

So why do cells need to communicate?

- Coordination of movement
bacterial movement towards a chemical gradient
green algae - colonies swimming through the water
- Coordination of metabolism -
insulin glucagon effects on metabolism
- Coordination of growth - wound healing, skin, blood and gut cells

Three basic types of intercellular signaling

I - Intracellular signal via gap junctions

- tight junctions that form a cytoplasmic bridge between cells
- allows the signaling molecules to be transferred directly between cells

II - Secreted extracellular messenger

- one cell "talking" to another
- ex. insulin glucagon, growth factors

III - Anchored extracellular messenger

- signaling occurs between cells in physical contact
- transforming growth factor

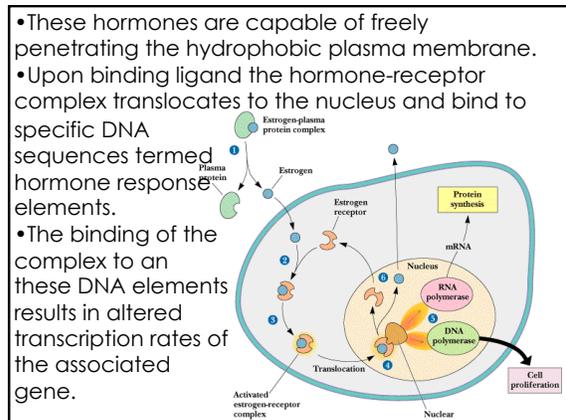
Hormones are chemical signals that reach their target via the blood stream.

- 1) Every different hormone binds to a specific receptor and in binding a significant alteration in receptor conformation results in a biochemical response inside the cell
- 2) This can be thought of as an allosteric modification with two distinct conformations; bound and free.
- 3) The binding of the hormone leads to a transduction of the hormone signal into a biochemical response.
- 4) Hormone receptors are proteins and are typically classified as a cell surface receptor or an intracellular receptor. Each have different roles and very different means of regulating biochemical / cellular function.

Intracellular Hormone Receptors

The steroid/thyroid hormone receptor superfamily (e.g. glucocorticoid, vitamin D, retinoic acid and thyroid hormone receptors)

- Protein receptors that reside in the cytoplasm and bind the lipophilic steroid/thyroid hormones.



Cell surface receptors

The cell surface receptors are a general classification of the proteins which specifically bind water soluble hormones.

- These receptors are very complex and varied. **A key component of this class of receptors is that they possess at least one transmembrane spanning domain.**
- From there all bets are off. The mechanism of the cell surface receptors varies depending on the type of hormone bound and the second messenger system involved.

- Each receptor binds specifically to ONE hormone at the appropriate hormone concentration.
- The association of hormone with its receptor is defined by the equilibria process $K_d = \frac{[R][L]}{[RL]}$ - the dissociation constant. Similar to K_m - measures affinity.
- The effective concentrations are very low 10^{-6} to 10^{-15} M
- Each receptor will have various but distinct second messenger systems associated with it.
- The specificity of action of an organism to a hormone (tissue and cell type) depends on which receptors are expressed in each cell and to which signaling pathway is linked to the receptor.

There are three phases to a water soluble hormone action

- 1) hormone or first messenger
- 2) receptor binding and initiation of the second messenger system
- 3) amplification and cascade of the second messenger system

1) Messengers

First messengers - secreted signaling molecules

- Hormones - secreted from endocrine glands (usually into the blood stream) effects are long distance
- Neurotransmitters - signaling molecules released in special regions of the cell and move across a synaptic cleft in neurons
- Local Mediators (paracrine - Greek for besides) similar effect to hormones but effect is very short. Signaling between neighboring cells

First messengers

Structure of 1st messengers vary greatly - we already know most of them

- 1) amino acid derivatives
 - tyrosine -> thyroid hormones T3/T4, epinephrine, dopamine
 - glutamate -> histamine
 - tryptophan -> serotonin
- 2) Peptides - usually made in pre/pro format, large families
 - insulin, glucagon, oxytocin, growth factors

First messengers

Structure of 1st messengers vary greatly - we already know most of them

- 3) Fatty acids and eicosanoids - can act as local mediators
 - TXA, LTE, phosphatidic acid, lysophosphatidic acid
- 4) Steroids - cholesterol derivatives
 - progesterone, estrogen, testosterone
 - site of action usually in nucleus w/ DNA binding protein
- 5) NO Nitric Oxide - small short lived gas molecule
 - responsible for dilation and Viagra

2) 1st messengers elicit cellular response by binding to receptors - signal transduction

three general methods of signaling

I. Ion channel system

- binding of ligand (1st messenger) to receptor opens a specific ion channel. The receptor itself may be the ion channel. Often called ligand gated channel. Ion flux leads to a significant biochemical change. Typically Ca^{+2} or K^{+}/Na^{+}

II. Receptors without enzymatic activity (Second messenger systems)

- 1st messenger binds to receptor and activates production of a new molecule - heterotrimeric G proteins

three general methods of signaling

III. Receptors with integral enzymatic activity

- 1st messenger binding activates an enzyme activity in the receptor itself - leads to new proteins interacting with intracellular portion and a new second messenger molecule.

