-4 gene was identified from a genetic analysis of a yeast strain defective in secretion. One *ras* related protein (r-*ras)* and probably 3 more *(rap* 1A, 1B and 2) interact with the same 116 kDa GAP as the three *ras* proteins. The mechanisms by which they regulate cell proliferation is yet to be investigated.

3.11 *Phosphoinositide cascade*

Phosphoinositide cascade is another ubiquitous cascade that is mediated by G-proteins and evokes many responses. This, like the adenylyl cyclase cascade, converts extracellular signals into intracellular ones. The intracellular messengers formed by activation of this pathway arise from phosphatidyl inositol 4, 5-bisphosphate (PIP_2) a phospholipid in the plasma membrane (figure 5). The binding of hormone such as

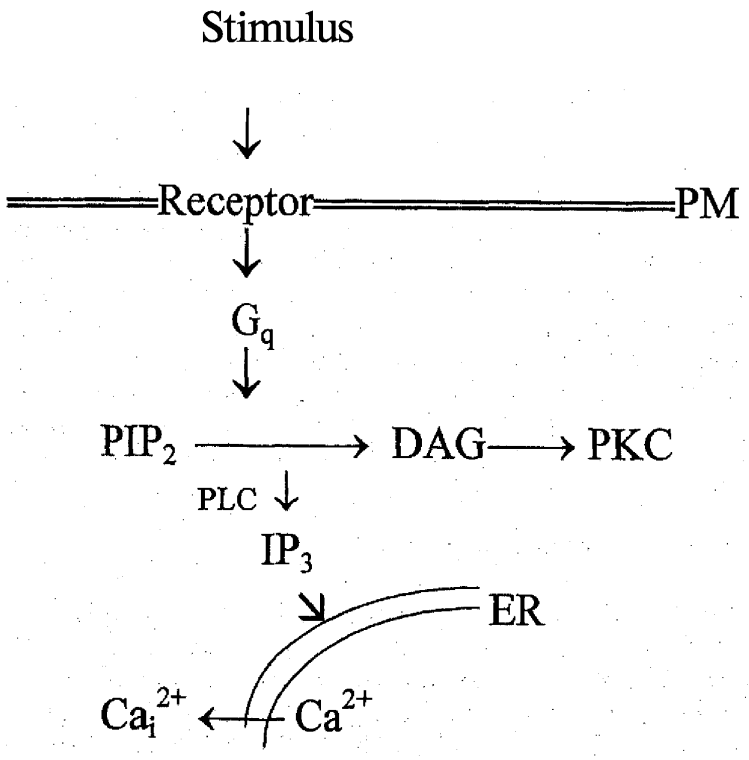

Figure 5. Phosphoinositide-derived second messengers in olfaction. Stimulus-activation of PLC is mediated by a G-protein (G_p) and catalyses the hydrolysis ofphosphatidylinositol-4,5-bisphosphate (PIP_2). IP_3 subsequently releases calcium from endoplasmic reticulum (ER), thereby elevating intracellular calcium levels. Calcium, together with diacylglycerol (DAG), may stimulate protein kinase C (C-kinase) (figure adopted from R C Bruch from page 121 of G-proteins as mediators of cellular signalling processes (eds.) M D Houslay and G Milligan, Wiley publishers, 1990).

vasopressin to a cell surface receptor leads to the activation of phospholipase C. This membrane bound enzyme hydrolyses the phosphodiester bond linking the phosphorylated inositol unit to the acylated glycerol moiety. Two messengers, inositol 1, 4, 5 triphosphate (I P_3) and diacylglycerol are formed by the cleavage of P I P_2.

PLCs are cytosolic enzymes that act on membrane inserted phosphoinositide substrates. Their enzymatic activity increases markedly when the calcium level is raised from 100 nm to 1 μm. A G-protein that specifically activates a PLC has been purified (Blank *et al* 1991; Sternweiss and Smrcka 1992). G_q increases the catalytic activity of the β_1 isoform of PLC and increases its affinity for Ca^{2+}. Several other PLC isoforms are also activated by G-proteins.

3.12 G-protein mediated signalling in olfaction

In vertebrates, peripheral chemosensory neurons in the olfactory epithelium detect olfactory stimuli, transduce stimulus encoded information into ion channel activity and transmit these ionic signals directly to the central nervous system. Peripheral olfactory neurons are bipolar cells with single, unbranched and unmyelinated axons collectively forming the olfactory nerve (Cranial nerve I) which synapses with second order neurons in the olfactory bulb. The single unbranched dendrites of the receptor cells project towards the external environment and terminate at the apical end in cilia or microvilli. Stimulus interaction with the apical dendrite cilia and microvilli of the primary chemosensory neurons initiates the subsequent transmembrane signal transduction events that lead to membrane depolarization, action potential generation and synaptic transmission (Lerner *et al* 1988).

