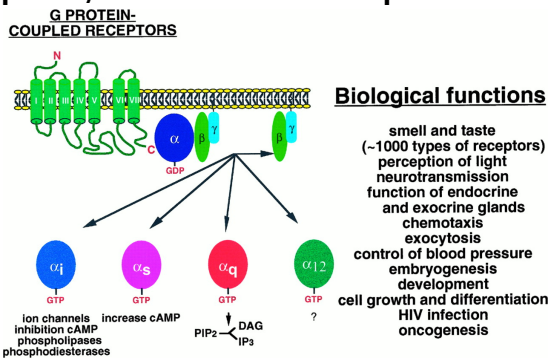
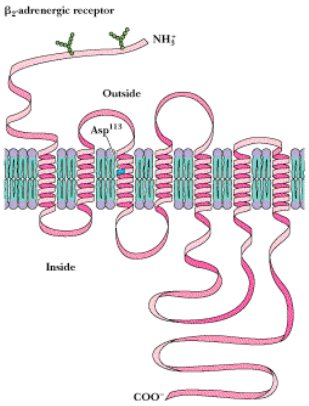
# G-Protein Coupled Receptors - Seven pass receptors / Heterotrimeric G proteins -

- many seven pass receptors with unknown number of ligands / hormones
- all receptors act through G proteins




There are several different classifications of receptors that couple signal transduction to G-proteins. These classes of receptor are termed G-protein coupled receptors, GPCRs.

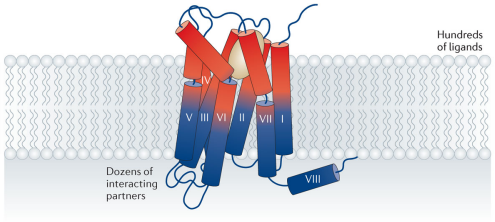
> 800 human GPCR genes account for >2% of human genome and are the target of 40-50% of all drugs on the market

- These proteins possess seven transmembrane spanning domains
- The cytosolic side has the N-terminal and is glycosylated
- Three ec loops and three cytosolic loops.
- Carboxy terminus is Intracellular
- Binding of hormone to the receptor initiate a twisting of two or more of the TM helixes.

## GPCR Classification

Largest single class of membrane proteins in genome. > 800 GPCRs (human) classified into five- seven major divisions (two non-human). Many orphan receptors.

- ~ 350 non-sensory (light, smell, taste) ligands
- Old mechanism was based on cAMP or Ca2+ signaling
- New system involves agonsit, sequence, structure and signaling partners. (Scripps GPCR Network)




## Classes

**Class A (Rhodopsin-Like)**: small molecules, neurotransitters, peptides, olfactory, visual, taste type 2, pheromone IV

**Class B (Secretin):** 15 genes glucagon, glucose insulinotropic peptide, vasoactive intestinal peptide..

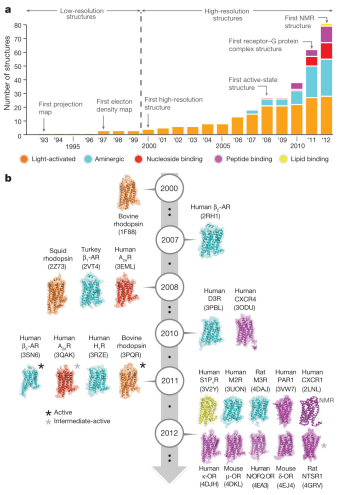
**Adhesion** – related to Class B, but include large N term (~320 aa) proteolysis domain.

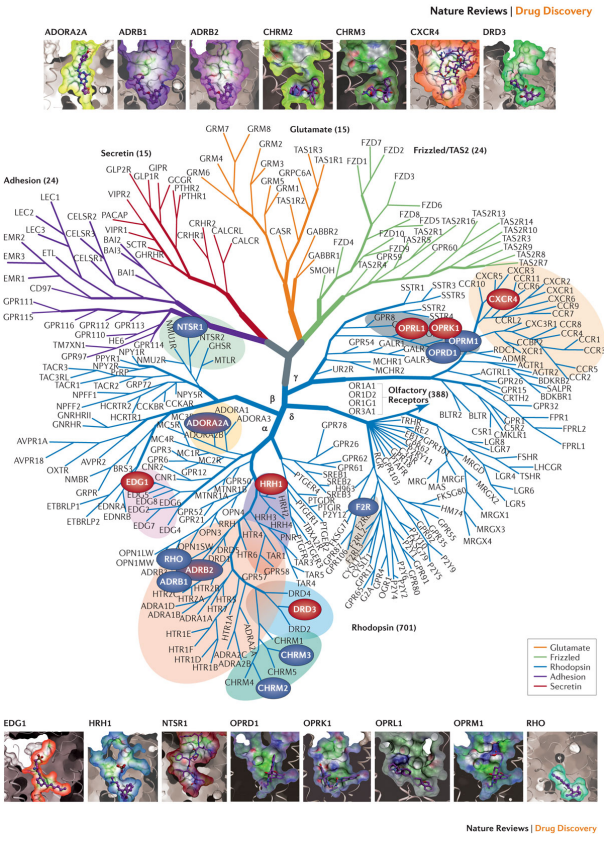
**Class C** (Metabotropic glutamate): Glu, GABA, Ca-sensing, taste type I, pheromones in rodents but not human