3) Second messengers Third phase of the signal transduction concept

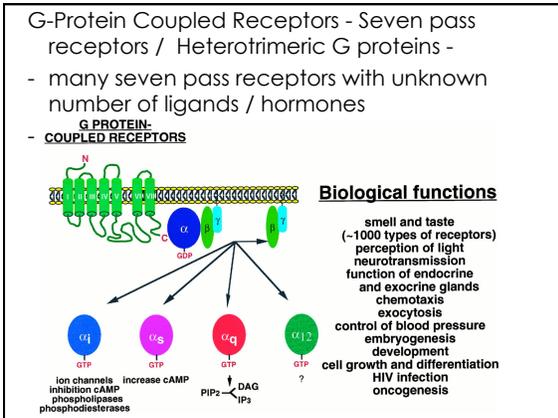
A second messenger is a molecule produced in response to a 1st messenger binding to a receptor. - Hallmark of a true signal is a specific and short lived response to an agonist

Leads to an amplification of original signal second messenger may directly effect target protein / DNA or mostly leads to a chain of second messengers with a wide variety of effects

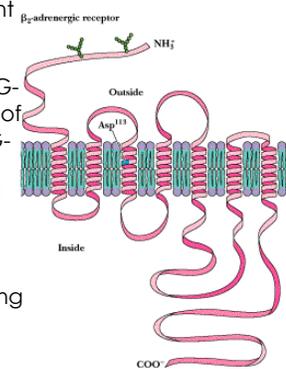
- specific control and response - cAMP
- Many different signaling proteins are involved (thousands). The design of the proteins organization comprise the signaling pathways

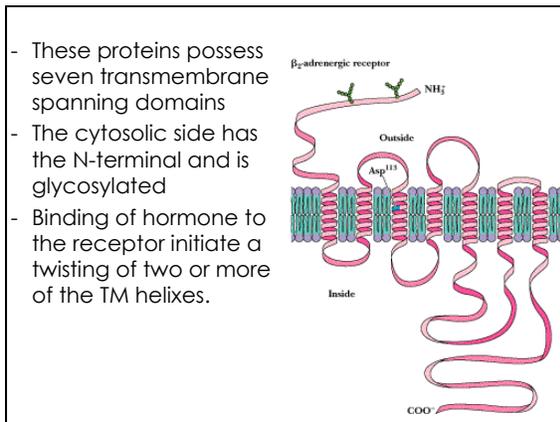
One of the first signaling systems identified was a visual pigment - retinal rod cell - rhodopsin

- first messenger is light
- receptor is retinal/rhodopsin - a kind of a G protein
- second messenger is cGMP
- Physiologic effect is the flux of ions -> membrane potential which triggers neurotransmitter release

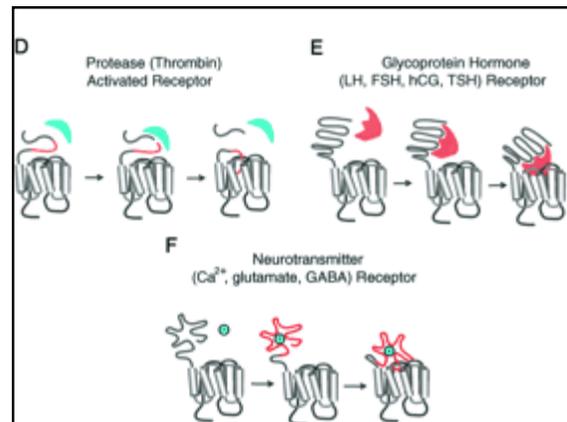
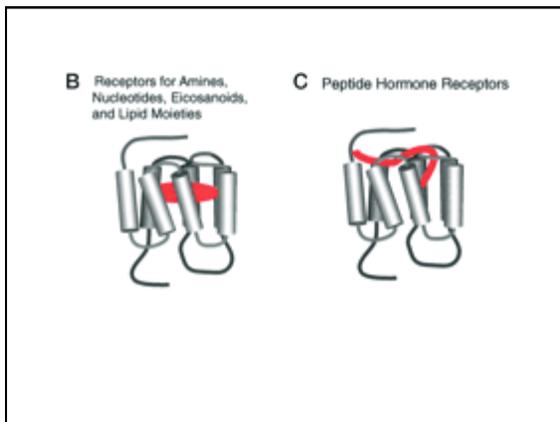
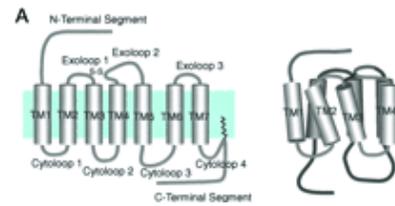


There are several different classifications of receptors that couple signal transduction to G-proteins. These classes of receptor are termed G-protein coupled receptors, GPCRs. Well over 1000 different GPCRs have been cloned, most being orphan receptors having no as yet identified ligand.





Ligand (epinephrine) binding takes place within the hydrophobic core, not the loops. Other agonists (ligands which bind and activate receptors i.e. hormones) act in other ways.

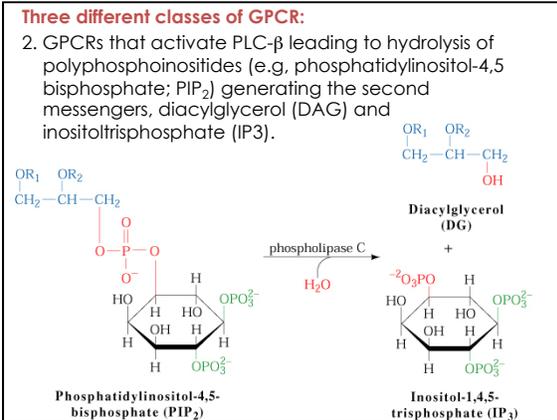


Long term activation / hormone levels leads to the deactivation of a receptor in two ways

- 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!

