

Cell-to-Cell Signaling Signaling hardware



Mechanisms of molecular transduction



- Phosphorylation and dephosphorylation
 - Addition and removal of phosphate group
- Activation of G proteins
- Molecular recognition by adapter proteins

Signaling paradigm



- All of the listed mechanisms work as binary switches (they are on or off)
 - Single protein can be **active or inactive** (and not half way)
- A series of these binary switches (proteins) makes a signaling cascade
 - It can be one or many of them in a series or in parallel
- Amplification and divergence

Phosphorylation

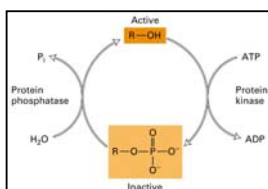


- Post-translational modification
- Addition of γ phosphate from ATP to side chains of residues in a polypeptide chain
- Regulates activity
 - Metabolic enzymes
 - Ion channels
 - Cell cycle progression
- Can turn the process on or off

Kinases and phosphatases



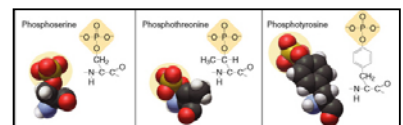
- **Kinases** phosphorylate (add phosphate group) and **phosphatases** dephosphorylate (remove phosphate group)



Phosphorylated residues

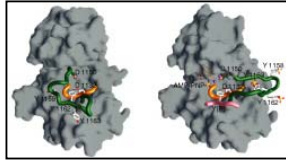


- 99% serine and threonine
- Tyrosine – regulation of several critical processes
- Bacteria use also histidine and aspartate
- Kinases can also have dual specificity



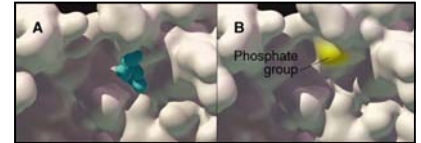
Effects of phosphorylation

- Change of conformation
- Alteration of interactions with substrates (steric interference)



Effects of phosphorylation

- Alteration of interactions with substrates
 - Block of substrate binding sites
 - Creation of binding sites
 - SH2/PTB domains recognize only phosphorylated ligands

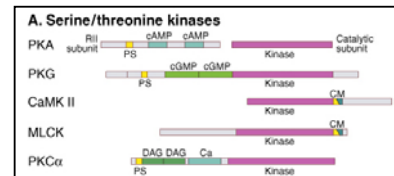


Protein kinases

- Enzymes!
- Catalyze the addition of γ phosphate from ATP
- Catalytic (kinase) domain usually forms a pocket for ATP binding

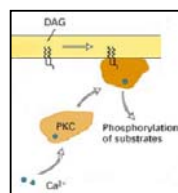
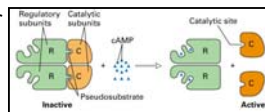
Protein kinases

- Very homologous in structure
 - Kinase domain joined to additional domains for binding, localization and regulation



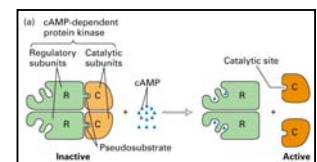
Protein kinases - regulation

- Phosphorylation by other kinases
- Activation by second messengers
- Regulatory subunit
 - Pseudosubstrate sequences
 - Regulatory subunits
 - Activating subunits
- Targeting to specific location



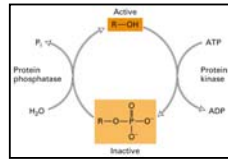
Regulation of PKA

- Pseudosubstrate sequence on regulatory subunit is plugging the catalytic site
- Binding of cAMP changes conformation of regulatory subunit and dissociation (activation)



Protein phosphatases

- Enzymes too!
- Remove phosphate from amino acid side chains
- Similar in structure, diverse in function