**Class D/E** fungal mating and cAMP non human receptors

**Class F** (Frizzled/smoothened): Cystein rich ligands and lipoglycoproteins. WNT and Hedghog signaling

- First structure was Rhodopsin, over 14 Class A structures determined, many more to be determined



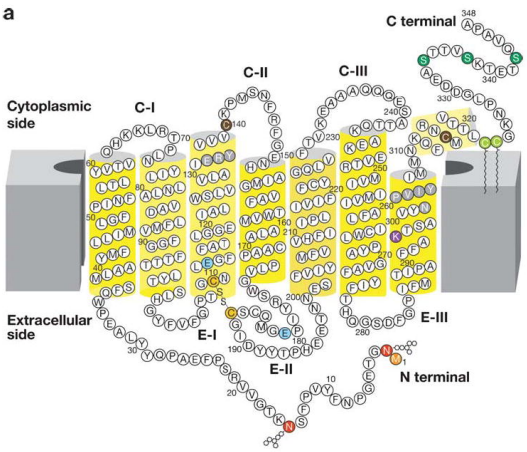
## Based on Rhodopsin

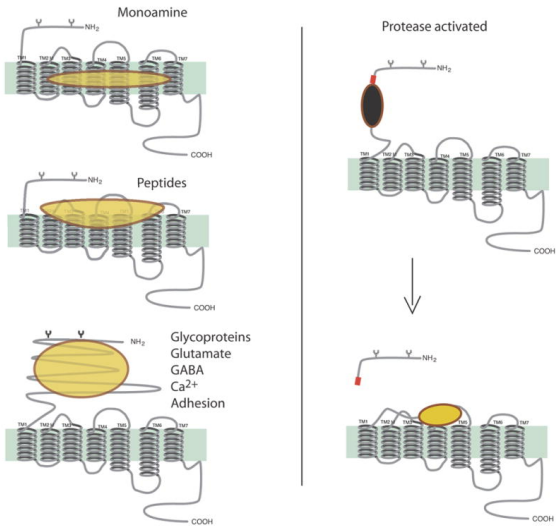
GPCR who's ligand is light (retinal bound to lysine via shiff base undergoes a cis/trans conversion)

First sequence then structure helped build insight to function of GPCR.

***Key features***:

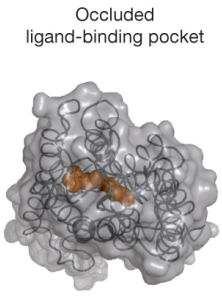
- Two dimensional – "snake" model
- Helical segment stability increased by Cys-Cys dark yellow.
- Extracellular glycosylation
- Green Ser – phosph sites
- Light green Cys palmitoylated
- Gray aa – support inactive to G-Protein conformation



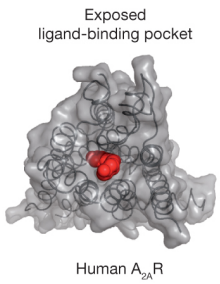




## Extracellular ligand binding pocket

Ligands bind at either the N terminus, within the surface of the N terminus-extra cellular loops or presumably through membrane – within the TM for hydrophobic ligands




Bovine rhodopsin



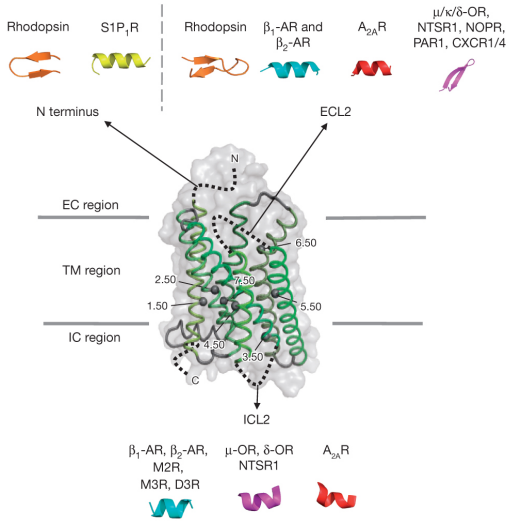

Human $A_{2A}R$

## N term and EC Loops

Top down view of GPCR – showing hidden or exposed ligand binding pocket. The two common types of binding pocked for water accessible ligands

