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Cell signaling

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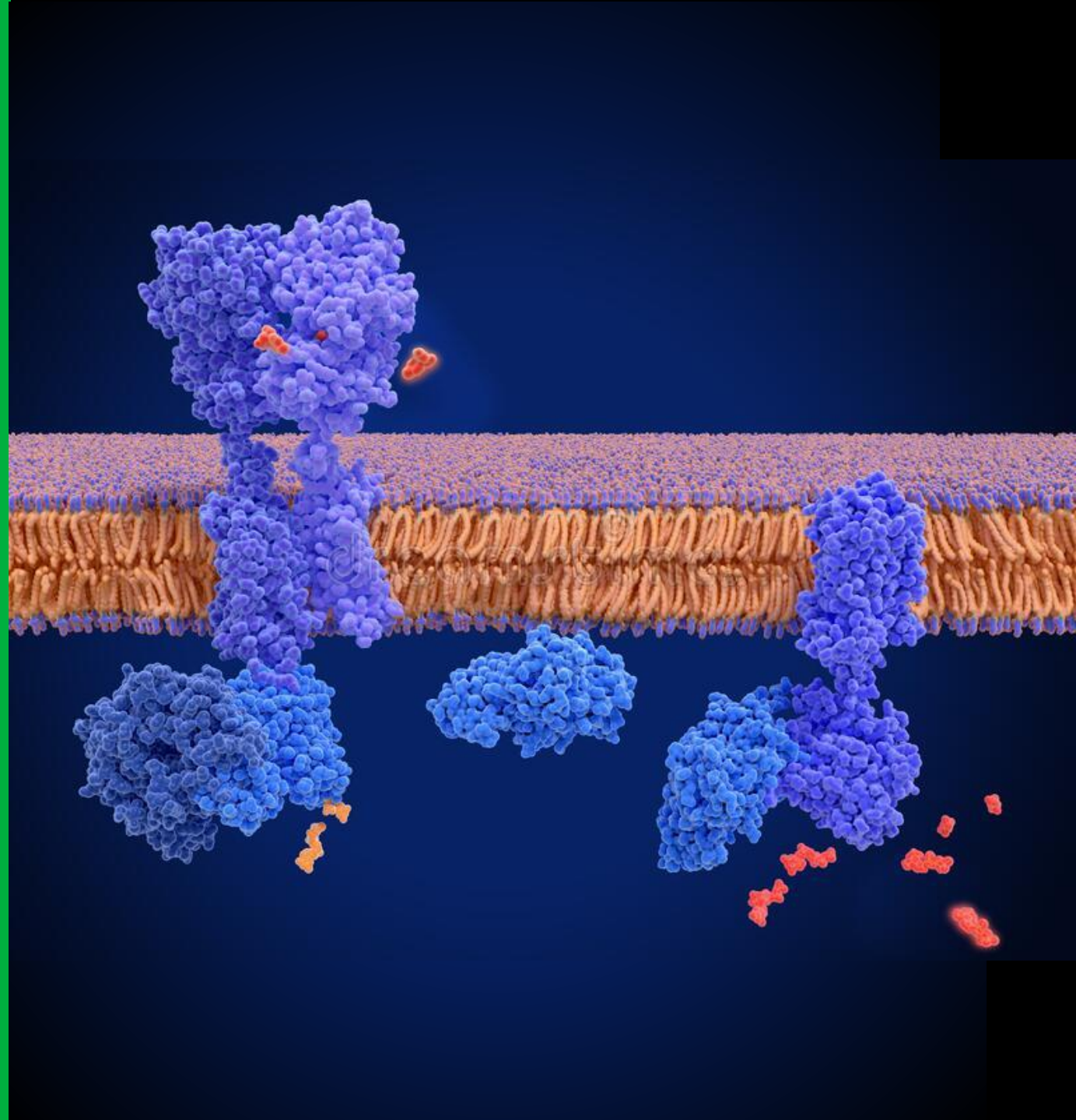


Outline

- Principles of cell-cell communication
- Signals – their origin and distance of action
- Intracellular signaling pathways
- Chemical nature of signals
- Examples of signals and their effects in the cell

Communication

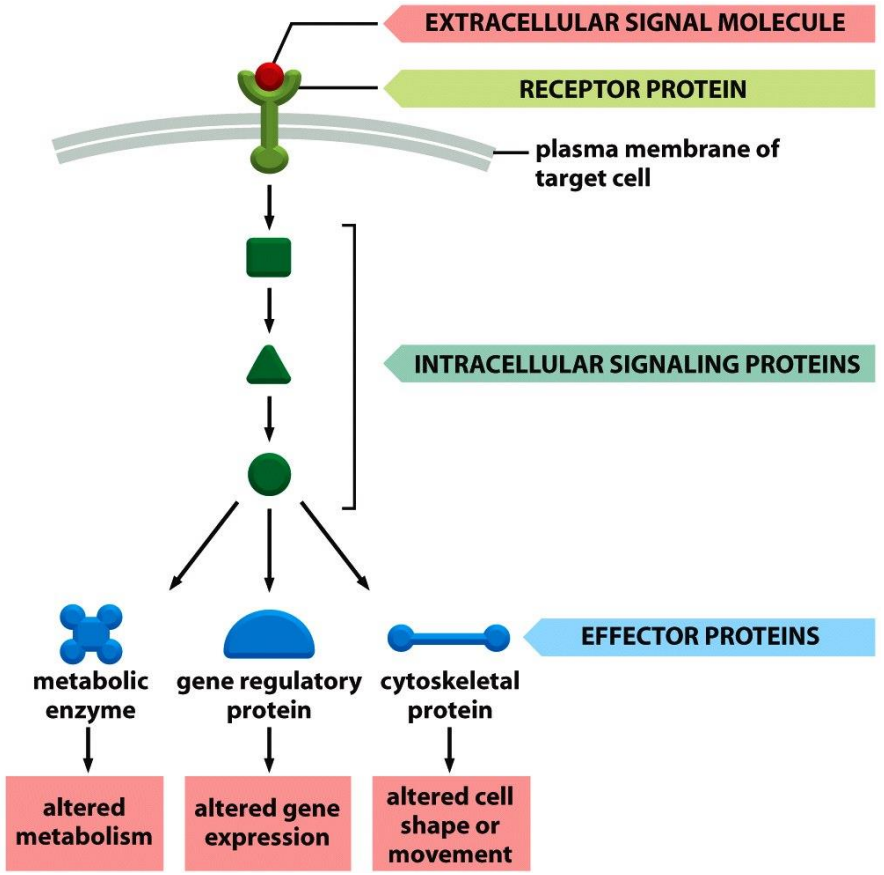
- Response to the presence of other cells – influence one another's behavior in accordance with the changes in their environment