Protein phosphatases

- Directed toward serine/threonine or tyrosine
- Dual specificity too
- In many cases the kinases have a built in mechanism for phosphatase activation
 - Prevents constant activation of kinase that is usually harmful to a cell or organism

G proteins

- Work as molecular switches
- Use a cycle of GTP binding and hydrolysis to switch the protein on and off
 - G protein is *on* when bound to GTP, *off* when bound to GDP

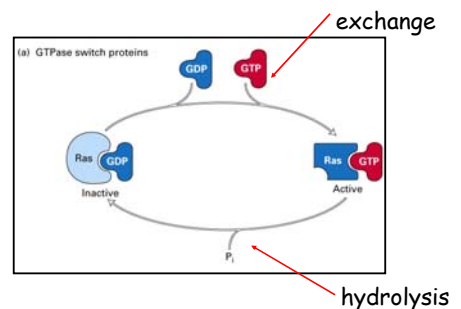
G proteins

- GTP/GDP change the conformation of G protein
 - GDP bound form does not bind effectors
 - GTP bound form interacts with and stimulates effector proteins
- **G proteins = GTPases**, have intrinsic enzymatic activity (means they are enzymes!)

G protein cycle

- Replacement of GDP with GTP turns the G protein (switch) on
- GTP hydrolysis turns the G protein (switch) off
- **Notice that the process is not symmetrical!**
 - Exchange turns the protein on
 - Hydrolysis turns the protein off

Basic G protein cycle



G protein cycle

- Hydrolysis of GTP to GDP that terminates the signal is done by G protein itself (**intrinsic GTPase activity**)



G proteins - **Big** and small

- Small – monomeric
- Trimeric (heterotrimeric)
- Elongation factors
- Dynamin related GTPases



Small or monomeric G proteins

- Have only one subunit
- Ex: Ras, Rho, Cdc42
- Control multiple aspects of cell metabolism and growth, also cytoskeleton



Trimeric G proteins = G proteins

- Have three subunits α , β , γ
- For practical purposes there are only two subunits α and $\beta\gamma$
- Membrane bound proteins
- Coupled to seven helix receptors



Subunit diversity

- 16 α , 6 β , 12 γ
- Alternative splicing provides even more diversity
- A particular combination of subunits couples to a particular seven helix receptor



Trimeric G proteins cycle

- When G protein is inactive it is bound to GDP and exists as a trimer
- The exchange of GDP for GTP activates G protein
- G protein dissociates into two subunits: α and $\beta\gamma$ dimer
 - GTP is bound to α subunit



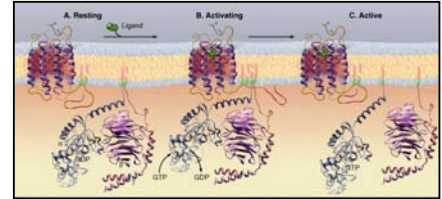
Trimeric G proteins cycle

- α subunit has an intrinsic GTPase activity and hydrolyses GTP to GDP
- This process terminates the signal
- α and $\beta\gamma$ reassociate into the trimer



G protein activation by seven helix receptor

- Activated receptor "speeds up" the exchange of GDP for GTP and the activation of G protein

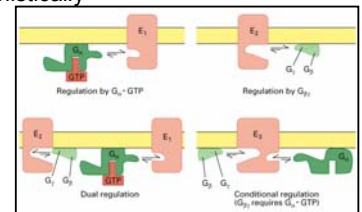


G protein effectors

- Each subunit can activate (inhibit) the effector enzyme and production of second messengers
- G proteins activate several signal transduction cascades

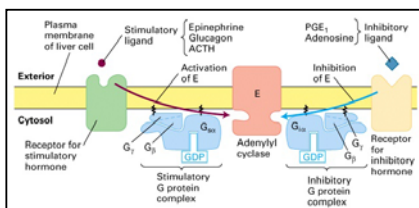
Both subunits, α and $\beta\gamma$ dimer, are active signaling molecules

- May act individually
- May act synergistically
- Or even antagonistically



Antagonistic action of G proteins

- Adenylyl cyclase can be differentially regulated by different G proteins



What can G proteins do?