Five members of the heterotrimeric family of signal transducing G-proteins have been identified in the olfactory epithelium. Both Gs and Gi were identified (the other two being G_{i2} and G_{i3}). A new G-protein identified in the olfactory neuroepithelium is the G_{olf} (figure 6) (Jones and Reed 1989).

4. Disease conditions associated with malfunctioning of G-proteins

4.1 *Chronic ethanol administration*

Acute exposure to ethanol has been shown by several investigators to lead to the stimulation of adenylyl cyclase activity (Gordon *et al* 1986), This occurs over a range of concentrations where ethanol increases lipid fluidity of the membranes. It is possible that this change provides the molecular basis for the stimulatory effect of ethanol.

Chronic exposure to ethanol has the opposite effect, that is, attenuating adenylyl cyclase activity in lymphocytes and platelets from alcoholic subjects. Part of this inhibitory effect may be due to the membrane lipid mediated adaptive response to chronic ethanol administration. This leads to cholesterol incorporation into membranes, which can lead to the attenuation of adenylyl cyclase activity (Whetton *et al* 1983). However, it is clear that such a lipid mediated effect cannot account for the magnitude of the diminished receptor-coupled adenylyl cyclase activity in cells treated chronically with ethanol. It was shown that chronic treatment of a neuroblastoma cell line (NG 108-15) with ethanol led to the reduced expresion of αG_s (Mochly-Rosen *et al* 1988). This was monitored by both reconstitution studies into cyc– cells and also by

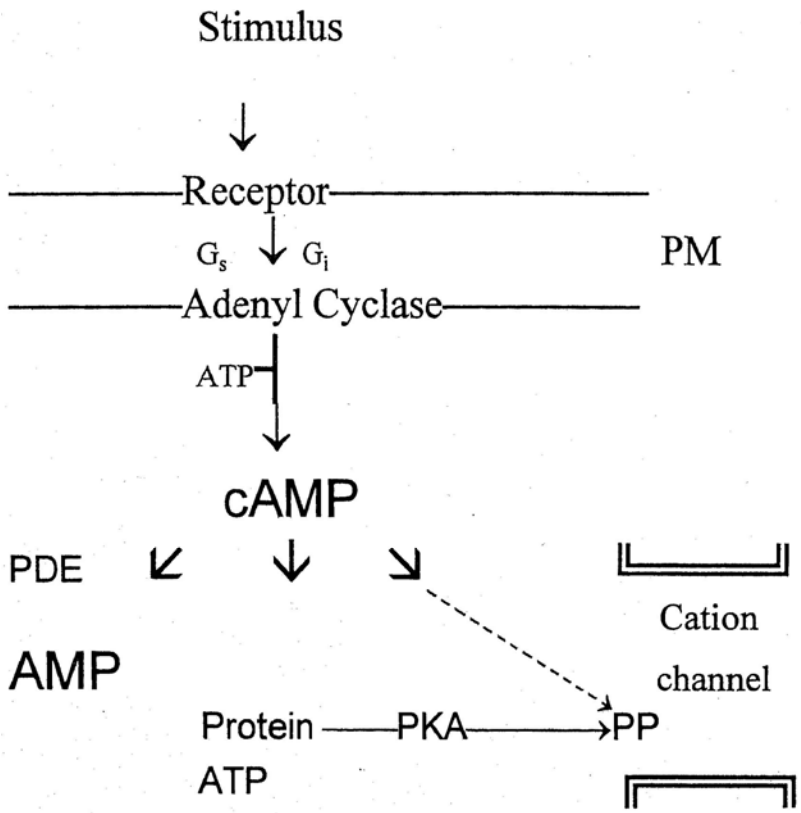

Figure 6. Second messenger role of cAMP in olfaction. Stimulus-activated elevation of cAMP in olfactory cilia leads to increases in memberane conductance by direct gating of cation channels. These channels may also be regulated by cAMP-dependent protein kinase (A-kinase), either by phosphorylation of channel proteins or by phosphorylation of an intermediary regulatory protein (dashed line). Dephosphorylation by, protein phosphatases, and degradation of cAMP by cyclic nucleotide phosphodiesterase, would lead to signal termination. (Figure adopted from R C Bruch, page 119 of G-Proteins as mediators of cellular signalling processes (eds.) M D Houslay and G Milligan, Wiley publishers, 1990).

