

Signal Transduction III

Signaling Intermediates – G Proteins

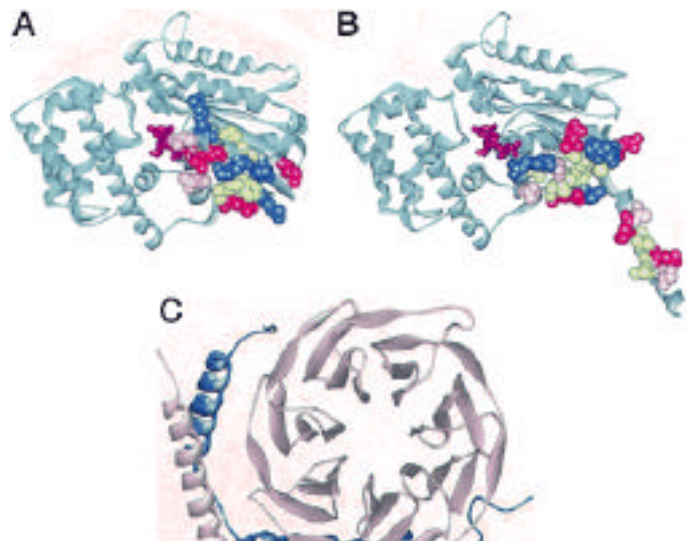
GDP/GTP Binding Proteins: Heterotrimeric and monomeric (small) G Proteins

Heterotrimeric G Protein α Subunits					
subfamily	$G\alpha$	typical receptor	intracellular effectors	message	expression
s	α_1	β -adrenergic receptor	\uparrow Adenylyl cyclase Open Ca^{2+} channels	\uparrow cAMP \downarrow MP	Ubiquitous
	α_{olf}	Odorant receptors	\uparrow Adenylyl cyclase	\uparrow cAMP	Olfactory epithelium
i/o/t	α_{1-3}	Somatostatin receptor	Open K^+ channels \downarrow Adenylyl cyclase	\uparrow MP \downarrow cAMP	Ubiquitous
	α_0	m2 acetylcholine receptor	Closed Ca^{2+} channels	\downarrow MP	Brain
	α_x	Unknown	\downarrow Adenylyl cyclase	\downarrow cAMP	Brain
	α_{11}	Rhodopsin	\uparrow cGMP-phosphodiesterase	\downarrow cGMP	Retinal rods
	α_{12}	Color opsins	\uparrow cGMP-phosphodiesterase	\downarrow cGMP	Retinal cones
	α_{gust}	Tastant receptors	Unknown	Unknown	Taste buds
	q	$\alpha_q, \alpha_{11}, \alpha_{14}, \alpha_{15}$	m1 acetylcholine receptor	\uparrow PI-PLC (β subtypes)	\uparrow IP_3 , DAG
12/13	α_{12}, α_{13}	Unknown	Unknown	Unknown	Ubiquitous

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

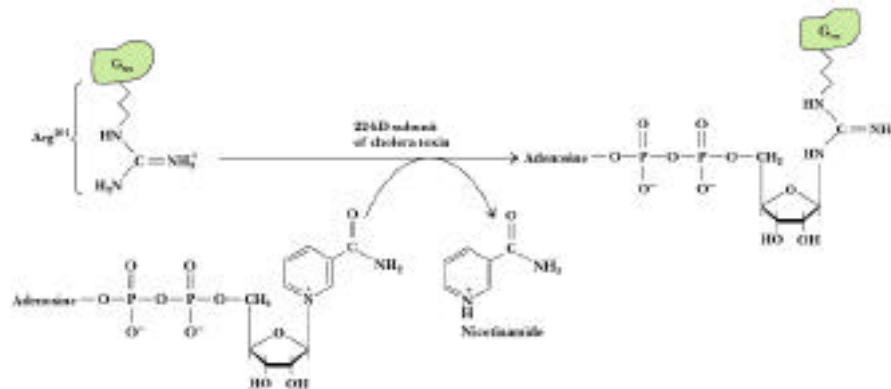
- In mammals, G protein α , β , and γ subunits are encoded by at least 16, 4 and 7 different genes, respectively.
- The α subunit binds and can slowly hydrolyze GTP.
 - 6 G protein classes in large families based on effectors and amino acid identity of the SU
 - 23 different known G α subunits
 - G α is N terminal modified with a fatty acid (palmitate)
- G $\beta\gamma$ - there are various forms of each subunit
 - stay bound together as a pair
 - some $\beta\gamma$ pairs have their own effectors once released from the α subunit
 - the β subunit has a CAAX box – geranylated or myristoylated at the C-terminus