- Prokaryotes: **quorum sensing** – population density coordinate motility, antibiotic production, spore formation, conjugation
- Yeast: **mating factor** – peptide, signal for mating
- Multicellular organisms: **homeostasis**, **development**, **coordination of activities** in response to the environment (food, photoperiodism, circadian rhythms, predators…)

Principles of cell communication

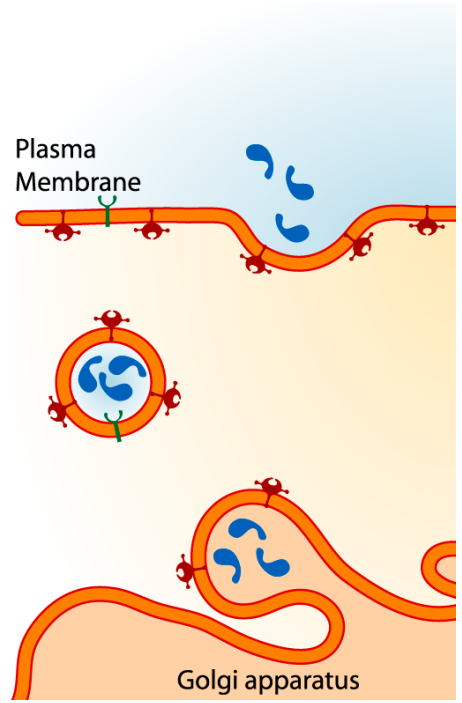
Principles of cell communication

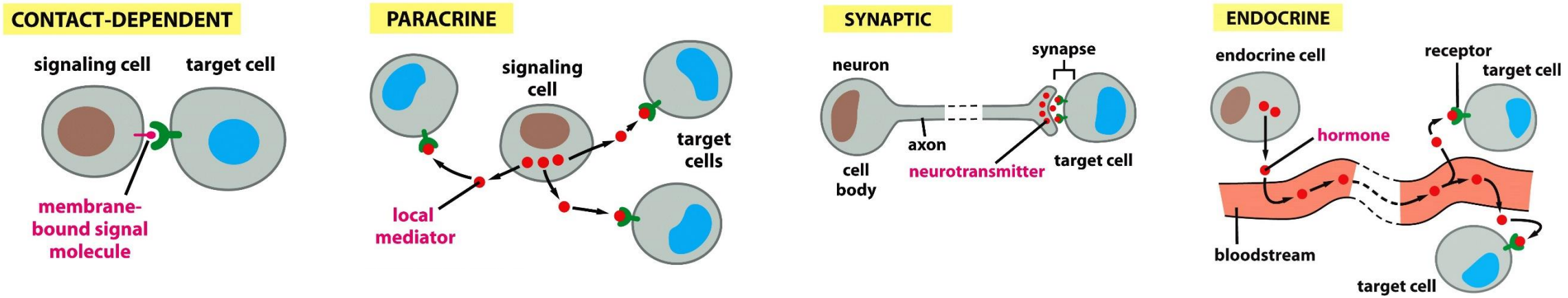
1. **Signaling molecule** (a ligand) produced by the signaling cell
2. Signaling molecule binds to a **receptor** at the target cell (at the membrane or internal)
3. Activated receptor induces internal **signaling pathways** leading to effectors = **signal transduction** (conversion of an extracellular molecular signal into a different form of messengers)
4. **Effectors** control the cell response



Signal production

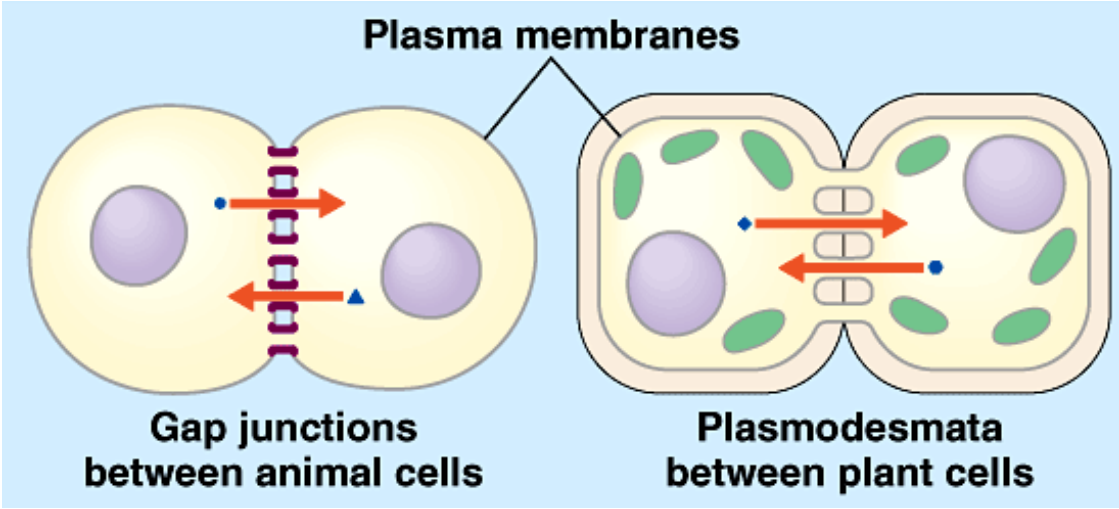
- Utilizes vesicular transport and exocytosis
- Signaling molecules **secreted** or presented **at the cell surface**
- **Different distance of activity**
  - Contact, local, distant