## N term and EC Loops

- N term can form a beta loop , 3-turn a helix to forma lid and binds with EC loops for hydrophobic ligands
- GPCR for water soluble ligands often have EC loop 2 form helices or sheets to fold over and help form "funnel" for ligands to fit into binding pocket and plays a role in selectivity of ligand
- Cys between TM and ECs help stabilize by limiting conformational changes during activation

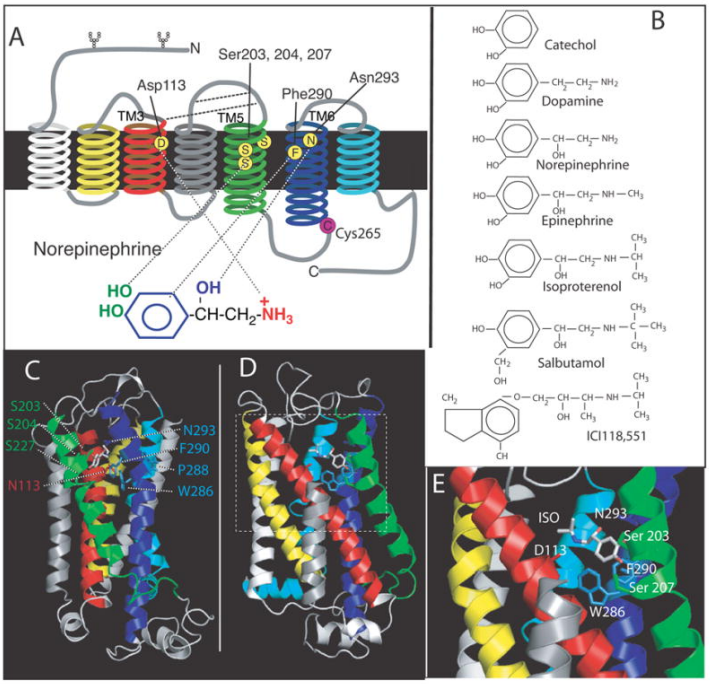


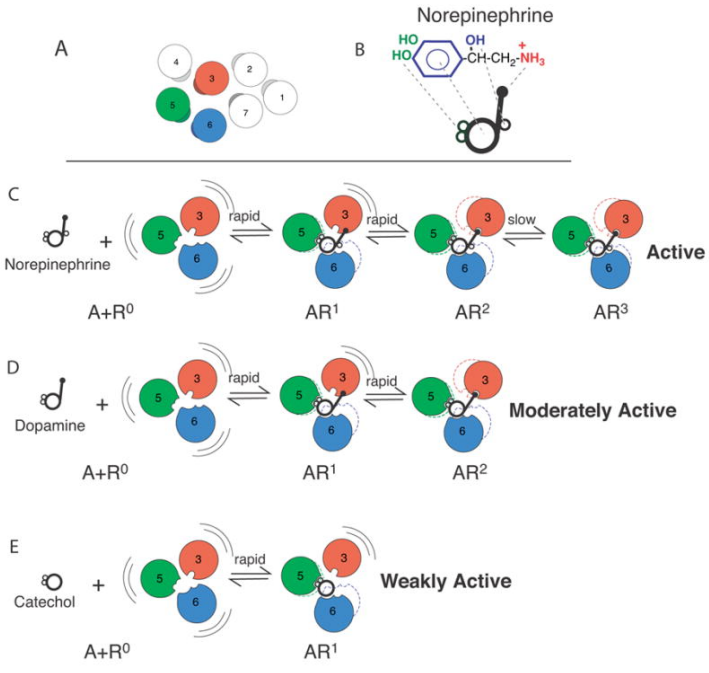

Mutations:

## Adrenergic Ligand Binding – a specific model

Agonist binding in $\beta_2$ AR ( beta 2 adrenergic receptor)

- Catacholamines bind as watersoluble interacting with EC loops and TM amino acids.
- Amine N – binds with ASP113 in TM3, catachole PH interacts with Ser TM5.
- Top down weak to strong agonists. ICI118,551 is an inverse agonist
- Isoproterenol docked into the $\beta_2$ AR showing the interactions




## Transmembrane Helices

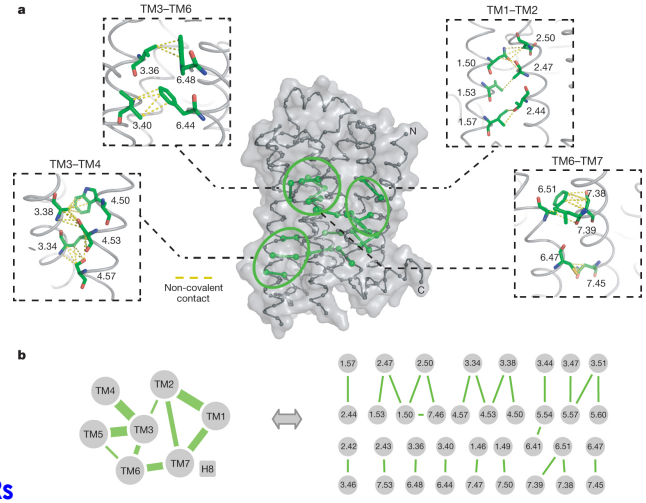
***TM helices serve to interact between ligand/EC domain and intracellular loops and C term where G proteins are bound.***