using a specific anti-peptide antibody. The lesion appeared to be due to decreased synthesis of αG_s than enhanced degradation, as the mRNA levels were also reduced comparatively. This reduction did not appear to reflect a general, non specific effect on either transcription or mRNA stability. It will be of interest to define the extent and mechanism(s) whereby ethanol alters the mRNA concentrations of αG_s and other species. Ethanol probably exerts complex and Pleiotropic effects upon cellular functioning,

4.2 *Diabetes and insulin -resistant states*

Treatment of rats with either streptozotocin or alloxan elicits the destruction of the β cells which secrete insulin. This induces diabetes and also causes insulin resistance in

liver and adipose tissues. In hepatocytes and adipocytes from such diabetic rats, there appears to be total loss of functional G_i. This has been assessed by the failure of either p(NH)ppG or GTP to inhibit adenylyl cyclase activity in membranes derived from diabetic, but not from normal animals. However, inhibitory coupling of receptors to adenylyl cyclase is not abolished in either intact hepatocytes or adipocytes from such diabetic animals. In streptozotocin induced diabetic rats, hepatocytes had marked decrease in α_{i2}, α_{i3}, a moderate decrease in α_3 and no changes in $\beta\gamma$ subunits (Bushfield *et al* 1990; Griffiths *et al* 1990). The α_{i2} from diabetic hepatocytes was found phosphorylated, a key event, which is suspected to be involved in impairing the function of α_{i2} in diabetics. Such a phosphorylation of α_{i2} is evidently absent in the hepatocytes from control animals. In alloxan induced diabetes, a functional decrease in all the α_i subunits has been noticed in hepatocytes, adipocytes and even in corpus striatum (Abbracchio *et al* 1989). In non insulin dependent diabetics, decreased amounts of α_{i2} and α_{i3} have been reported in the platelet membranes (Livingstone *et al* 1991). The apparent loss of Gi function in these various diabetic conditions could possibly arise due to (i) reduced or loss of expression of G_i, (ii) expression of an inactive form of G_i and (iii) Inactivation of G_i by post translational modification such as phosphorylation. Since all these possibilities can be operative, as such, the exact mechanism involved still remains elusive, though these studies suggest that G-proteins do have a role in the pathogenesis of diabetes.

4.3 *Growth hormone secreting pituitary adenomas*

Secretion from somatotrophic cells is known to be controlled by alterations in intracellular concentrations of cAMP. A number of human growth hormone secreting adenomas have been shown to have abnormally high intracellular concentrations of cAM P and very high ratios of secretion of GH in the absence of stimulation by growth hormone releasing hormone (GHRH) (Landis *et al* 1989).

When the adenylyl cyclase activity of GH secreting adenomas was studied in isolated membranes, it was found hyper-responsive to stimulation by Mg^{2} +. This suggests that either G_S or adenylyl cyclase in these tumour cells has adopted a fully activated conformation. It appears that in the G_S activated group a mutant form of αG_s is produced which adopts an active conformation (Landis *et al* 1989). The molecular basis which gives rise to mutant form of αG_s is likely to be of interest and should shed light on how the structure of this protein is related to its functioning. However, such a mutant form of αG_s is not common in all hyper-secreting adenomas, but observed in a substantial group (Clementi *et al* 1990).

Experiments with a number of GH-secreting pituitary tumours which constitutively activate adenylyl cyclase have led to the cloning and sequencing of αG_s genes. Two specific types of mutations have been noticed in αG_s leading to its constitutive activation (Landis *et al* 1989). One mutation occurs at position 227 where glutamine is replaced by arginine. The position of glutamine here is analogous to that of Glu 61 in $p21^{ras}$ and lies in the GTP binding domain. This mutation in $p21^{ras}$ leads to the production of an oncoprotein with reduced GTPase activity (Bos 1989). Thus G-proteins appear to share a common GTP-binding domain where cognate mutations lead to similar functional effects. The second mutation occurs at position 201 where arginine is mutated to cysteine or histidine. These mutations are not within the GTP-binding domain. In $p21^{ras}$ mutation of this residue functions as a target for

ADP-ribosylation by cholera toxin. Either cholera toxin mediated APD-ribosylation or point mutations at position 201, leads to constitutive activation of this mutant G-protein and to a reduced GTPase activity. Another possibility is the αG_s could be an oncoprotein in those cell types where increased synthesis of cAMP leads to cellular proliferation as seen in case of pituitary somatotrophs (Castrillo *et al* 1991).