Fig. 1. 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 GTP-bound G α subunit (A) and the inactive GDP-bound G α (B). Notice the N-terminal helix is visible only in the GDP-bound structure. The G α subunit is silver, and the bound nucleotides are magenta (the colored pict will be on the web if you are interested). The G $\beta\gamma$ contact



sites on G_s are indicated by space-filled residues. Polar residues are pink, hydrophobic residues are yellow, basic residues are blue, and acidic residues are red. The relative orientations of the contact sites in the switch interface of G_s·GTP are very different from the G_s·GDP and result in decreased binding. (C) The G_s dimer. The G_s subunit, in metallic pink, forms a seven-bladed propeller structure that contains a water-filled pore. The G_i subunit, in blue, is an α helical structure that lies along the bottom of G_s. The N termini of G_s and G_i form a parallel coiled coil. When the subunits dissociate, G_s is free to activate a number of effectors. (Journal of Biological Chemistry, Volume 272, Number 7, Issue of February 14, 1997 pp. 3871-3874.)

- GTP vs GDP bound G_s
 - 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 site is shifted into the protein – increased mobility than when it is tethered into the membrane
 - do not change (WD40 is a rigid propeller with a 40 repeat of tryptophan and aspartate [WD40] structure)
 - acts as a “lever” to pry open G_s GDP binding site when interacting with an activated receptor
- G_s family proteins are modified by cholera toxin
 - ADP ribosylation of G_s
 - inhibition of GTP hydrolysis leaves G_s active
 - cholera caused by bacterium *Vibrio cholerae* in water supplies
 - responsible for cholera epidemics 22,000 British died between 1831 – 1832
 - increased cAMP in the intestine activates the Na⁺/K⁺ channels and results in loss of water into the gut – diarrhea and eventually death

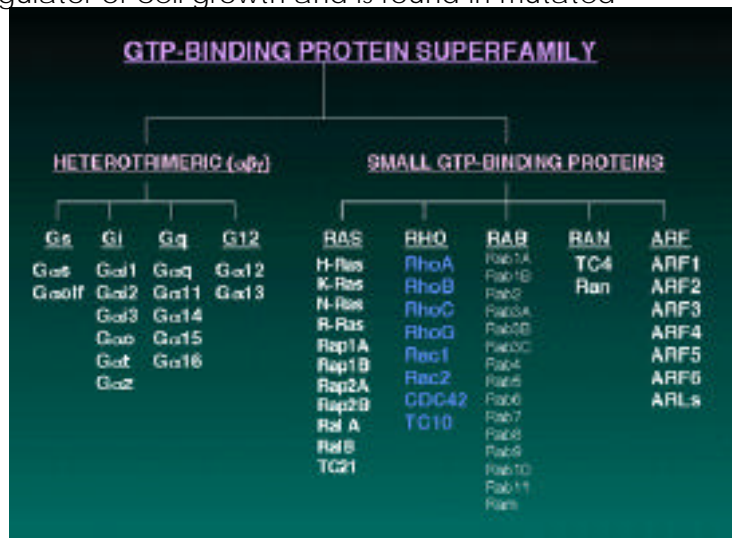


- most members of the G_{i/o} family are ribosylated by pertussis toxin
 - caused by whooping cough bacteria
 - uncouples G_s and G_i from receptor and leaves the $\beta\gamma$ subunit in the inactive form

Small G proteins

Small GTPases are monomeric guanine nucleotide-binding proteins of 20-25 kDa molecular mass. 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. These are grouped in five subfamilies (Ras, Rho, ADP-ribosylation factors (ARF), Rab, and Ran)

Ras is important because it is a key regulator of cell growth and is found in mutated oncogenic forms in a large number of human tumors. When specific residues in Ras are mutated it becomes constitutively active (insensitive to GAP action) and causes cell transformation. The first signaling pathway involving Ras to be discovered was the Raf/MEK/ERK cascade of protein kinases that leads to the stimulation of certain transcription factors. Other pathways besides Raf/MEK/ERK contribute to malignant transformation. These other Ras effectors include p120GAP, which associates with p190RhoGAP, RalGDS, which targets Ral and other proteins, RIN1, which enhances the transforming ability of Bcr/Abl, and phosphatidylinositol (PI) 3-kinase, which generates PIP₃, an activator of the protein kinases Akt/PKB and PDK1. All these proteins have demonstrated or potential roles in the control of cell growth, morphology, and apoptosis.