Three different classes of GPCR:

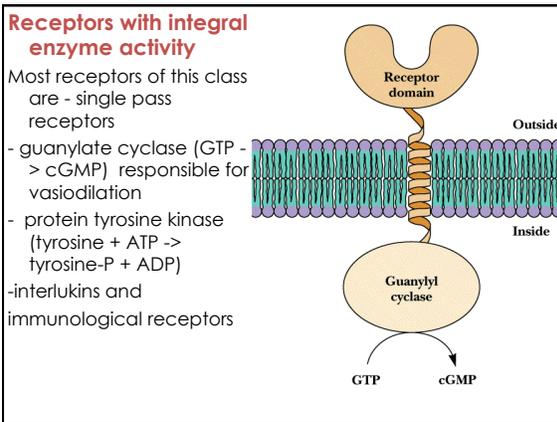
1. GPCRs that modulate adenylate cyclase activity.
 - adenylate cyclase modulating receptors - β -adrenergic, glucagon and odorant molecule receptors.
 - In the case of odorant molecule receptors the increase in cAMP leads to the activation of ion channels.
 - In contrast to increased adenylate cyclase activity, the α -type adrenergic receptors are coupled to inhibitory G-proteins that repress adenylate cyclase activity upon receptor activation.



Three different classes of GPCR:

3. A novel class of GPCRs are the photoreceptors. This class is coupled to a G-protein termed transducin that activates a phosphodiesterase which leads to a decrease in the level of cGMP.

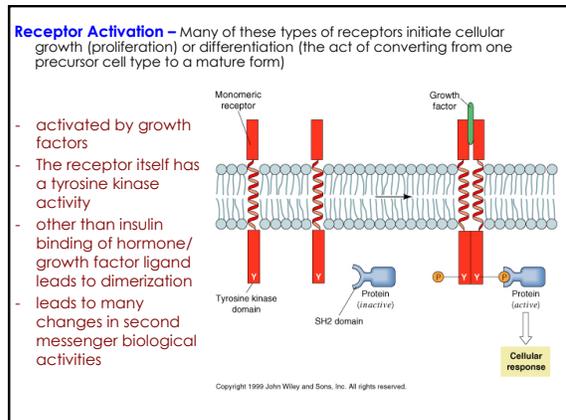
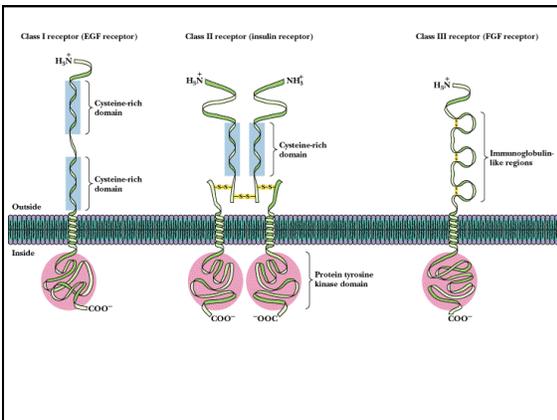
- The drop in cGMP then results in the closing of a Na⁺/Ca²⁺ channel leading to hyperpolarization of the cell.
- Vitamin A is key in detecting the light and initiating these changes.



Each receptor has three basic domains:

- 1 - Extracellular domain**
 - this is the ligand binding domain /often glycosylated
 - often heavy in cysteine rich domains and immunoglobulin like domains
- 2 - Transmembrane domain**
 - usually a single alpha helix (rich in hydrophobic amino acids)
- 3 - Intracellular domain**
 - the intracellular portion becomes activated by tertiary structural changes
 - many times autophosphorylates intracellular domain (tyrosine-P)
 - once activated, many different protein: bind to intracellular domain

A 3D ribbon diagram of a receptor protein, showing the extracellular domain in red, the transmembrane domain in blue, and the intracellular domain in green.



Many intracellular receptor binding sites for other proteins based on Rous Sarcoma Virus

- Src is a transforming causing protein with 3 distinct domains Src Homology
- SH1 - contains tyrosine kinase of Src specific for this protein
- SH2 - binds phosphotyrosine
- SH3 - believed to bind to cytoskeleton or portion of plasma membrane that is high in proline amino acid residues

Therefore once the receptor is activated and phosphorylated there will be a slug of proteins recruited to the cell membrane.

- This may result in the receptor phosphorylation of the protein (PLC γ)
- Another action is that a number of critical components are brought together where they can form a functional complex and initiate a series of events (Ras MAPK)

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 16, 4 and 7 different genes, respectively.

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

The α subunit binds and can slowly hydrolyze GTP.