- In the unbound state, TMs are somewhat flexible and binding of agonist brings conformational change.
- Top down view of TM and Catacholamine
- Three TMs bind to ligand
- R1 is TM5 & TM6 bound by catechol ring
- R2 state happens when Asp 133 in TM3 binds amine
- R3 involves OH of TM6 Asn293
- R3 is fully active receptor

## TM contacts are plentiful

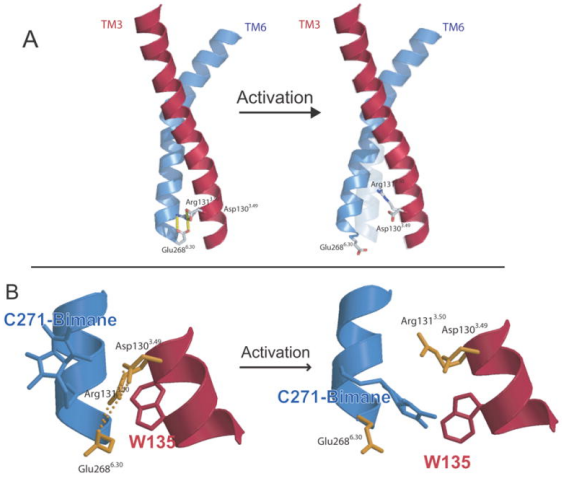
Using modeling and existing structures, Class A GPCRs have many (24 inter-TM) contacts involving 36 amino acids. Mutations of 14 of these are critical and result in loss of receptor function

- Typically non covalent (not disulfide) interactions
- The conserved set of contact amino acids suggest evolutionarily conserved structural scaffold of the GPCR membrane TM fold.
- TM1-2; TM 3-4; TM 3-5 and TM 3-6-7 all possess maintained contacts
- Lipid ligands can bind within the TMs causing a similar disruption/stabilization of active receptor conformation as seen b2AR




## Scaffold of non-covalent contacts in GPCRs

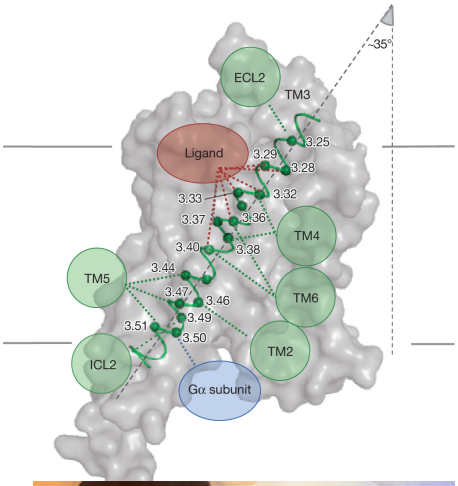
a) 24 TM contacts made of 36 conserved aa of □2AR.
b) Top down and TM interaction schematic of contact aa




## Close up view – Ionic Lock TM3-TM6

**On the intracellular "end" of TM3 and 6 are pairs of ionic aa for binding**

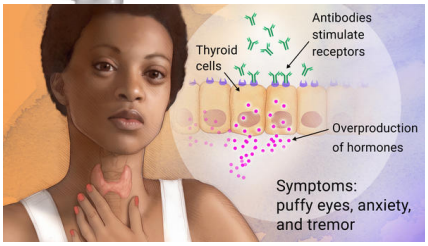
- TM3 Asp 130 and Arg 131 lock with TM6 Glu268
- Maximal receptor (R3) activation requires ionic lock is disrupted for final R3 conformation
- Full agonists are able to break this lock and place the receptor in fully active state – interact with G proteins




## TM3 is a structural and functional hub

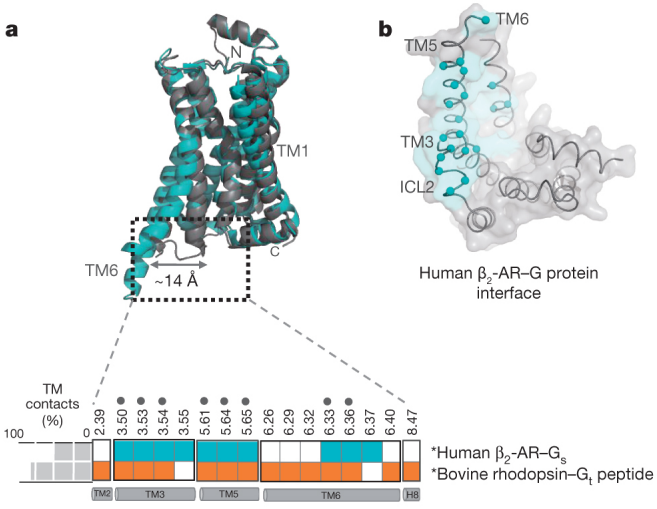
Only TM with significant til that maintains inter-TM contacts and disulphide with ECL2