4.4 *S49 lymphoma cell line*

A mutant *cyc*$^-$ derived from this cell line provided the means for the first unequivocal demonstration of a G-protein namely G_s. The underlying selection mechanism which has proved to be so useful is that, elevation of intracellular cAMP levels is cytotoxic to these cells. Thus mutants in cAMP metabolism and in protein kinase-A have been identified. One such mutant is the *cyc* $^-$ cell line which fails to express G_S while still expressing β-adrenoreceptor and adenylyl cyclase. As such its plasma membranes offer a useful reconstitution system for G_s.

It will be interesting to see if further mutations are found in G-proteins of particular aberrant cells. Another mutant cell line called UNC (Haga *et al* 1977) has been identified with an aberrant G_s protein. This aberrant protein was found capable of stimulating adenylyl cyclase when activated directly using either Na(Al)F or the p(NH)ppG, but fails to mediate β-adreno receptor. Sequence analysis of the αG_s gene from these cells shows a single point mutation ARG-PRO at position 372 located at the C-terminal end of these proteins. This also supports the suggestion that the receptor interaction domain of G-protein α-subunits is at the C-terminal end. Another mutant form of S49 cells is the cell line called H21a. The form of G_S expressed in these cells is capable of coupling to β-adrenoreceptor but it is incapable of coupling to adenylyl cyclase (Miller *et al* 1988). Sequence analysis of the αG_s from such a cell line shows a point mutation at residue 226 where glycine is replaced by alanine. It will be of interest to see if mutations are found in G-proteins of particular aberrant cells.

4.5 *Diseases caused by mutations in G- protein-coupled receptors*

4.5a *G-Protein-coupled receptor linked diseases in humans:* The following diseases are known to have mutations or gene rearrangements in receptors

Disease	Receptor	Mode of inheritance	Type and function of mutation (−) = Loss of function (+) = Gain of function
Retinitis pigmentosa	Rhodopsin	AD	(−) apoptosis of rod cells
Retinitis pigmentosa	Rhodopsin	AR	(−) null mutations
Stationary night blindness	Rhodopsin	AR	(+) missense mutations
Colour blindness	Red and green opsins	X-linked	(−) X chromosome rearrangements
Nephrogenic DI	V2-receptor	X-linked	(−)
Isolated glucocorticoid deficiency	ACTH-receptor	AR	(−)
Hyperfunctioning thyroid adenomas	TSH-receptor	Somatic mutation	(+) missense
Familial precocious puberty	LH-receptor	AD male limited	(+) missense
Familial hypocalciuric hypercalcemia	Ca^{2+} sensing receptor	AD	(−) missense
Neonatal severe hyperparathroidism	Ca^{2+} sensing receptor	AR	(−) missense

4.5b *G protein-coupled receptor linked diseases or phenotypes in other animals*

Receptor	Phenotype	Type and function of mutation (−) = Loss of function (+) = Gain of function
MSH receptor	Coat colour in mice (extension locus)	(+)
GHRH receptor	Little mouse	(−)

(Further information on this can be obtained from http://receptor.mgh.harvad.edu/GCRs-disease.html using internet facility. The information shown in these two tables is downloaded from this site.)

5. Conclusion

Any molecular defect in the structure of G-protein is enough to induce a wrong signal, altering the defined function of Grproteins. For any cell, in order to be able to explicitly allow right signals and transduce them inside for a right response depends on the molecular integrity of G-proteins. Allowing inappropriate responses will contribute to abnormal cell function and hence new insights into the functioning of G-proteins will help unravel the mechanisms of cellular network of complex inflow of information processing and response. Recent studies showing the crystal structure of G-proteins as well as the biochemical data strongly suggest that there are some common and some variable features in the molecular architecture of GTP binding proteins with their effectors. More effectors for G-proteins are being discovered and it is emerging that G-proteins interact with more than one effector to generate more than one signal.

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References

Abbrachio M P, DiLuca M, DiGiulo A M, Cattabeni F, Tenconi B and Gorio A 1989 Denervation and hyper-innervation in the nervous system of diabetic animals: III Functional alterations of G-proteins in diabetic encephalopathy; *J. Neurosci. Res.* **24** 517-522

Adari H, Lowy D R, Willumsen B M, Der C J and McCormick F 1988 Guanosine Triphosphatase Activating Protein (GAP) interacts with the $p21^{ras}$ effector binding domain; *Science* **240** 518-521

Arshavsky V Y and Bownds M D 1992 Regulation of deactivation of photoreceptor G-protein by its target enzyme and cGMP; *Nature (London)* **357** 416-417