Direct communication

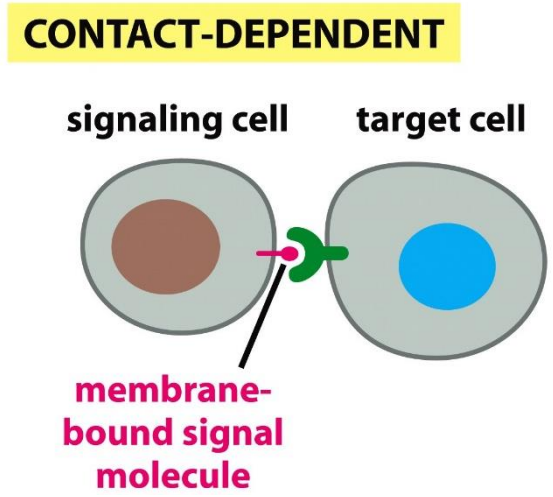
- **Gap junctions** in animal cells
- **Plasmodesmata** in plant cells
- Channels allowing exchange of ions and small soluble molecules (not macromolecules)

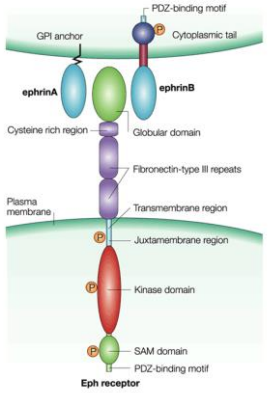


- **Bidirectional signaling, follows concentration gradient**
- Signal molecules can spread to cells that would not otherwise receive it (synchronization of activities)

Contact-dependent signaling

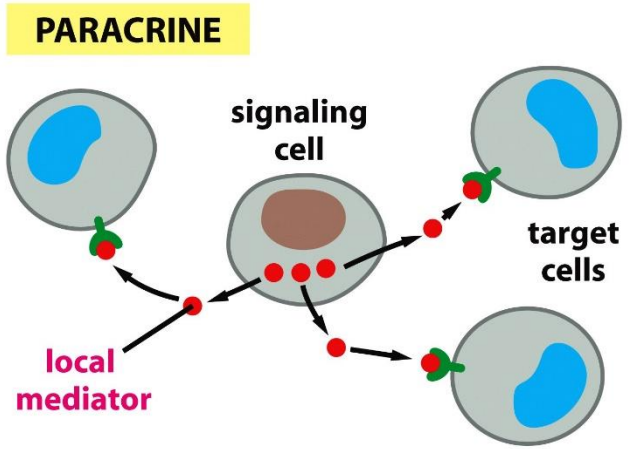
- Juxtacrine signaling
- **Signaling molecule immobilized in the membrane** of the signaling cell
- **Contact with the receptor** of the target cell
- Mediates contact of cells
- Important in development and immune responses
  - Notch, Programmed death-ligand 1, ephrin signaling

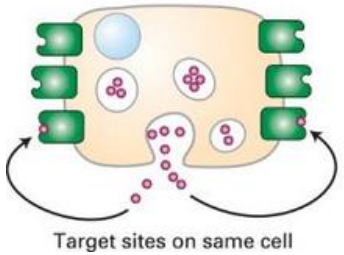




Paracrine signaling

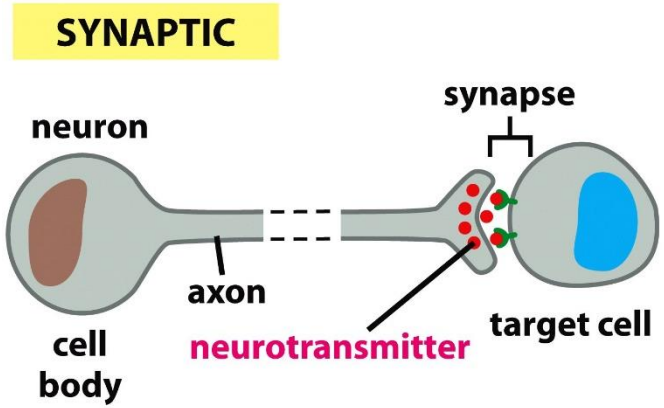
- **Secreted ligands, local mediators**
  - Signaling molecules diffuse locally in the extracellular space
- Signals **activate receptors of neighboring cells**
  - E.g., regulation of inflammation or proliferation during wound healing
- vs. **autocrine signaling:** “self-activation”; triggers response in the producing cell; common in cancer cells

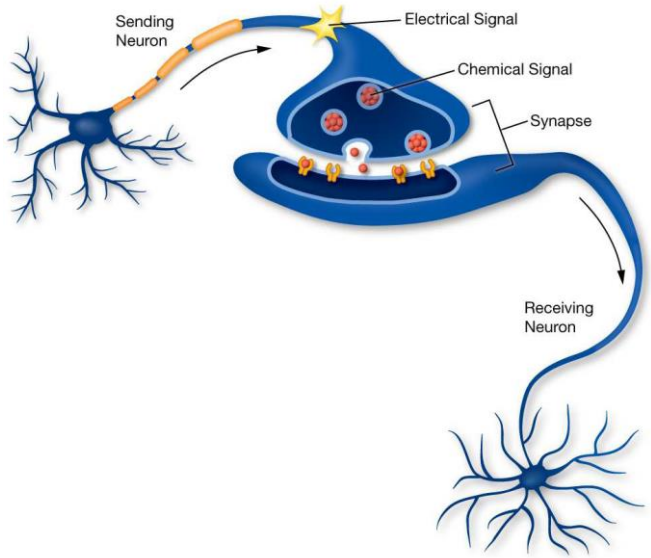




Synaptic signaling

- **Neurons** transmit **electrical impulses** along axons
- Long distances, fast, dedicated structures
- **Synapses** – sites of cell-cell communication
- Release of **neurotransmitters** – electrical signal converted into chemical