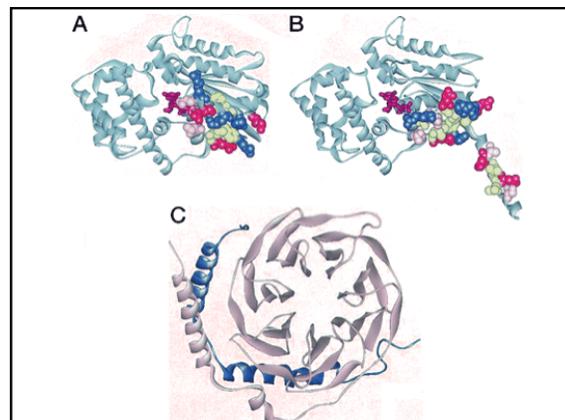
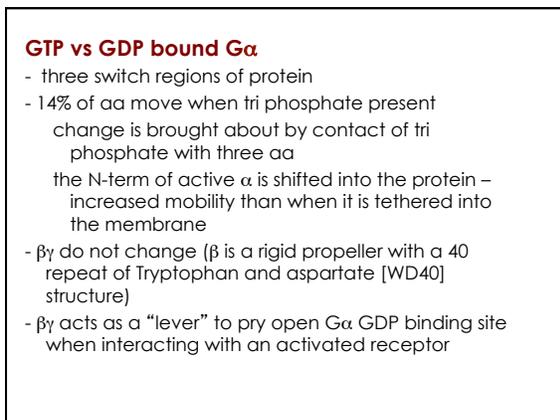
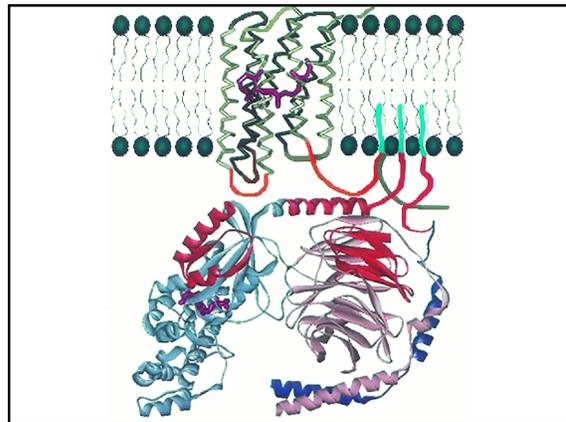
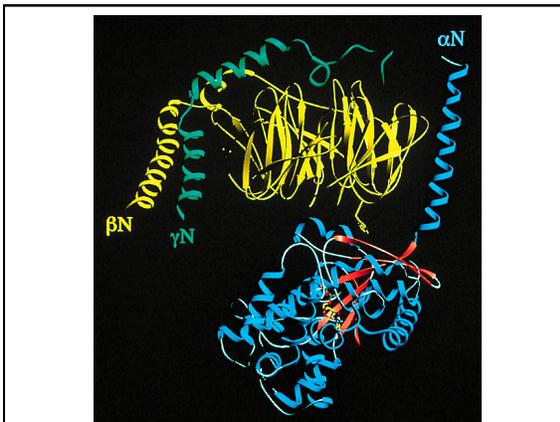
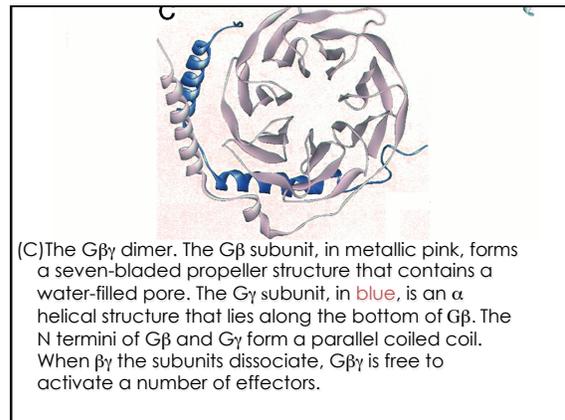
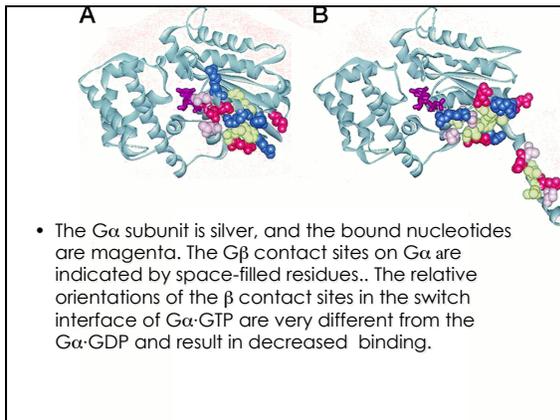
- 6 G protein classes in large families based on effectors and amino acid identity of the α SU
- 23 different known $G\alpha$ subunits
- $G\alpha$ is N terminal modified with a fatty acid (palmitate)

- $G_{\beta\gamma}$ - 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 γ subunit, has a CAAX box - geranylated or myristoylated at the C-terminus

Heterotrimeric G Protein α Subunits					
subfamily	$G\alpha$	typical receptor	intracellular effectors	message	expression
s	α_1	β -adrenergic receptor	\uparrow Adenyl cyclase Open Ca^{2+} channels	\uparrow cAMP \downarrow MP	Ubiquitous
	α_{olf}	Odorant receptors	\uparrow Adenyl cyclase	\uparrow cAMP	Olfactory epithelium
i/o/t	$\alpha_{1-3}, \alpha_{1-2}, \alpha_{1-3}$	Somatostatin receptor	Open K^+ channels \downarrow Adenyl cyclase	\uparrow MP \downarrow cAMP	Ubiquitous
	α_0	m2 acetylcholine receptor	Closed Ca^{2+} channels	\downarrow MP	Brain
	α_2	Unknown	\downarrow Adenyl 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

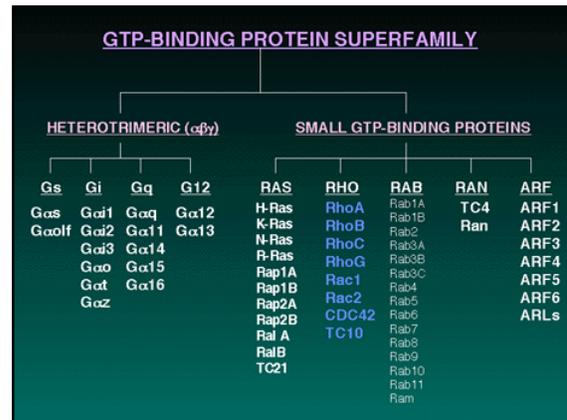
- Upon GTP binding to G, the G-binding site is rearranged and the subunits dissociate. Ribbon diagrams of G protein subunits shown are the activated GTP γ S-bound $G\alpha$ subunit (A) and the inactive GDP-bound $G\alpha$ (B).

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



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 tumors (>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 β -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 + 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

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)

G-Protein Regulators

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

G-Protein Regulators

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.

Protein Kinases

Phosphorylation/dephosphorylation Protein phosphorylation is one of the most important mechanisms of cellular responses to growth, stress metabolic and hormonal environmental changes. Most mammalian protein kinases have highly a homologous 30 to 32 kDa catalytic domain.