Asano T, Katada T, Gilman A G and Ross E M 1984 Activation of the inhibitory GTP binding protein of adenylate cyclase, Gi, by β-adrenergic receptors in reconstituted phospholipid vesicles; *J. Biol. Chem,* **259** 9351-9354

Backlund P S Jr, Simonds W F and Spiegel A M1990 Carboxy-methylation and CO OH -terminal processing of the brain G-protein ã subunit; *J. Biol. Chem.* **265** 15572-15576

Bar- Sagi D and Feramisco J K 1985 Micro injection of the *ras* oncogene protein into PC-12 cells induces morphological differentiation; *Cell* **42** 841-848

Bernstein G, Blank J L, Jhon D Y, Exton J H, Rhee S G and Ross E M 1992 Phospholipase C-β is GTPase activating protein for $G_{q/111}$, its physiologic regulator; *Cell* **70** 411-418

Blank J L, Ross A H and Exton J H 1991 Purification and characterization of two G-proteins that activate the β-l isozyme of phosphoinositide-specific phospholipase C. Identification as members of the G_q class; *J. Biol. Chem.* **266** 18206-18216

Blank J L, Brattain K A and Exton J H 1992 Activation of cytosolic phosphoinositide phospholipase C by G protein $\beta\gamma$ subunits; *J. Biol. Chem.* **267** 23069-23075

Bos J L 1989 *ras* oncogene in human cancer: a review; *Cancer Res.* **49** 4682-4690

Bubis J and Khorana H C 1990 Sites of interaction in the complex, between β and γ subunits of transducin; *J. Biol. Chem.* **265** 12995-12999

Bushfield M, Griffiths S L, Murphy G J, Pyne N J, Knowler J T, Milligan G, Parker P J, Mollner S and Houslay M D 1990 Diabetes induced alterations in the expression, functioning and phosphorylation state of the inhibitory guaninenucleotide regulatory protein $G_{i\text{-}2}$ in hepatocytes; *Biochem. J.* **271** 365-372

Cali J J, Balcueva E A, Rybalkin I and Robishaw J D 1992 Selective tissue distribution of G-protein γ-subunits including a new form of the γ subunit identified by cDNA cloning; *J. Biol. Chem.* **267** 24023-24027

Castrillo J L, Theill L E and Karin M 1991 Function of the homeodomain protein G H F_1 in pituitary cell proliferation; *Science* **253** 197-200

Clapham D E and Neer E J 1993 New roles for G-proteins $\beta\gamma$ dimers in transmembrane signalling; *Nature* (*London*) **365** 403-406

Clementi E, Malgareti N, Meldolesi J and Taaramelli R1990 A new constitutively activating mutation of the G_S protein α-subunit gsp oncogene is found in human pituitary tumours; *Oncogene* 5 1059-1064

Darchen F, Zahraovi A, Hammel F, Monteils M P, Tavitian A and Scherman D 1990 Association of the GTP-binding protein Rab 3A with bovine adrenal chromaffin granules; *Proc. Natl. Acad. Sci. U.S.A* **87** 5692-5696

Denhardt D T 1996 Signal transducing protein phosphorylation cascades mediated by Ra/Rho proteins in the mammalian cell: the potential for multiplex signalling; *Biochem. J.* **318** 729-747

Der C J, Finkel T and Cooper G M 1986 Biological and biochemical properties of human ras^H genes mutated at codon 61; *Cell* **44** 167-176

Dohlman H G, Thorner J, Caron M G and Lefkowitx R J 1991 Model systems for the study of seven transmembrane segment receptors; *Annu. Rev. Biochem.* **60** 653-688

Gaudet R, Bohm A and Sigler P B 1996 Crystal structure at 2.4 A resolution of the complex of transducin $\beta\gamma$ and its regulator; phosducin; *Cell* **87** 577-588

Gibbs J B, Schaber M D, Allard W J, Sigal IS and Scolnick G M 1988 Purification of *ras* GTPase activating protein from bovine brain; *Proc. Natl. Acad. Sci. USA* **85** 5026-5030

Gordon A S, Coller K and Diamond I 1986 Ethanol regulation of adenosine receptor-stimulated cAMP levels in a clonal neural cell line: An in vitro model of cellular tolerance; *Proc. Natl. Acad. Sci. USA* **83** 2105-2118

Griffiths S L, Knowler J T and Houslay M D 1990 Diabetes induced changes in guanine-nucleotide regulatory protein mRNA detected using synthetic oligonucleotide probes; *Eur. J Biochem.* **193** 367-374