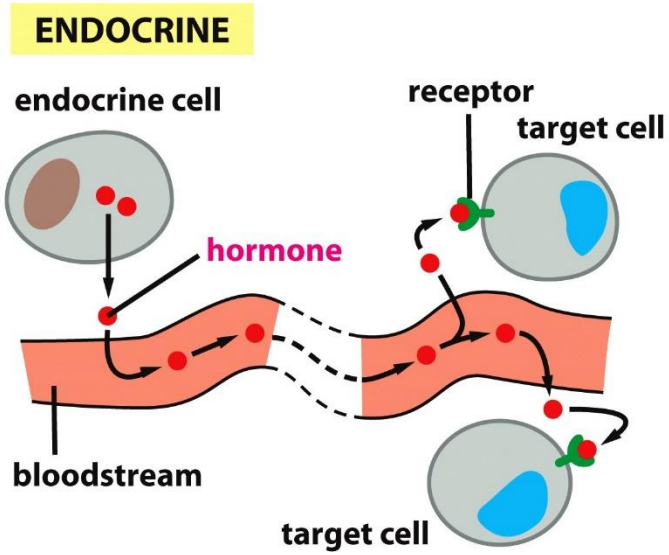




Endocrine signaling

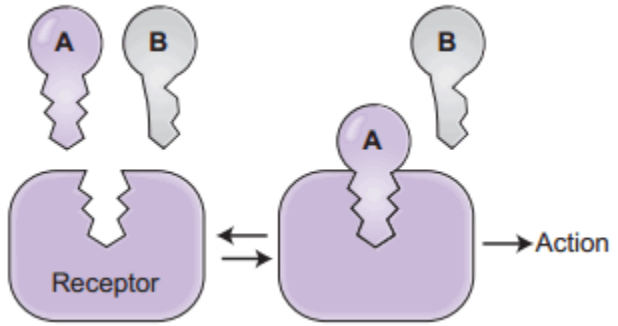
- **Long distance signaling**, whole organism
- Slower but longer-acting
- Via bloodstream in animals, phloem in plants

- Systemic coordination of activities (different responses in different cells)

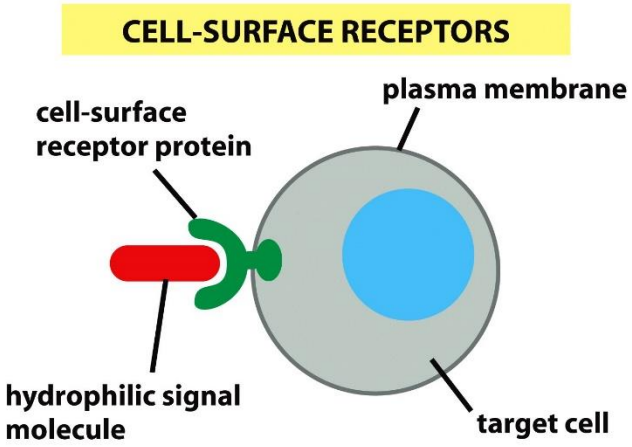


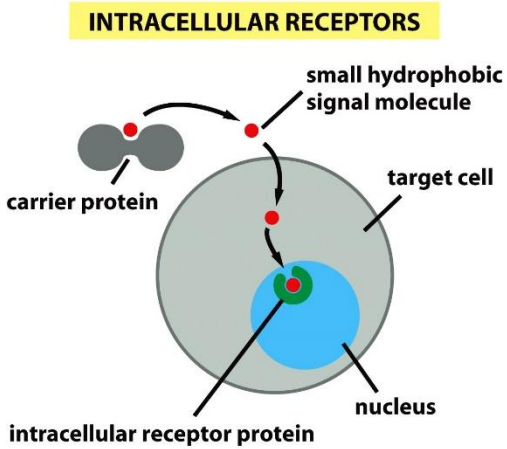
Receptor activation and cell responses

- **Receptors** are activated by **specific ligands**
  - Receptors bind ligands with high affinity – ligands can act at very low concentrations
  - Expression (presence, concentration) of the receptor dictates sensitivity of the cell to the signal
- **Cell surface** and **intracellular receptors**



Receptors & ligands:
"Lock & key" mechanism





Not all ligands activate the receptor

Endogenous agonists: growth factors, hormones, neurotransmitters

Agonists: chemicals binding and activating the receptor

Antagonists: chemicals blocking the receptor

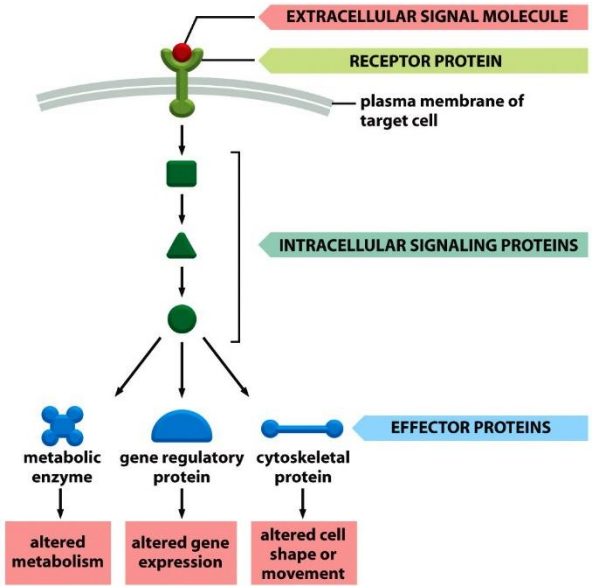
- might have higher affinity than endogenous agonists = concentration and affinity-dependent effects

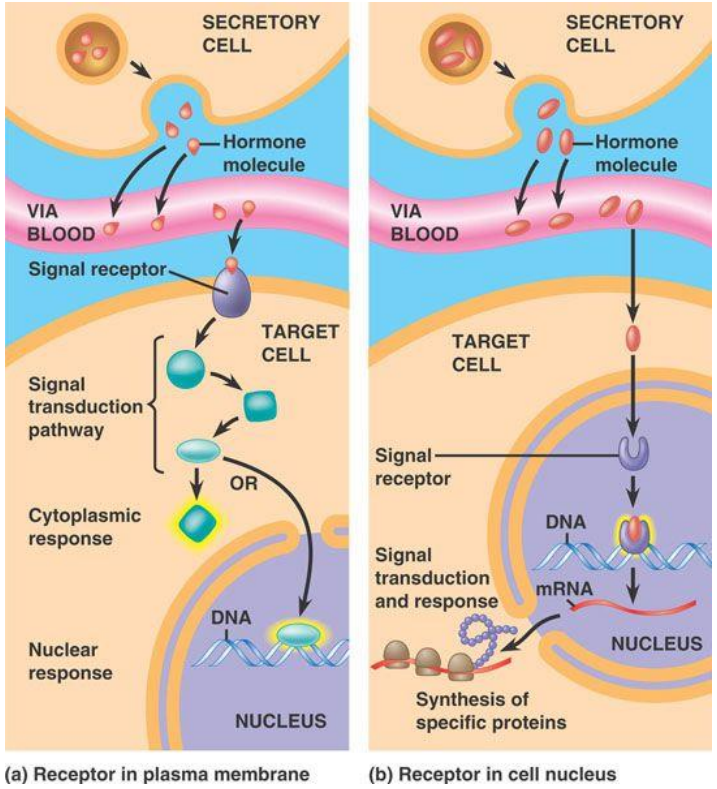
Receptor activation and cell responses

- Downstream signaling pathways induce effectors and context-dependent response

Major effectors:

- Gene regulatory proteins
- Enzymes
- Cytoskeletal proteins



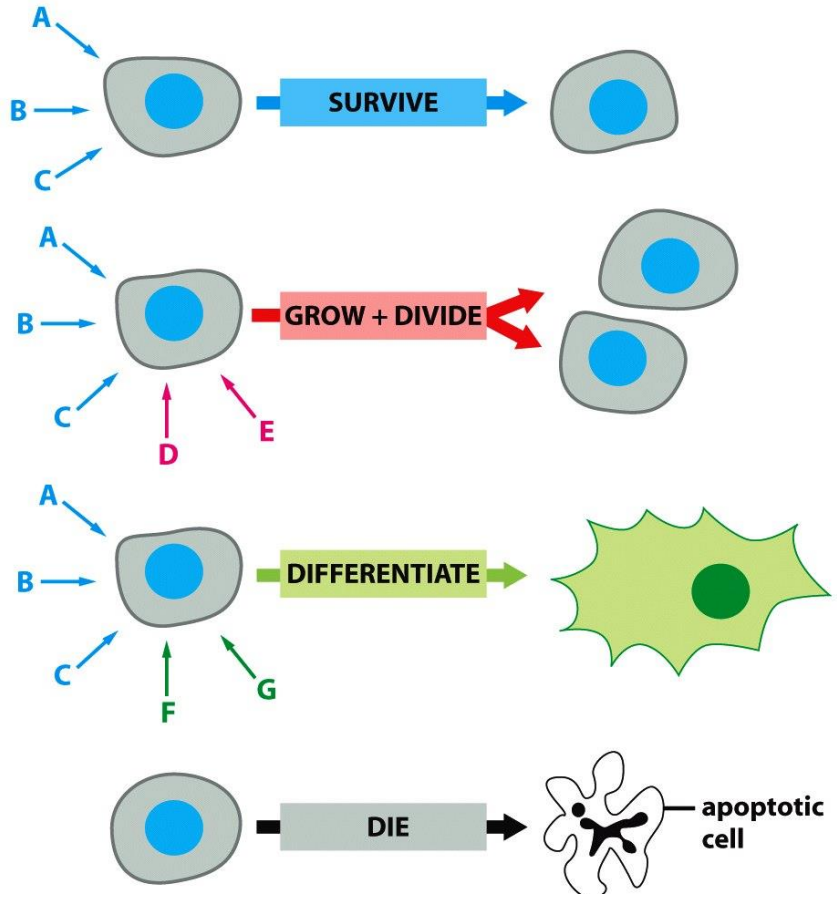


(a) Receptor in plasma membrane (b) Receptor in cell nucleus

Transcription, metabolism, movement, differentiation, cell death…

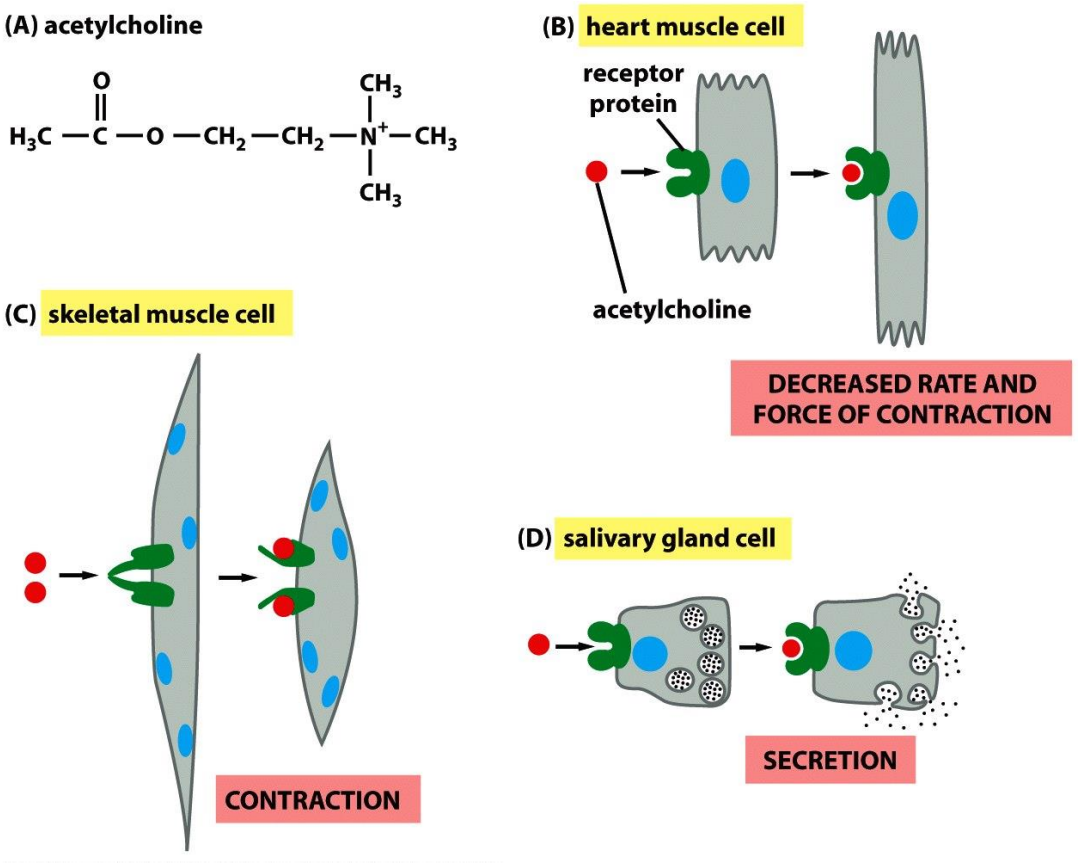
Cell responds to a spectrum of signaling molecules

- **Different sets of receptors** at different cells
- **Combination of signals** shape the final response