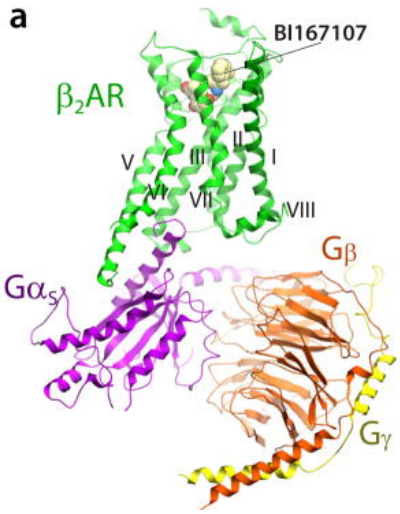
- Ligand, G-Protein and other TMs make this a central anchor for communicating twists – conformational changes from ligand to G alpha subunit
- Some autoimmune diseases make antibodies against the EC /TM3 domain stabilizing the active conformation
- Graves disease is due to autoantibodies that stimulate thyroid T3/T4 release



## Role or intracellular loops (ICL)

- Note TMs that interact with G protein
- Intracellular exposed amino acids bind to G proteins and are modified by fatty acids, acetate, and are phosphorylated by kinases like arrestin.




a) Top -Active (Cyan) and inactive (grey) contacts between TMs
TM3, 5 and 6 contacts on GPCR – dots are common aa contacts with g proteins (bottom)
b) "bottom up" cytoplasmic view of GPCR and contacts (c)

**Long term activation / hormone levels leads to the deactivation of a receptor in two ways**

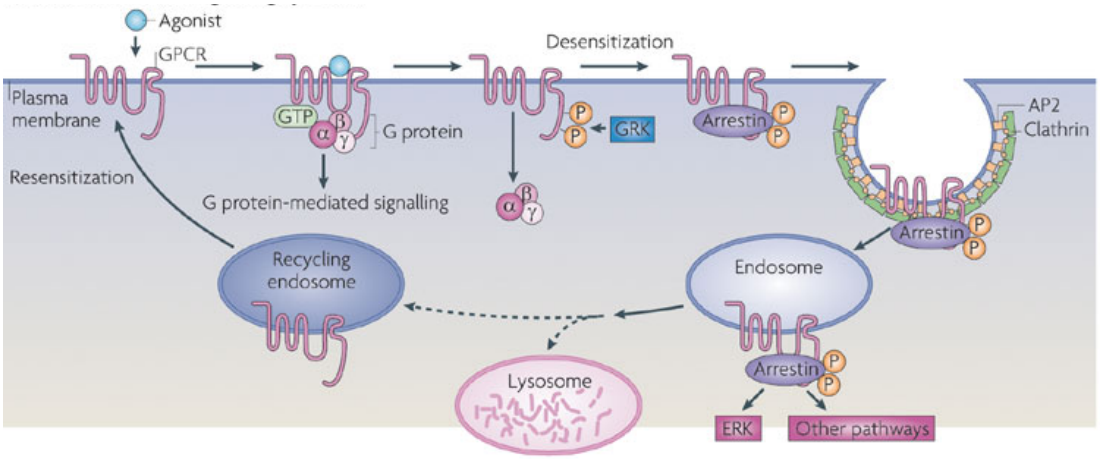
- The receptor is phosphorylated by protein kinases at the C – terminal domain. The result is a decrease in the interaction with the G proteins
- The receptors are removed (endocytosis) from the cell surface and either the hormone is degraded and the receptor returned or the receptor is degraded never to be seen again!

## Arrestin mediated signaling of GPCR

Desensitization begins within seconds of agonist exposure due to phosphorylation of GPCR by second messenger kinases including PKA and PKC (with or without ligand. Specific active receptor kinases only phosphorylate GPCR when ligand is bound.

Phosphorylation diminishes G-protein coupling and recruits arrestin to receptor limiting G-protein coupling to receptor (desensitization)

While sequestration is due to internalization of GPCR due to endocytosis.