- **Most common method of reversible modification**
- activation and localization
- **Up to 1/3 of cellular proteins can be phosphorylated**
- **Leads to a very fast response to cellular stress, hormonal changes, learning processes, transcription regulation**
- **Different than allosteric or Michealis Menten regulation**

- Phosphorylation stabilized thermodynamically
- only half available energy used in adding phosphoryl to protein
- change in free energy forces phosphorylation reaction in one direction
- Phosphatases reverse direction
- The rate of reaction of most phosphatases are 1000 times faster
- Phosphorylation occurs on Ser/The or Tyr
- What differences occur due to the addition of a phosphoryl group?

- Regulation of protein phosphorylation varies depending on protein
 - some turned on or off
 - most kinases are regulated
 - phosphatases generally not regulated
 - can lead to large amplification of original signal
- General classes of protein kinases, based on substrate (both sequence and target amino acid phosphorylated), homology and regulation mechanisms (thousands of kinases)

Protein Kinase A (PKA)

Activated by cyclic Adenosine Monophosphate (c-AMP)

- Recognizes specific sequences in substrate
 - Arg-Arg- X - Ser/Thr - Z
 - X = small aa, Z = hydrophobic aa (not Tyr)
- Called consensus sequence
- Important in regulation by hormones and neurotransmitters
 - Epinephrine (adrenaline)
- c-AMP produced from ATP by adenylyl cyclase (AKA adenylate cyclase)

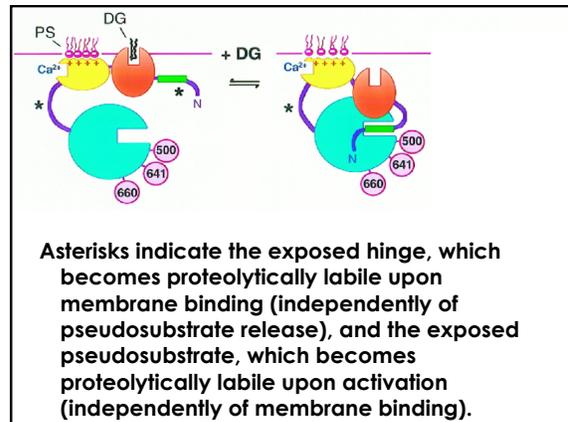
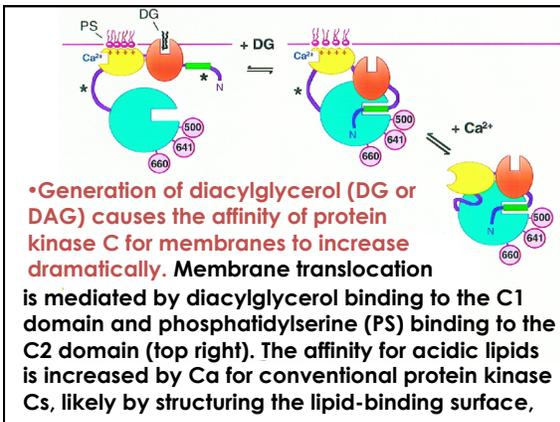
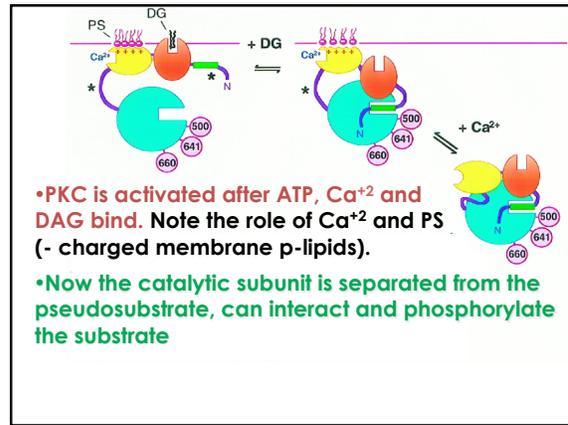
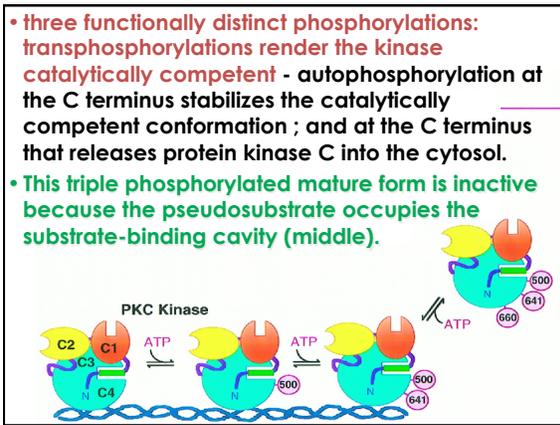
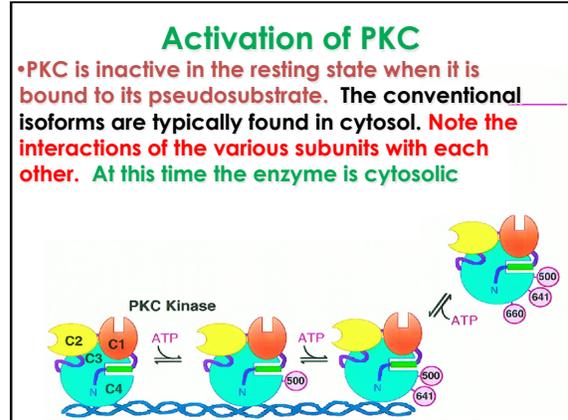
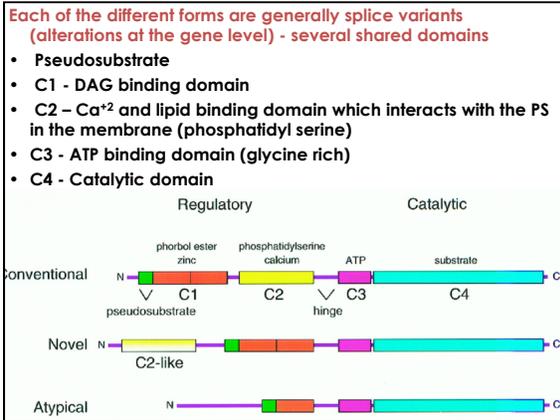
- PKA is a heterotetramer, not linked together by peptide bond
- Regulatory subunits - Arg-Arg- Gly - Ala - Ile
- Pseudosubstrate - binds deep in cleft between catalytic subunits
- Competitive inhibitor at active site
- Binding of c-AMP to R subunits shifts Pseudosubstrate away from active site
- Catalytic subunits now active
- Degradation of c-AMP to AMP by another enzyme leads to removal of c-AMP from R subunits and reformation of inactive heterotetramer