One signal triggers different responses

- Acetylcholine
  - Inhibitory effects in heart muscle cells
  - Stimulation of skeletal muscle cells
  - Stimulation of salivary gland cells
- Differences in receptors
  - Some ligands bind to more receptors
- **Differences downstream of the receptor – signal processing**
  - Components of the signaling pathways, expression profiles and effector proteins

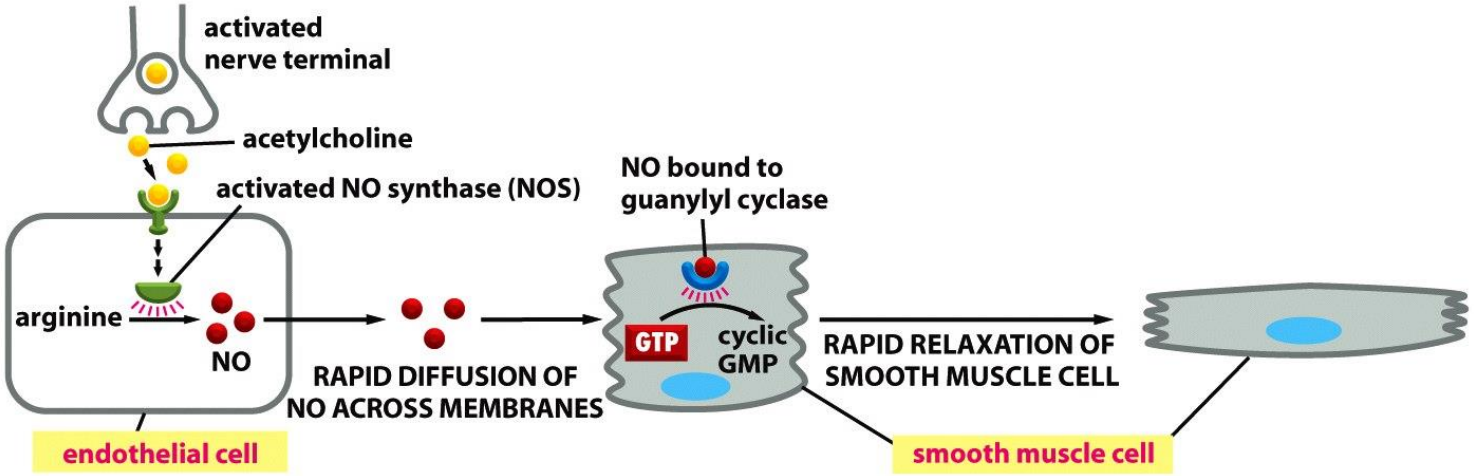


Ligands binding to intracellular targets

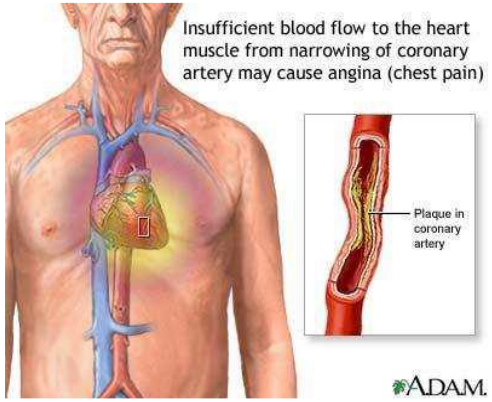
- Diffusing **gaseous** and **hydrophobic** signaling molecules

Nitric oxide

- From arginine – **NO synthases**
- Binds to **guanylyl cyclase** → **cyclic GMP** → cGMP activates effectors
- Fast response
- Acts locally (+ H_2O, O_2 → nitrates, nitrites)
- cGMP breaks down by **phosphodiesterase**



Nitroglycerin



- Treatment of angina episodes
- **Converted to NO** → relaxed blood vessels

Viagra (Sildenafil)

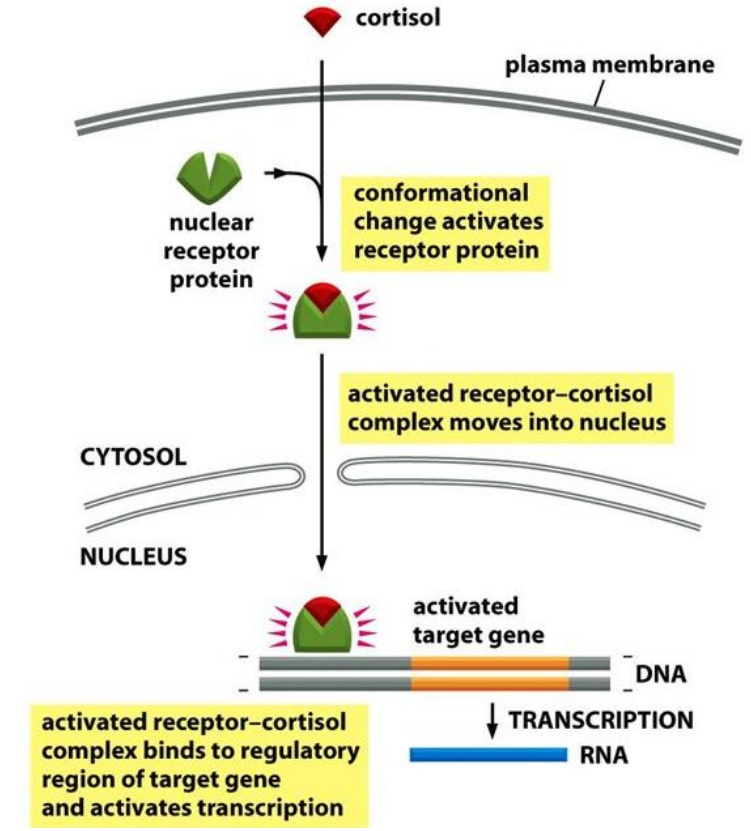
- **Inhibits cGMP phosphodiesterase** in the penis → prolonged activity of cGMP = relaxed blood vessels