Gupta S K, Diez E, Heasley L E, Qsawa S and Johnson G L1990 A G-protein mutant that inhibits thrombin and purinergic receptor activation of phospholipase A_2; *Science* **249** 662-666

Haga K and Haga T 1992 Activation by G-protein βy subunits of agonist or light dependent phosphorylation of muscarinic acetylcholine receptors and Rhodopsin; *J. Biol. Chem.* **267** 2222–2227

Haga T, Ross E M, Anderson H J and Gilman A G 1977 Adenylyl cyclase permanently uncoupled from hormone receptors in a novel variant of S49 mouse lymphoma cells; *Proc. Natl. Acad. Sci. USA* **74** 2016–2020

Houslay M D and Milligan Graeme 1990 *Molecular Pharmacology of Cell regulation.* Vol 1. *G proteins as mediators of cellular signalling processes* (Brisbane: John Wiley)

Itoh H, Toyama R, Kozasa T, Tsukamoto T, Matsuoka M and Kaziro T 1988 Presence of three distinct molecular species of G; protein α subunit; *J. Biol. Chem.* **263** 6656-6664

Itoh H, Tung R T, Sugimoto T, Kobayashi I, Takahashi K, Katada T, Ui M and Kurachi Y 1992 On the mechanism of G-protein $\beta\gamma$ subunit activation of the muscarinic κ+ channel in guinea pig atria cell membrane. Comparison with the ATP-sensitive K^+ channel; *J. Gen. Physiol.* **99** 961-983

Jelsema C L and Axelrod J 1987 Stimulation of Phospholipase A_2 activity in bovine rod outer segments by the $\beta\gamma$ subunits of Transducin and its inhibition by the α subunit; *Proc. Natl. Acad. Sci. USA* **84** 3623-3627

Jones D T and Reed RR 1989 G_{olf}:An olfactory neuron specific-G protein involved in odorant signal transduction; *Science* **244** 790-795

Jones T L Z, Simonds W F, Merendino J J Jr, Brann M R and Spiegel A M 1990 Myristoylation of an inhibitory GTP-binding protein α-subunit is essential for its membrane attachement; *Proc. Natl. Acad, Sci. USA* **87** 568-570

Kaziro Y, Itoh H, Kozasa T, Nakafuku M and Satoh T 1991 Structure and function of signal transducing GTP binding proteins; *Annu. Rev. Biochem.* **60** 349-400

Kim D, Lewis D L, Gaziadi L, Neer J E, Bar-sagi D and Clapham D E 1989 G-protein *βγ* subunits activate the cardiac muscarinic K^+ channel via Phospholipase A_2; *Nature* (*London*) **337** 557-560

Kim S, Ang S L, Bloch D B, Bloch K D, Kawahara Y, Tolman C, Lee R, Seidman J G and Neer E J 1988 Identification of cDNA encoding an additional α-subunit of a human GTP-binding protein. Expression of three α_i sub-types in human tissues and cell lines; *Proc. Natl. Acad. Sci. USA* **85** 4153-4157

Kleus C, Scherubl H, Hescheler J, Schutz G and Wittig B 1992 Different *β* subunit determine G protein interaction with transmembrane receptors; *Nature* (*London*) **358** 424-426

Kleus C, Scherubl H, Hescheler J, Schutz G and Wittig B1993 Selectivity in signal transduction is determined by *γ* subunit of heterotrimeric G proteins; *Science* **259** 832-834

Koch C A, Anderson D, Moran M F, Ellier C and Pawson T 1991 SH2 and SH3 domains: Elements that control interactions of cytoplasmic signalling proteins; *Science* **252** 668-674

Kozasa T, Itoh H, Tsukamoto T and Kaziro Y 1988 Isolation and characterization of the human G_S α gene; *Proc. Natl Acad. Sci. USA* **85** 2081-2085

Landis C A, Masters S B, Spada A, Pace A M N, Bourne H R and Vallar L1989 GTPase inhibitory mutations activate the α-chain of G_S and stimulate adenylyl cyclase in human pituitary tumours; *Nature* (*London*) **340** 692-696

Lee R H, Ting T D, Lieberman B S, Tobias D E, Lolley R N and Ho Y K 1992 Regulation of retinal cGMP cascade by phosducin in bovine rod photoreceptor cells. Interaction of phosducin and transducin; *J. Biol. Chem.* **267** 25104-24112

Lerner M R, Reagen J, Gyorgyi T and Roby A 1988 Olfaction by melanophores: What does it mean?; *Proc. Natl. Acad. Sci. USA* **85** 261-264