The Rho subfamily of GTPases. These proteins come in three major subtypes, namely Rho, Rac, and Cdc42, which control the actin cytoskeleton in distinct ways. One of these is p160Rho kinase, which alters myosin light chain phosphorylation, thus regulating myosin filament assembly and F-actin bundling. Another is the enzyme (PI-4P 5-kinase) that synthesizes the regulatory lipid PIP₂. This lipid affects many proteins, which are essential for Rho- and Rac-mediated actin changes. Another major role for the Rho proteins is the regulation of gene transcription. There are an astonishingly large number of GEFs for Rho proteins with a lack of information on their specific roles and regulation.

The ARF subfamily. The first of these was discovered as a factor required for the ADP ribosylation of the β -subunit of the heterotrimeric G protein Gs by cholera toxin. Subsequently it was found that ARFs comprised three classes and were critical components of several vesicular trafficking pathways. Structures of the various GEFs for ARFs all contain a domain present in Sec7, a yeast gene involved in protein secretion. This domain encodes the GEF activity, and the proteins also contain pleckstrin homology and other domains that bind PIP₂ and are responsible for membrane binding.

The Rab GTPases There are at least 30 different members. These play key roles in the secretory and endocytic pathways and are located in distinct cellular compartments. Rabs facilitate the formation of v-SNARE-t-SNARE complexes, which are integral components of vesicle trafficking. It is proposed that Rabs act by recruiting specific docking factors (Exocyst, Rabaptins) from the cytosol to facilitate pairing of the SNAREs. In line with other small GTPases, Rabs are active in the GTP form, and several Rab-binding proteins (Rabphilin, Rabaptin 5) keep them in this form and thus influence vesicle fusion.

The Ran GTPase These small G proteins play a central role in protein and RNA trafficking in and out of the nucleus. It is one of the most abundant GTPases, and cells contain either one or a few isoforms. Macromolecules travel in and out of the nucleus through nuclear pore complexes (NPCs) and utilize different receptors and carriers. However, almost all the receptors interact with GTP-Ran and are regulated by the Ran GTPase cycle. One

GEF for Ran is RCC1 (regulator of chromosomal condensation), which is strategically placed inside the nucleus. One Ran GAP isoform is post translationally modified by a ubiquitin-like addition, which targets it appropriately to the cytoplasmic entrance to the NPC. The NPC is a very complicated structure with many proteins still uncharacterized. Important are the repeat-containing nucleoporins, which form "tracks" by which transport substrates pass through the NPC. Ran functions to trigger the assembly or disassembly of transport complexes, and an important factor is probably the difference in the concentration of GTP Ran between the nucleus and cytoplasm.

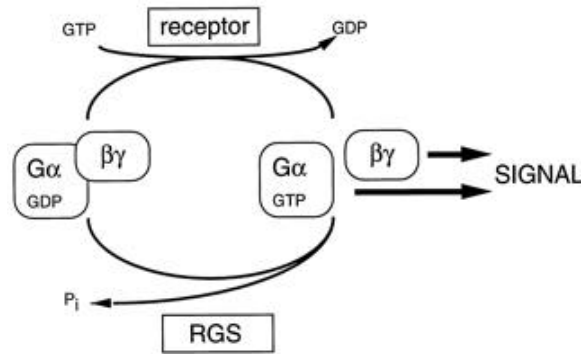
Key points:

- Small G proteins are members of the Ras super-family
- These proteins are similar to an α subunit
- Molecular mass of 19 to 27 (p21)
- The activation of some are well know such as ras
- Others like RhoA (Ras Homology A) are less known and may be activated by the heterotrimeric G proteins.
- The functions of small G proteins are varied as the number of types of small G proteins

G-Protein Regulators

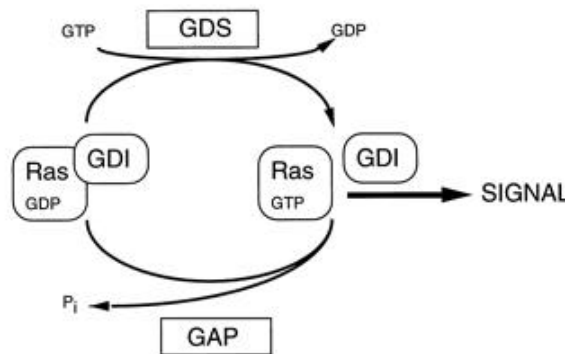
In the resting state G proteins are usually in the GDP bound state. Specific proteins activate G proteins

- 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
- 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.