Ligands binding to intracellular targets

- Diffusing **gaseous** and **hydrophobic** signaling molecules

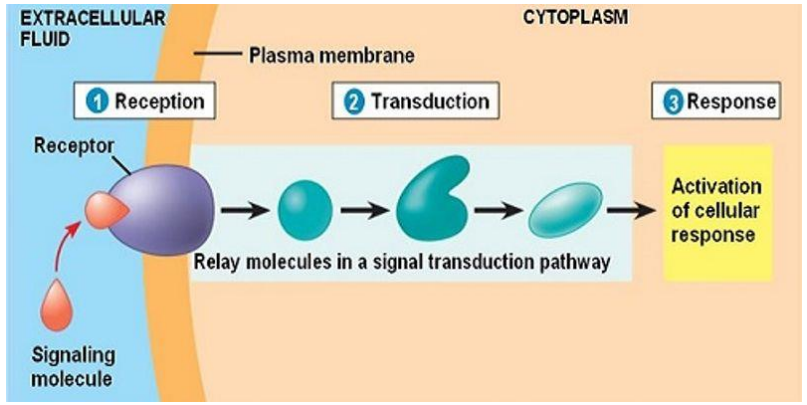
Steroid and thyroid hormones

- Bind to intracellular **nuclear receptors**
- Nuclear receptors contain DNA-binding domain
- Active nuclear receptors can bind to DNA and **regulate transcription**



Intracellular signaling pathways

Complexity of signal transduction



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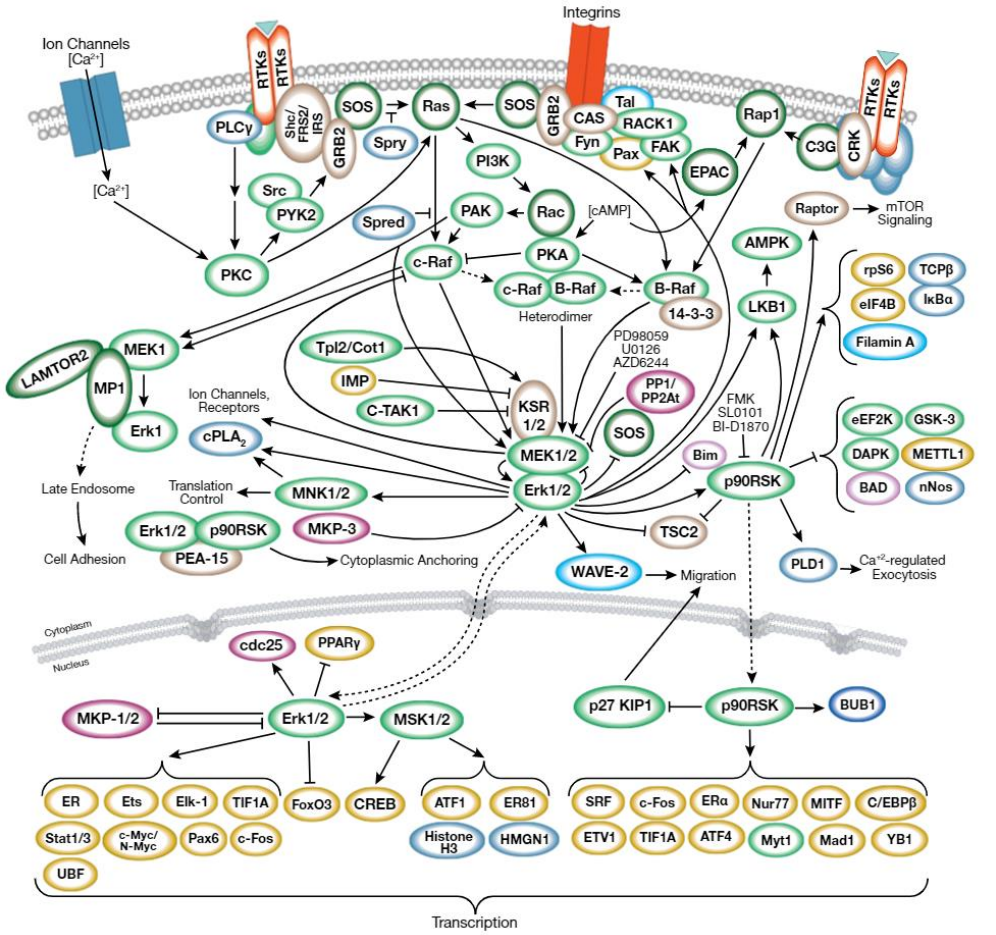