Linder M E, Middleton P, Hepler J R, Taussig R, Gilman A G and Mumby S M 1993 Lipid modifications of G-proteins: α-subunits are palmitoylated; *Proc. Natl Acad. Sci. USA* **90** 3675-3679

Livingstone C, McLellan A R, McGregor M A, Wilson A, Connel J M C, Small M, Milligan G, Paterson K R and Houslay M D 1991 Altered G-protein expression and adenylate cyclase activity in platelets of non-insulin dependent diabetic (NIDDM) male subjects; *Biochem. Biophys. Acta. Mol Basis Dis.* **1096** 127-132

Logothetis D E, Kurachi Y, Galper J, Neer E J and Clapham D E 1987 The *βγ* subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart; *Nature* (*London*) **325** 321-326

Logothetis D E, Kim D, Northup J L, Neer E J and Clapham D E 1988 Specificity of action of guanine nucleotide binding regulatory protein subunits on the cardiac muscarinic K + channel; *Proc. Natl. Acad Sci. USA* **85** 5814-5818

Lupas A N, Lupas J M and Stock J B 1992 Do G-protein subunits associate via a three-stranded coil?; *FEBS Lett.* **314** 105-108

Maltese W A and Robishaw J D 1990 Isoprenylation of C-terminal cysteine in a G-Protem γ-subumt; *J. Biol. Chem.* **265** 18071-18074

Masters S B, Sullivan K A, Miller R T, Beiderman B, Lopez N G, Ramachandran J and Bourne H R 1988 Carboxyl-terminal domain of G_S α specifies coupling of receptors to stimulation of Adenylyl cyclase; Science **241** 448-453

Matsuoka M, Itoh H, Kozasa T and Kaziro Y 1988 Guanine nucleotide binding proteins that enhance choleragen ADP-ribosyl transferase activity; *Proc. Natl Acad. Sci. USA* 85 5388-5391

Miller R T, Masters S M, Sullivan K A, Beiderman B and Bourne H R 1988 Mutation that prevents GTP dependent activation of the α chain of G_s; *Nature* (*London*) **334** 712-715

Mochly-Rosen D, Chang F H, Cheever L, Kim M, Diamond I and Gordon A S 1988 Chronic ethanol causes heterologous desensitization of receptors by reducing α_s messenger RNA; *Nature* (*London*) 333 848-850

Mollard G V F, Megney G A Baumert M, Perm M S, Hanson T J, Burger P M, Jahn R and Sudhof T C 1990 *rab* 3 is a small GTP-binding protein exclusively localized to synaptic vesicles; *Proc. Natl. Acad. Sci. USA* **87** 1988-1992

Mumby S M, Casey P J, Gilman A G, Gutowski S and Sternweiss P C 1990a G-protein γ-subumts contain a 20-carbon isoprenoid; *Proc. Natl. Acad. Sci. USA* **87** 5873-5877

Mumby S M, Heukeroth R O, Gordon J I and Gilman A G 1990b G-protein α-subunit experiments, myristoylation and membrane association in COS cells; *Proc. Natl. Acad. Sci. USA* **87** 728-732

Muntz K H, Sternweiss P C, Gilman A G and Mumby S M 1992 Influence of *y* subunit prenylation on association of guanine nucleotide binding regulatory proteins with membranes; *Mol. Biol. Cell* **3** 49-61

Neer E J 1994 G proteins: Critical control points for transmembrane signals; *Protein Sci.* **3** 3-14

Neer E J 1995 Heterotrimeric G-Proteins: Organizers of transmembrane signals; *Cell* **80** 249-257

Noel J P, Hamm H E and Sigler P B 1993 The 2-2 A crystal structure of transducin-α complexed with GTPγS; *Nature* (*London*) **366** 654-661

Pennington S R 1994 *GTP binding proteins: Protein Profile* Vol 1. (Review on G-Proteins) (New York: Academic press) pp 169-234

Pitcher J A, Inglese J, Higgins J B, Arriza J L, Casey J, Kim C, Benovii J L, Kwatra M M, Caron G M and Lefkowitz R J 1992 Role of *β*γ subunits in targetting the *β*-adrenergic recetpor kinase to membrane bound receptor; *Science* **257** 1264-1267

Pronin A N and Gautam N 1992 Interaction between G protein *β* and y subunit types is selective; *Proc. Natl. Acad. Sci. USA* **89** 6220-6224

Rahtnatullah N and Robishaw J D 1994 Direct interaction of the α and *y* subunits of G proteins; *J. Biol. Chem.* **269** 3574-3580