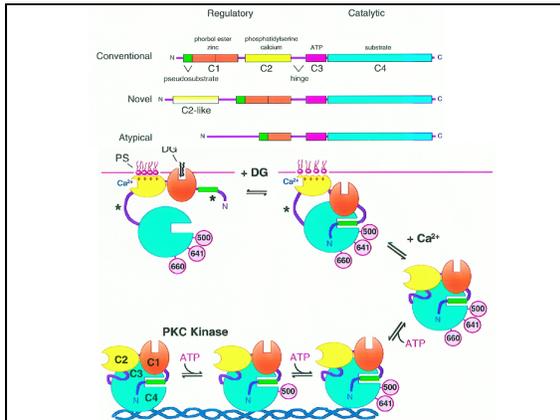
Protein Kinase C (PKC)

- Ser/Thr protein kinase
 - Monomer - pseudosubstrate part of whole protein
 - Activated by increases in cellular Ca^{+2} and Lipid activator (DAG)
 - Diacylglycerol (DAG) - tumor promoter made by other enzymes in response to hormonal changes.
- Very transient molecule, often use phorbol esters (PMA) to study
- No real stringent consensus sequence - usually Arg rich targets

Over 23 isoforms are based in three categories

- 1) **conventional PKC** - Ca^{+2} and Lipid regulated **α , β_1 , β_2 and γ isoforms**
- 2) **novel PKC** - only Ca^{+2} activated
- **δ , ϵ , η , ϕ and μ forms**
- 3) **Atypical PKC** - not regulated by either Ca^{+2} or DAG possibly activated by sphingosine **ζ and τ** .





Protein Tyrosine Kinases (PTK)

Phosphorylates at a tyrosine residue only
Several kinds of cancer are mutated versions of tyrosine kinases

2 classes; receptor or cytosolic

Receptor tyrosine kinases

- receptor of hormones/growth factors
- found on both sides of the cell membrane
- extracellular portion binds hormone and alters conformation through the membrane and the cytosolic portion
- now the kinase part of the receptor is active

Protein Tyrosine Kinases (PTK)

Cytosolic or non-receptor

- Part of the Src family - mutated form originally found in rous sarcoma virus

Usually regulated by other tyrosine kinases (receptor kinases)

- many different soluble tyrosine kinases
- most have SH2 or and SH3 domains
- Murine lymphoma (leukemia) formed when tyrosine kinase of Abl is uncontrolled

Protein Kinase B (PKB/AKT)

• Newer type of kinase - not much known (1996)
Protein kinase B is now better known as Akt and is a serine threonine kinase that was first found from a virus that induces T-cell lymphomas in rats.

- Prevents apoptosis, induces glucose uptake by increasing Glut 4 translocation to the membrane, regulates glucose metabolism through phosphorylation of glycogen synthase kinase and can alter protein expression by phosphorylation of ribosomal kinases.

Protein Kinase B (PKB/AKT)

- Activated by growth factor receptors such as insulin and epidermal growth factor.
- Binds and is activated by the phospholipid Phosphoinositol 3,4,5 trisphosphate. Binds at the PH domain - pleckstrin homology
- Catalytic domain similar to PKC and PKA
- Exact mechanism of activation remains unclear

Mitogen Activated Protein Kinases

MAP kinases (MAPK) were identified by virtue of their activation in response to growth factor stimulation

- ERKs for extracellular-signal regulated kinases.
 - Also microtubule associated protein-2 kinase (MAP-2 kinase), myelin basic protein kinase (MBP kinase),
- P38
- JNK
- Big MAP Kinase
- Ribosomal S6 protein kinase (RSK-kinase: i.e. a kinase that phosphorylates a kinase)

Each of the three forms of MAP kinases ERK, JNK and p38 are activated by different mechanisms.

- ERK (also commonly referred to as MAPK) is activated by G proteins and most growth factors.
- JNK and p38 are activated by cellular stress such as UV, heat and osmotic changes.

The targets for each of the MAP kinases vary greatly and can be found in the **cytosol** (where metabolism, cytoskeletal and other responses are initiated) or in the **nucleus** (where transcription factors are activated, ultimately leading to altered gene production).