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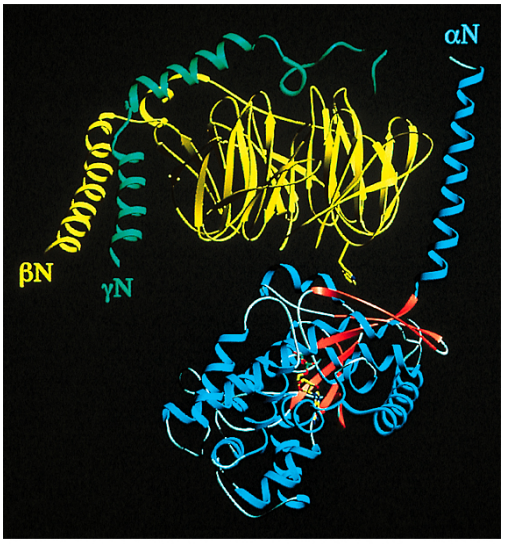
## Signaling Intermediates - G Proteins

GDP/GTP Binding Proteins: Heterotrimeric and monomeric (small) G Proteins

Heterotrimeric G proteins function to relay information from cell surface receptors to intracellular effectors.

- In mammals, G protein, and  subunits are encoded by at least 21, 6 and 12 different genes, respectively.



Courtesy of Mark Wall and Stephen Sprang, University of Texas Southwestern Medical Center.

The α subunit binds and can slowly hydrolyze GTP.

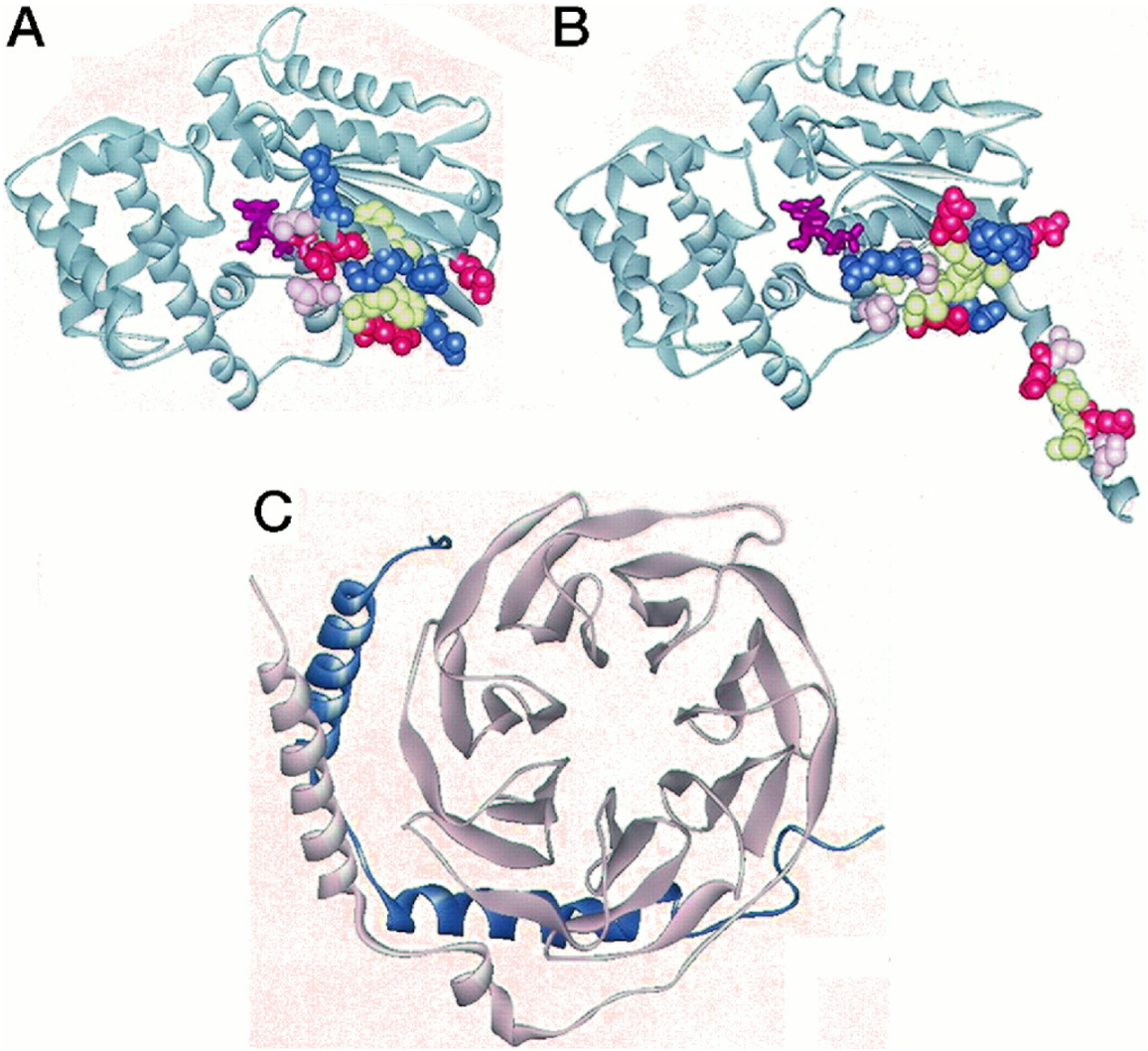
4 G protein classes (Gi/Go, Gq, Gs and G12/13) placed in large families based on effectors and amino acid identity of the □ SU