Raport C J, Dere B and Hurley J 1989 Characterization of the mouse rod transducin α subunit gene; *J. Biol. Chem.* **264** 7122-7128

Rhee S G 1991 Inositol phospholipid specific phospholipase C: interaction of the y_t isoform with tyrosine kinase; *TIBS* **16** 297-301

Sanford J, Codina J and Birnbaumer L 1991 γ-subunits of G-proteins, but not their α or *β* subunits are polyisoprenylated. Studies on post-translational modifications using in vitro translation with rabbit reticulocyte lysates; *J. Biol. Chem.* **266** 9570-9579

Savarese T M and Fraser C M 1992 In vitro mutagenesis and the search for structure-function relationships among G proteins-coupled receptors; *Biochem. J.* **283** 1-19

Seeburg P H, Colby W W, Capon D J, Goeddel D V and Levinson A D 1984 Biological properties of human C-Ha-ras 1 genes mutated at codon 12; *Nature* (*London*) **312** 71-75

Simon M I, Strathmann M P and Gautham N 1991 Diversity of the G proteins in signal transduction; *Science* **252** 802-808

Simonds W F, Butrynski J E, Gautham N, Unson C G and Spiegel A M 1991 G protein *βy* dimers; *J. Biol. Chem.* **266** 5363-5366

Spiegel A M, Backlund P S Jr, Butrynski J E, Jones T L Z and Simonds W F 1991 The G protein connection. Molecular basis of membrane association; *TIBS* **16** 338-341

Spiegel A M, Shenker A and Weinstein L S 1992 Receptor-effector coupling by G-proteins: implications for normal and abnormal signal transduction; *Endocriol. Rev.* **13** 536-565

Sternweiss P C and Smrcka A V 1992 Regulation of phospholipase C by G-proteins; *TIBS* **17** 502-506

Strathmann M P, Wilkie T M and Simon M 11990 Alternative splicing produces transcripts encoding two forms of the α subunit of GTP binding protein (G_o); *Proc. Natl. Acad. Sci. USA* **87** 6477-6481

Tang W J and Gilman A G 1991 Type specific regulation of adenylyl cyclase by G protein *βy* subunits; *Science* **254**1500-1503

Tang W J and Gilman A G 1992 Adenylyl cyclases: Minireview; *Cell* **70** 869-872

Trahey M and McCormick F 1987 A cytoplasmic protein stimulates Normal N *ras* p21 GTPase but does not afect oncogenic mutants; *Science* **238** 542-545

Vogel U, Dixon RAF, Schaber M D, Diehl R E, Marshall M S, Scolnick G M, Sigal IS and Gibbs J B 1988 Cloning of bovine GAP and its interaction with oncogenic *ras* p21; *Nature (London)* **335** 90-93

Watson A J, Katz A and Simon M 11994 A fifth member of the mammalian G-protein jS-subunit family;*J. Biol. Chem.* **269** 22150-22156

Whetton A D, Gordon L M and Houslay M D 1983 Elevated membrane cholesterol concentration inhibit glucagon stimulated adenylyl cyclase; *Biochem. J.* **210** 437-449

Wieland T, Nürnberg B, Ulibarri I, Kaldenberg-Stasch S, Schultz G and Jakobs K H 1993 Guanine nucleotide specific phosphate transfer by guanine nucleotide binding regulatory protein *βγ* subunits: Characterization of the phosphorylated amino acid; *J. Biol. Chem.* **268** 18111-18118

Willumsen B M, Norns K, Papageorge A G, Hubert N L and Lowy D R1984 Harvey murine sarcoma virus p21 *ras* protein: Biological and biochemical significance of the cysteine nearest the carboxy terminus; *EMBO J.* **3** 2581-2585

Willingham M C, Pastan I, Shih T Y and Scolnick E M 1980 Localization of the src gene product of the Harvey strain of MSV to plasma membrane of tranformed cells by electron microscopic immunocytochemistry; *Cell* **19**1005-1014

Wittinghofer A and Nassar N 1996 How Ras-related proteins talk to their eifectors; *TIBS* **21** 488-491

Yamane H C, Farnsworth C C, Xu H, Howald W, Fung B K K, Clarke S, Gelb M H and Glomset J A 1990 Brain G-protein *y* subunits contain an all trans geranylgeranyl- cysteine methyl ester at their carboxyl termini; *Proc. Natl. Acad. Sci USA* **87** 5868-5872

Yeramian P, Chardin P, Madaule P and Tavitian A1987 Nucleotide sequence of human rho cDNA clone 12; *NucleicAcids Res.***15**1869-1874

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