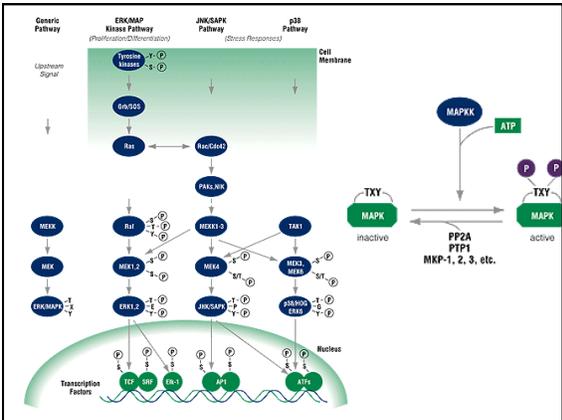
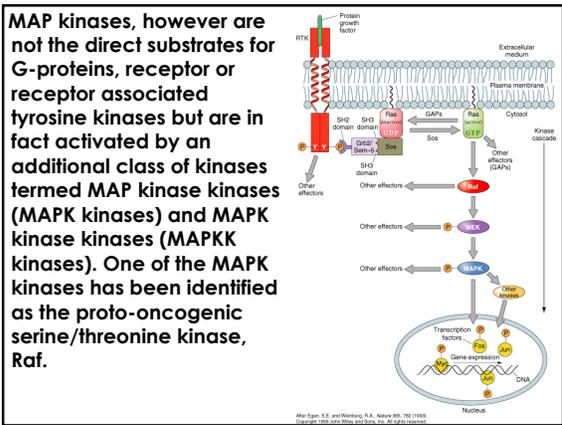
- p90Rsk (ribosomal kinase)
- C-Jun (transcription factor)
- Heat shock proteins
- proto-oncogenes Fos, Myc and Jun
- members of the steroid/thyroid hormone receptor super family of proteins.

Maximal MAP kinase activity requires that both tyrosine and threonine residues are phosphorylated.

MAP kinase activation was first observed in response to activation of the EGF, PDGF, NGF (epidermal, platelet and nerve growth factors) and insulin receptors,

Other cellular stimuli include: as T cell activation (which signals through the Lck [lick] tyrosine kinase)

And - phorbol esters (that function through activation of PKC), thrombin, epinephrine and lysophosphatidic acid (LPA)(all hormones that function through G-proteins) also rapidly induce tyrosine phosphorylation of MAP kinases.

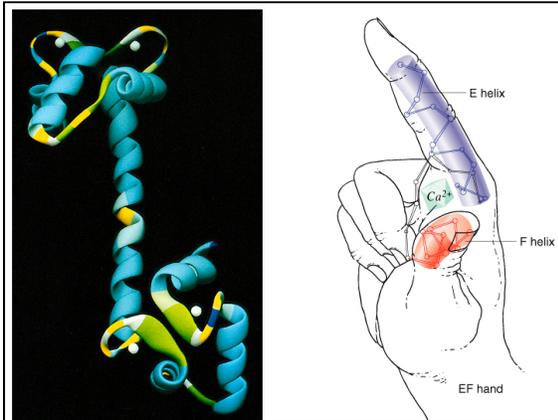


Ca²⁺/CaM dependent protein kinaseII (CaM-KII)

Another class of protein kinases that are activated by increases in calcium.

CaM-K binds tightly to calmodulin and thus is responsive to transient changes in intracellular calcium.

Calmodulin is a small (17 kDa) protein that binds 4 calcium ions by the four EF hand which bind Ca⁺² with high affinity



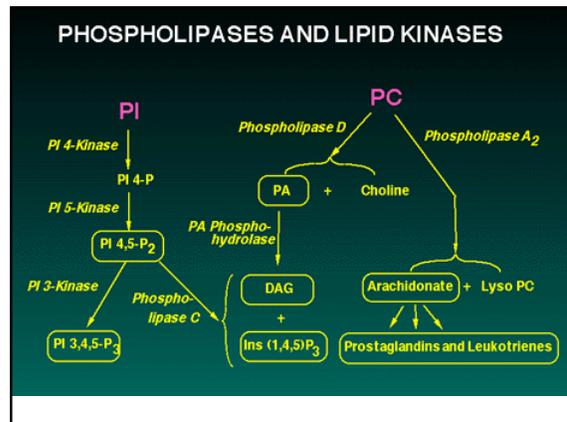
CaM-KII is a Ser/Thr protein kinase that binds and phosphorylates a wide variety of proteins. The protein is found in nearly all tissues and is a key component of Ca²⁺ signaling but is particularly enriched in neural tissue (up to 2% of the total protein in the hippocampus).

CaMKII is a complex of about 12 subunits

CaM-KII is a Ser/Thr protein kinase that binds and phosphorylates a wide variety of proteins. The protein is found in nearly all tissues and is a key component of Ca²⁺ signaling but is particularly enriched in neural tissue (up to 2% of the total protein in the hippocampus).

Ca binding to calmodulin activates the kinase by relieving its pseudosubstrate in a fashion similar to that of PKA.

- In the inactive state there is a strong interaction between the inhibitory and kinase domains
- Ca/Cam allows the catalytic domain to phosphorylate the inhibitory domain
- Enzyme stays active even after calcium is removed, prolonging the duration of kinase activity



Phospholipase C

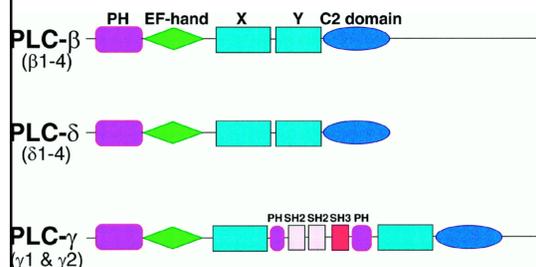
The lipase activity regulates PIP₂ signaling and is used by many different hormones. -So how is PLC regulated?

