

Model intracellular signaling pathway

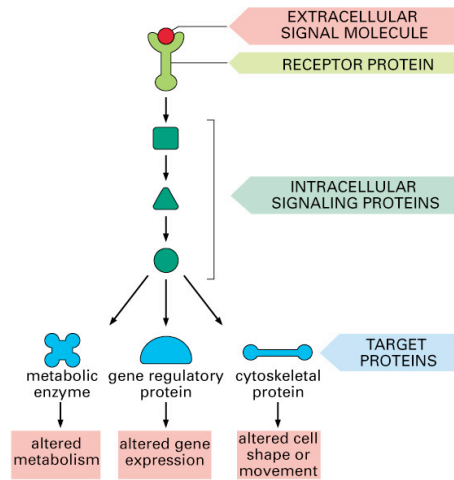


Figure 15-1. Molecular Biology of the Cell, 4th Edition.

Classes of receptors

- Surface receptors (ion channel, G-protein, enzyme)
- Intracellular (cytoplasmic or nuclear)

Schemes

- Endocrine – hormones in the blood, distant targeting cells
- Paracrine – secretory cell to adjacent target cell
- Synaptic – neurotransmitters

Pathways

- G-proteins
- Second messengers
- Kinase cascades

Cells adjust their sensitivity to a signal, and usually turn the signal off over time

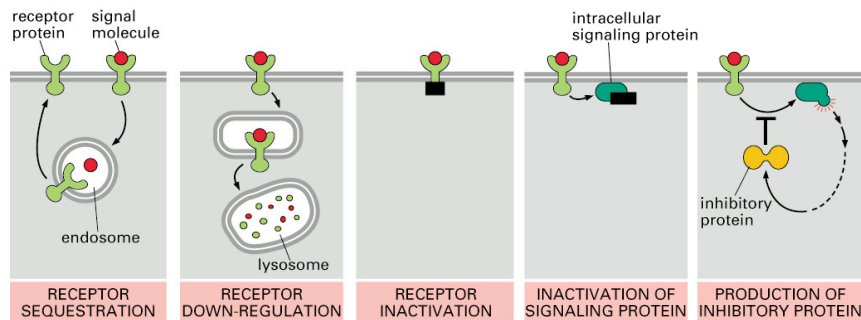


Figure 15-25 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Figure 15-25 part 2 of 2. Molecular Biology of the Cell,

Nuclear Receptors

- Intracellular
- Regulate gene expression in response to small lipophilic ligands derived from endocrine organs, metabolism, diet, environment
- DNA binding domain – Zn fingers, 4 highly conserved Cys residues
- Bind as either homo or heterodimers (GR-GR, RXR-TR)
- Stable DNA binding depends on Half-Site spacing (3-4-5 rule)
- In absence of ligand, receptor is bound to DNA through CoNR box (IxxII) and recruits co-repressors (N-CoR, SMRT)
- When ligand is present, receptor still bound to DNA through NR box (LxxLL) and recruits co-activators (CBP/p300, etc.)