- >20 different known Gα subunits
- Gα is N terminal modified with a fatty acid (palmitate)
- Two subdomains – GTPase and α-helical

- $G_{\beta\gamma}$ - there are various forms of each subunit
  - stay bound together as a pair
  - some βγ pairs have their own effectors once released from the □ subunit
  - the γ subunit$_\gamma$ has a CAAX box – gerenylated or myristoylated at the C-terminus

**Heterotrimeric G Protein α Subunits**

| subfamily | Gα | typical receptor | intracellular effectors | message | expression |
|---|---|---|---|---|---|
| s | $\alpha_1$ | β-adrenergic receptor | ↑ Adenylyl cyclase<br>Open $Ca^{2+}$ channels | ↑ cAMP<br>↓ MP | Ubiquitous |
| | $\alpha_{olf}$ | Odorant receptors | ↑ Adenylyl cyclase | ↑ cAMP | Olfactory epithelium |
| i/o/t | $\alpha_{i-1}$, $\alpha_{i-2}$, $\alpha_{i-3}$ | Somatostatin receptor | Open $K^+$ channels<br>↓ Adenylyl cyclase | ↑ MP<br>↓ cAMP | Ubiquitous |
| | $\alpha_0$ | m2 acetylcholine receptor | Closed $Ca^{2+}$ channels | ↓ MP | Brain |
| | $\alpha_z$ | Unknown | ↓ Adenylyl cyclase | ↓ cAMP | Brain |
| | $\alpha_{t1}$ | Rhodopsin | ↑ cGMP-phosphodiesterase | ↓ cGMP | Retinal rods |
| | $\alpha_{t2}$ | Color opsins | ↑ cGMP-phosphodiesterase | ↓ cGMP | Retinal cones |
| | $\alpha_{gust}$ | Tastant receptors | Unknown | Unknown | Taste buds |
| q | $\alpha_q$ $\alpha_{11}$, $\alpha_{14}$ $\alpha_{15}$ | m1 acetylcholine receptor | ↑ PI-PLC (β subtypes) | ↑ $IP_3$, DAG | Ubiquitous |
| 12/13 | $\alpha_{12}$, $\alpha_{13}$ | Unknown | Unknown | Unknown | Ubiquitous |




- Upon GTP binding to G, the G-binding site is rearranged and the subunits dissociate. Ribbon diagrams of G protein subunits shown are the activated GTPγS-bound G□ subunit (A) and the inactive GDP-bound Gα (B).

  Notice the N-terminal helix is visible only in the GDP-bound structure

- The Gα subunit is silver, and the bound nucleotides are magenta. The Gβ□contact sites on Gα are indicated by space-filled residues.. The relative orientations of the □ contact sites in the switch interface of Gα·GTP are very different from the Gα·GDP and result in decreased binding.

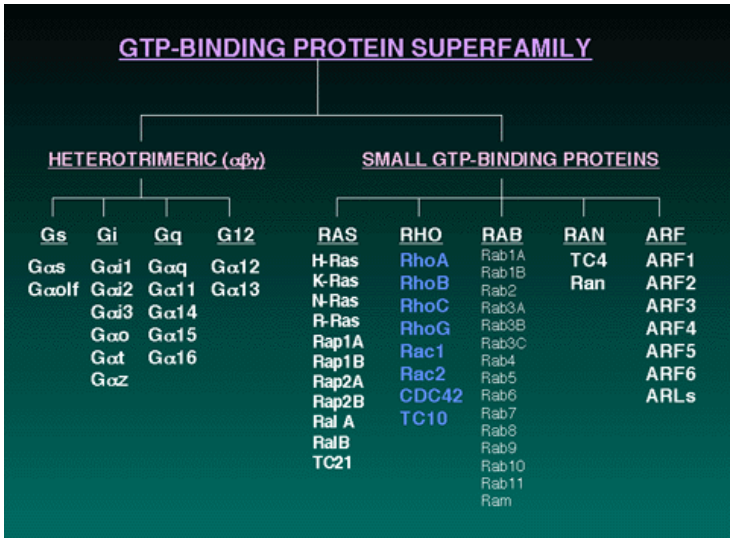
(C)The G□□ dimer. The G□ subunit, in metallic pink, forms a seven-bladed propeller structure that contains a water-filled pore. The Gγ subunit, in blue, is an □□helical structure that lies along the bottom of Gβ. The N termini of Gβ and Gγ form a parallel coiled coil. When □□ the subunits dissociate, Gβγ is free to activate a number of effectors.