Which signaling systems depends on PLC isozyme

- 8 different in 4 families PLC proteins / genes
- PLC γ - activated by growth factors & insulin -> SH2 SH3 domains
- PLC β enzymes activated by Gq proteins some by $\beta\gamma$ subunits
- PLC δ - is not well defined

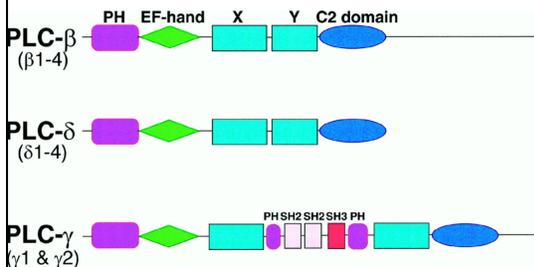
Differences in structural organization of forms of phospholipase C

- X and Y domains conserved throughout the PLC family - make up the catalytic domains



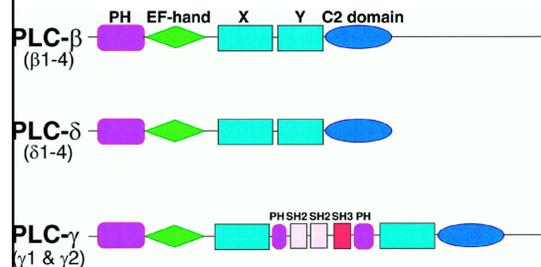
Differences in structural organization of forms of phospholipase C

- Each isozyme contains a PH (plekstrin homology domain) for binding inositol lipids



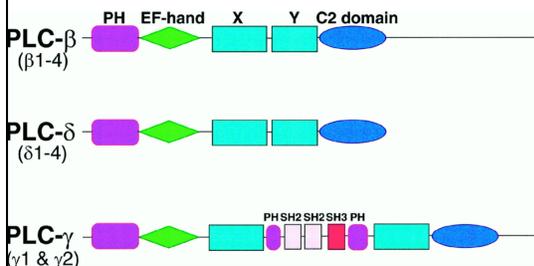
Differences in structural organization of forms of phospholipase C

- Calcium binding occurs through EF hand domain for each form and a C2 domain also interacts with calcium



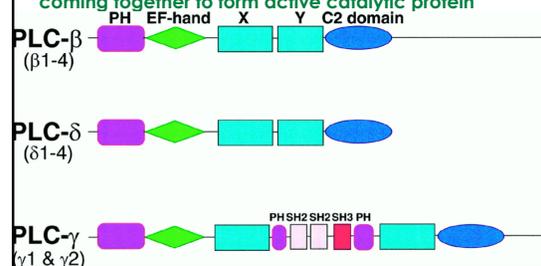
Differences in structural organization of forms of phospholipase C

- PLC γ - has additional SH domains (SH2 and SH3) for interactions with phosphotyrosine signaling



Differences in structural organization of forms of phospholipase C

- Calcium is required for activity, binding both at C2 domain and at the active site via histidine residues. Inositol lipids bind to protein, Ca^{+2} lead to X and Y domain coming together to form active catalytic protein



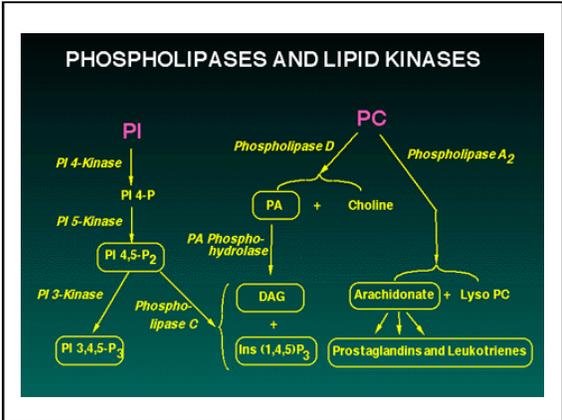
Activation of PLC beta form

- Activated by hormones which signal through Gq
 - Hormones include angiotensin II, alpha adrenergic agonists, acetylcholine
 - Lipase acts as GAP for G protein alpha subunit
 - G proteins interact at the C terminal
- beta gamma subunits of G proteins also can activate PLC beta

Activation of PLC gamma

- receptors which bind growth factors lead to PLC gamma activation
 - Interactions between intracellular domain of Receptor and PLC occurs by the SH2 domains
 - Once interacting PLC gamma becomes phosphorylated by the receptor on tyrosine residues - increases PLC interactions with other proteins
 - SH3 domain targets activated PLC to cytoskeletal proteins (actin myosin)
- In immune cells soluble tyrosine kinase can activate by phosphorylation of PLC

PLC inactivation: Additional phosphorylation of PLC leads to inhibition and down regulation of PLC signal - by either PKA or PKC



The phospholipase A2 (PLA2) enzymes hydrolyze fatty acid from the sn-2 position of phospholipid with the concomitant production of lysophospholipid.
 Mammalian cells contain structurally diverse forms of PLA2 including **secretory PLA2 (sPLA2)**, **calcium-independent PLA2**, and the 85-kDa **cytosolic PLA2 (cPLA2)** (1-4). PLA2 functions in the digestion of dietary lipid, microbial degradation, and regulation of phospholipid acyl turnover either in a housekeeping role for membrane repair or for the production of inflammatory lipid mediators.
 Phospholipase activity was first described in pancreatic juice and cobra venom at about the turn of the century.

- Another Phospholipase - PLD**
- Mammalian isoform- specific for phosphatidylcholine (PC)
 - Plant phosphatidylethanolamine (PE)
 - Animal form is activated by RhoA, PKC and ARF.
 - Involved in PC derived Diacylglycerol (DAG) - thus activating atypical and novel PKC isoforms
 - Many hormones activate this enzyme
 - PLD is believed to be responsible for chronic activation of PKC AND lipid rearrangement for signaling to exocytosis and Raf activation

Second Messengers
cAMP - adenylate cyclase and phosphodiesterase (caffeine inhibited) - '
Ca²⁺ - intracellular (mito and ER) and extracellular
Phosphoinositols
Bioactive lipids
 - DAG - PIP₂/PIP_x
 - PA / LPA - AA (arachadonic acid)