G-Proteins

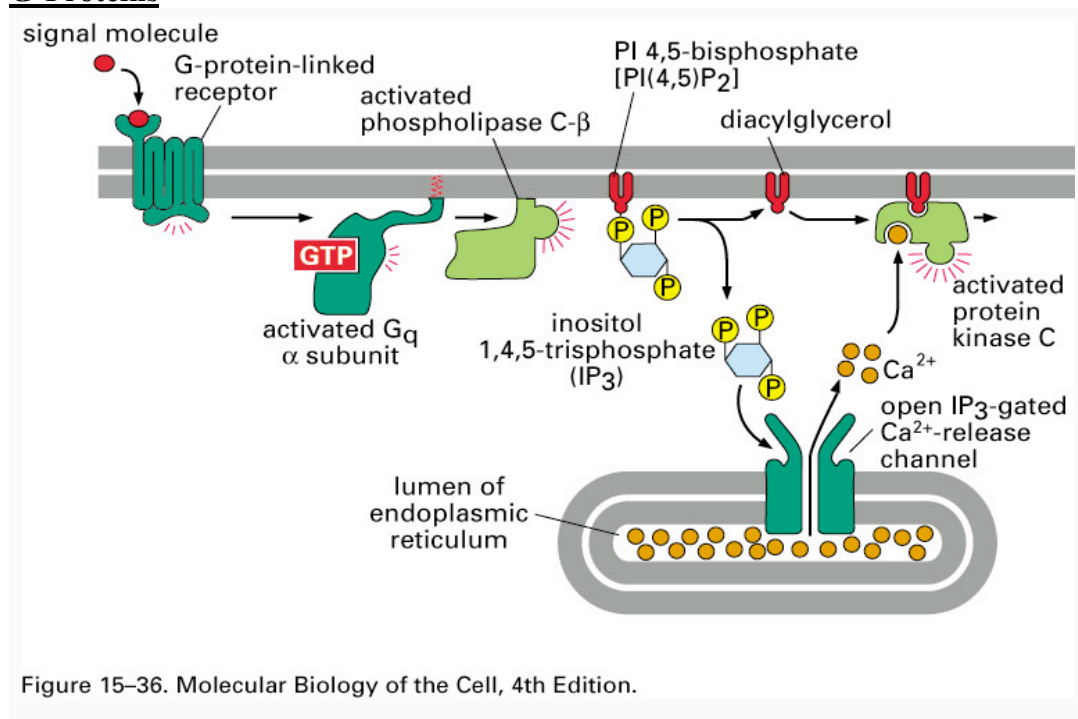


Figure 15–36. Molecular Biology of the Cell, 4th Edition.

- 7 TMD receptor gene family (4th largest)
- Heterotrimeric (α , β , γ) configuration – identity of G protein equated with identity of α subunit
- Regulated by factors influencing GTP/GPD exchange or GTP hydrolysis
- Activate or inhibit “effectors” in response to agonists
- G Protein Families:
 - G_s Adenylyl cyclase (stimulation), Ca²⁺ and Na⁺ channels
 - G_i Adenylyl cyclase (inhibition), PLC- β , cGMP
 - G_q PLC- β
 - G_{12/13} Rho
- Activation/Deactivation Cycle

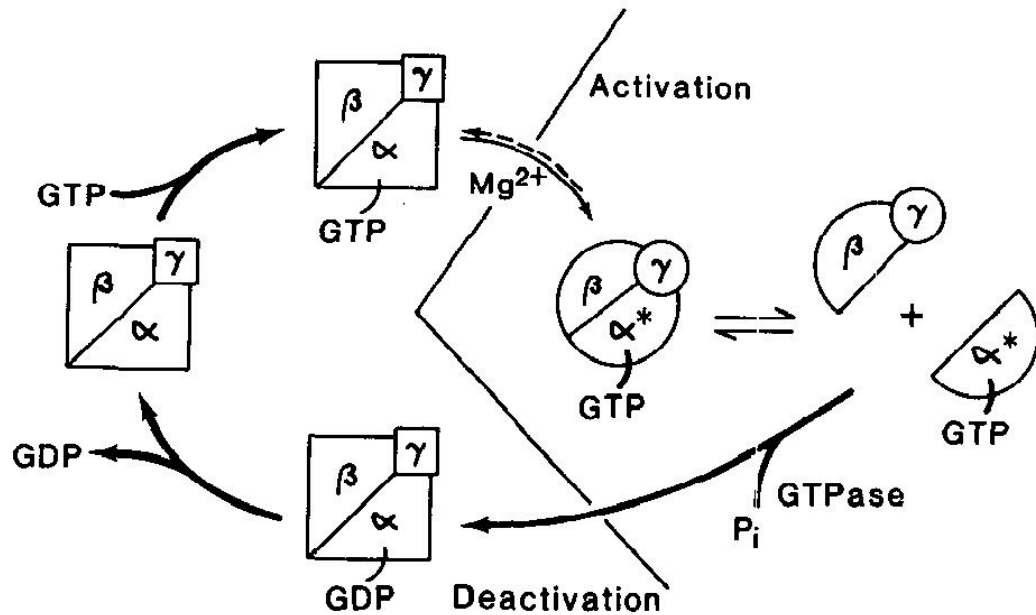
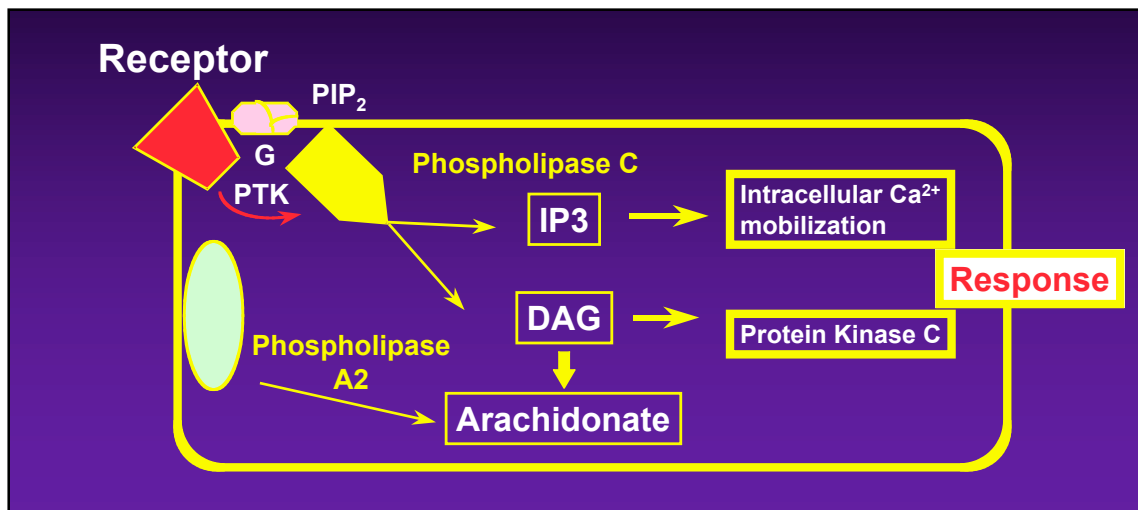