**GTP vs GDP bound Gα**

- three switch regions of protein
- 14% of aa move when tri phosphate present
  change is brought about by contact of tri phosphate with three aa
  the N-term of active □ is shifted into the protein – increased mobility than when it is tethered into the membrane
- βγ do not change (β is a rigid propeller with a 40 repeat of Tryptophan and aspartate [WD40] structure)
- βγ acts as a "lever" to pry open Gα GDP binding site when interacting with an activated receptor

## Small G proteins

- Monomeric guanine nucleotide-binding proteins of 20-25 kDa molecular mass. (p21)
- These proteins are similar to an a subunit
- They play major roles in the regulation of growth, morphogenesis, cell motility, axonal guidance, cytokinesis, and trafficking through the Golgi, nucleus, and endosomes.
- The first small GTPase to be discovered was Ras, and there are now many members of the Ras superfamily of GTPases.




## All in the family - Ras

- Found in large number of turmors (>90% pancreatic cancers)
- Several effectors - most commonly mentioned - Raf (part of a MAPK activation pathway), p190RhoGAP, RIN1, which enhances the transforming ability of Bcr/Abl, and phosphatidylinositol (PI) 3-kinase
- Mutation of glycine decreases the hydrolytic activity of the GTPase and leaves Ras active

## All in the family - Rho

- Divided in three major subtypes, namely Rho, Rac, and Cdc42,
- Lead to alteration in cytoskeletal restructuring for growth and response to stress
- Effectors include RhoA Activated Kinase (Rock) and phospholipase D
- Many regulators exists

## All in the family - ARF

- The first of these was discovered as a factor required for the ADP ribosylation of the -subunit of the heterotrimeric G protein Gs by cholera toxin.
- were critical components of several vesicular trafficking pathways
- Also involved in insulin signaling
- Strong activator of phospholipase D
- contain pleckstrin homology and other domains that bind $PIP_2$ and are responsible for membrane binding

## All in the family - Rab

- Play key roles in the secretory and endocytic pathways.
- Rabs facilitate the formation of v-SNARE·t-SNARE complexes, which are integral components of vesicle trafficking
- May act by recruiting specific docking factors (Exocyst, Rabaptins) from the cytosol to facilitate pairing of the SNAREs.

## All in the family - Ran

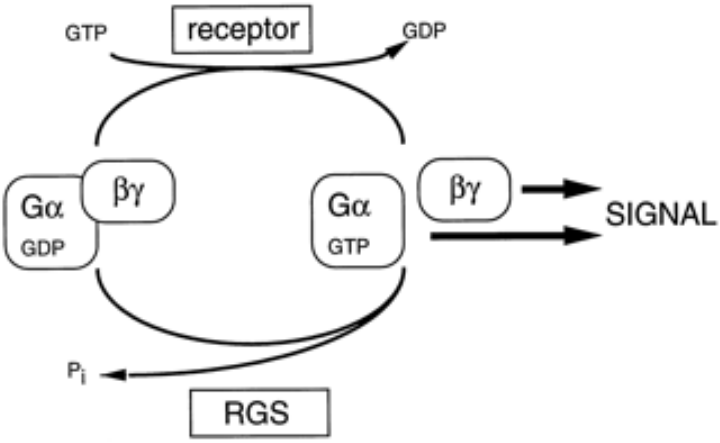
- Play a central role in protein and RNA trafficking in and out of the nucleus
- Rabs facilitate the formation of v-SNARE·t-SNARE complexes, which are integral components of vesicle trafficking
- May act by recruiting specific docking factors (Exocyst, Rabaptins) from the cytosol to facilitate pairing of the SNAREs.

**G-Protein Regulators**

- Slow basal level GTPase activity
- Two switch regions
  - Switch I - binds Mg+2 and GAP proteins
  - Switch II - GTP binding site
- GTP binding reorders the two switch regions and destroys the effector binding site

In the resting state G proteins are usually in the GDP bound state. Specific proteins activate G proteins

- Receptors act as activators for heterotrimeric G proteins
- Specific proteins called either Guanine dissociation stimulator (GDS) also called Guanine exchange factor (GEF)




The activity of both the heterotrimeric and small G proteins are altered by other proteins.

- The normal GTPase (hydrolytic) activity is slow. It can take several hours for the reaction to be complete
- For the hetero G proteins, the effectors (the proteins which a unit interacts with) increase the GTPase activity

The activity of both the heterotrimeric and small G proteins are altered by other proteins.

- Small G proteins have specific proteins that do this GTPase Activating Proteins (GAP)
- Regulation of the GAPs and GEFs are still under very intense study and many of these proteins are likely to be oncogenes.