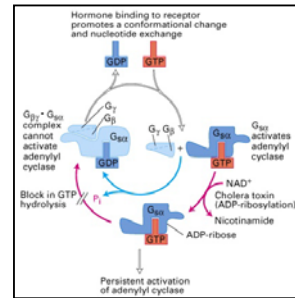
- Activate enzymes and production of second messengers
 - Activate adenylyl cyclase and production of cAMP
 - Activate phospholipase C and breakdown of membrane phospholipids (phospholipid second messenger)
- Activate transcription factors
- Modulate ion channels, pumps and exchangers
- Affect cytoskeleton

G proteins can be modified by bacterial toxins

Cholera toxin

- Addition of ADP-ribose to α subunit in Gs
- Normal activation of adenylyl cyclase
- No hydrolysis - signal transduction pathway remains activated
 - ChTX sensitive - Gs
 - ChTX insensitive - all others

Cholera toxin modifies Gs



G proteins can be modified by bacterial toxins

Pertussis toxin

- Addition of ADP-ribose to α subunit
- Prevents release of GDP
- Inhibits dissociation of subunits
 - PTX sensitive - G_i and G_o
 - PTX insensitive - G_q and G₁₂ families

Mechanisms of molecular transduction

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- › Phosphorylation and dephosphorylation
 - › Addition and removal of phosphate group
- Molecular recognition by adapter proteins

Adapter proteins

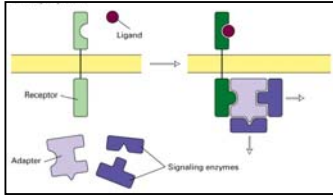
- Contain various protein-binding motifs that promote formation of multiprotein complexes (puzzle fit)
 - They are **no enzymes**
 - They don't directly activate effector proteins

Adapter proteins

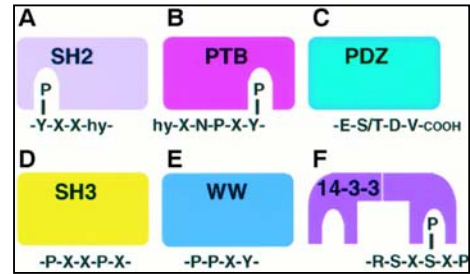
- Often seen domains
 - SH2 – src homology domain
 - PTB - phosphotyrosine binding motif
 - PH – plextrin homology
 - SH3
 - PDZ
- The same adapter protein can have different domains in different places

Adapter proteins

- Use lock and key strategy
- Specificity is provided by different locks and keys

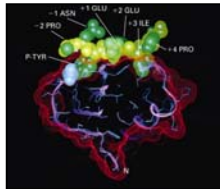


Diversity of adapter proteins



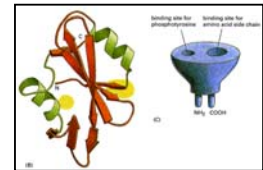
SH2 domains

- Src homology 2 from the homology with the region of highly conserved cytosolic kinase encoded by src gene
- Highly conserved polypeptide domain
- Participates in protein-protein interactions



Phosphotyrosine Binding Domain (PTB)

- Specificity of binding is determined by very specific residues next to the phosphotyrosine



- Tyr-hydrophobic-X-hydrophobic

SH3 domains

- Another protein-protein interaction domain
- Present in a large number of proteins involved in intracellular signaling
- β -barrel of 5-6 anti-parallel β -strands
- Facilitate interactions between proteins by extended conformation and increase of contact area

SH3 domains

- Form pockets for proline residues
- Selectively bind to proline rich sequences in target proteins
- Loss of binding can lead for example to a constitutively active Src molecule and cancer