Figure 1 Regulatory cycle of a G protein. Squares and semisquares represent inactive conformations as they relate to modulation of effector functions. Circles and semicircles represent activated forms of the G protein. Activation is both GTP and Mg^{2+} dependent and is stabilized by subunit dissociation to give an activated $G\alpha^*$ -GTP complex plus the $G\beta\gamma$ dimer. Hydrolysis of GTP by the $G\alpha$ subunit deactivates it, increases its affinity for $G\beta\gamma$, and leads to reassociation to give an inactive holo-G protein with GDP bound to it. Reinitiation of the activation system requires release of GDP and renewed binding of GTP. Specificity of action is encoded in $G\alpha$. Different $G\alpha$ subunits associate with a common pool of $G\beta\gamma$ dimers.

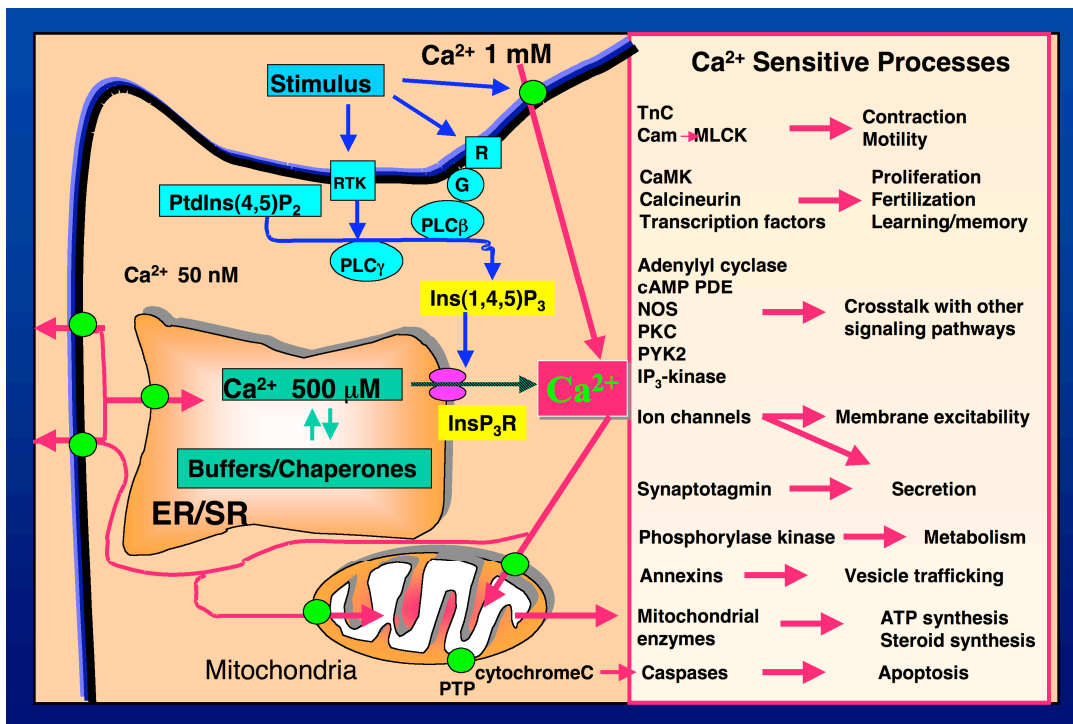
Phospholipase C



- Hydrolysis of PIP_2 by PLC generates second messengers IP_3 and diacylglycerol (DAG)

- Hormone = first messenger
- Hormone binds plasma receptor resulting in activation of IP₃ (2nd messenger)
- Criteria for second messenger:
 - Agonist must stimulate PLC
 - Change in IP₃ should precede or occur at the same time as response to agonist
 - Inhibition of IP₃ metabolism should increase action of agonist
 - Biological effect of agonist should be mimicked by addition of IP₃
- 10 mammalian isoforms (β, γ, δ)
- Catalytic domain: X and Y; Scr homology domains (SH2 and SH3); Pleckstrin homology domain (PH) mediates binding to PIP₂; C2 domain mediates Ca²⁺ dependent binding to lipid
- G proteins activate PLC-β: G_q α subunit, G protein βγ subunit

Calcium Signaling



- IP₃ mediated [Ca²⁺]_i signaling is ubiquitous
- InsP₃ mediated [Ca²⁺]_i signaling requires pumps, channels and buffers
- InsP₃ mediated [Ca²⁺]_i signals are local and global

Tyrosine Phosphorylation

- How does a growth factor transmit a signal across the membrane?

- Ligand binding on the outside alters the interaction of subunits of the receptor (e.g. dimerization)
- Oligomerization of receptors critical for transmitting the signal
- How does the receptor get activated?
 - Oligomerization results in transphosphorylation of the receptor
 - As long as the receptor remains phosphorylated, ligand no longer needed
- How does the active receptor subunit transmit the signal to intracellular targets?
 - Intracellular signaling by protein kinases achieved by assembly of stable signaling complexes

Classes of cell surface receptors

(C) ENZYME-LINKED RECEPTORS

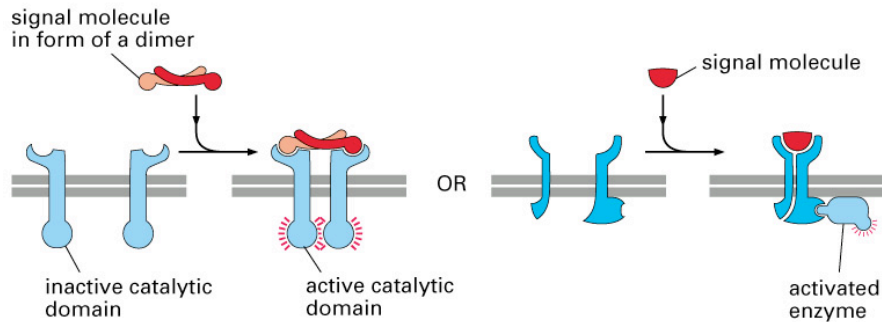


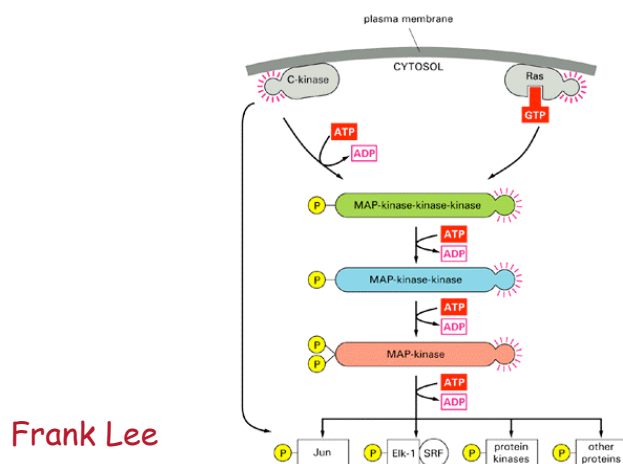
Figure 15-15 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Morrie Birnbaum

Serine and Threonine Phosphorylation

Types of intracellular signaling pathways

Kinase cascades



Frank Lee

- Protein kinases are bean shaped, with a smaller N-terminal lobe and a larger C-terminal lobe.
- Active site resides in a cleft between the two lobes (ex. PKA)

- *PKC*
 - Integrates DAG and calcium signals (activated by growth factors like PDGF and insulin)
 - Modular structure (C1: N-term catalytic domain, C3/4 – C term love, AI- autoinhibitory domain ->inactive in resting state since AI binds active site)
 - Increase in cytosolic calcium sensed by C2 domain (aspartate rich region, binds Ca^{2+}). C2 domain also serves to target PKC to the membrane
 - C1 domain binds DAG and also promotes membrane translocation of PKC. DAG also increases affinity of C2 for phospho serine in the membrane
 - Binding of C1 and C2 to DAG and Ca^{2+} promote dissociation of AI peptide from active site -> augment catalytic activity
- Mitogen activated protein kinase (MAPK)

Raf	->	MEK	->	ERK	->	Elk-1
MAP3K		MAP2K		MAPK		

 - ERK is insulin stimulated protein kinase phosphorylated at Thr-183 and Tyr-185
 - In resting cells, ERK present in inactive unphosphorylated form
 - MEK – dual specificity kinase (phosphorylates both Thr and Tyr). Activated by phosphorylation of its two Ser residues
 - Raf activated by Ras, a small GTPase. Upon stimulation by growth factors, Ras is activated and binds GTP. Ras:GTP complex binds and activates Raf.
- Kinase transducing signals
 - Can allow substantial amplification of signal
 - Allow for multiple layers of regulation – each kinase in a cascade can serve as a target for regulation
 - Allow multiple points of crosstalk with other signal transduction pathways