Receptors

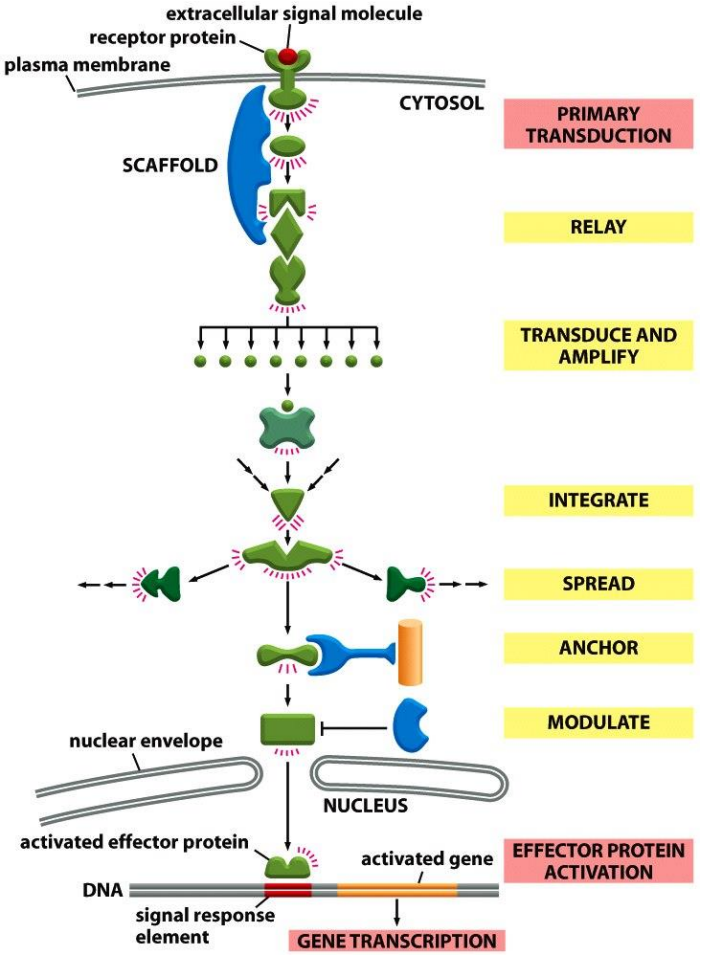
Signal transduction

Effectors

Response

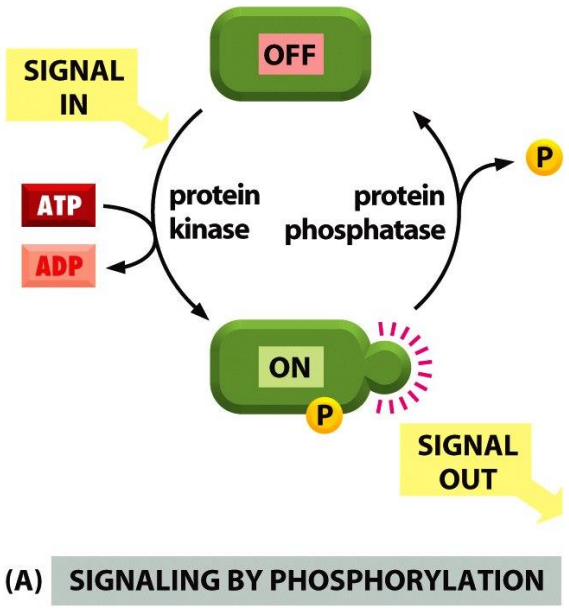
Complexity of signal transduction

- Primary transduction of the signal
- Relay of signaling proteins – often function as molecular switches
- **Amplification and processing of the signal**
- Some signaling proteins shared – crosstalk of signaling pathways
- Outcome: **One signaling molecule can induce many effectors**



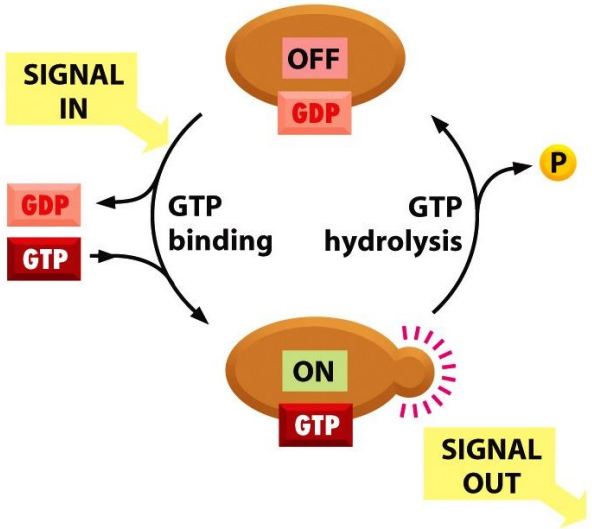
Molecular switches

- Signaling proteins often **switch between “on” & “off” states**
- Signal turns signaling proteins active: **phosphorylation/GTP-binding**
  - This might lead to deactivation of other signaling proteins (counterbalance between pathways)



(A) SIGNALING BY PHOSPHORYLATION

- Protein kinase covalently binds phosphate group
- Protein phosphatase catalyze dephosphorylation

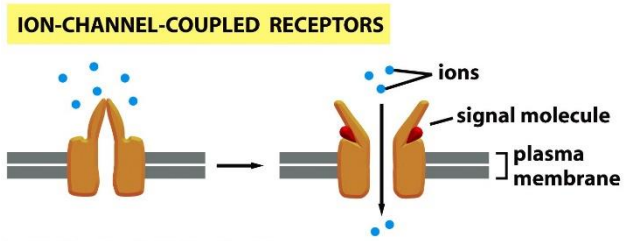


(B) SIGNALING BY GTP-BINDING

- Signal triggers exchange of GDP for GTP
- Hydrolysis of GTP inactivates the signaling protein

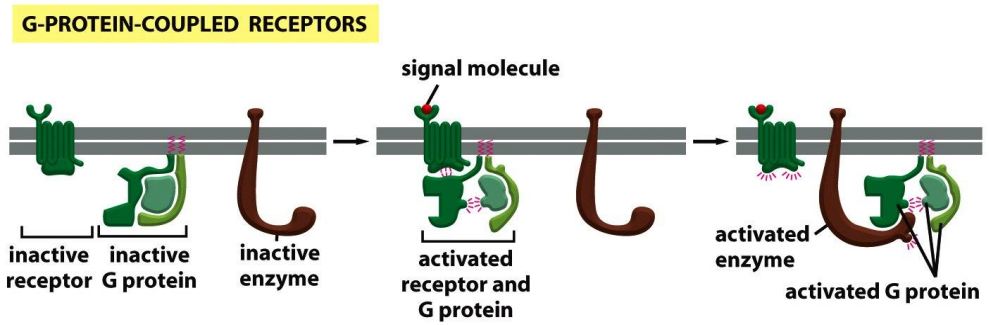
Major classes of cell-surface receptors

1)



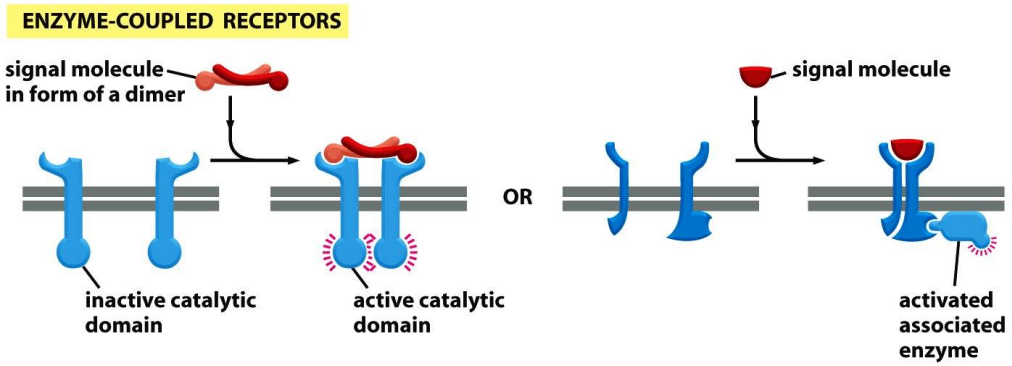
– Regulate ion-channel gating

2)



– Control activity of enzymes or channel gating

3)



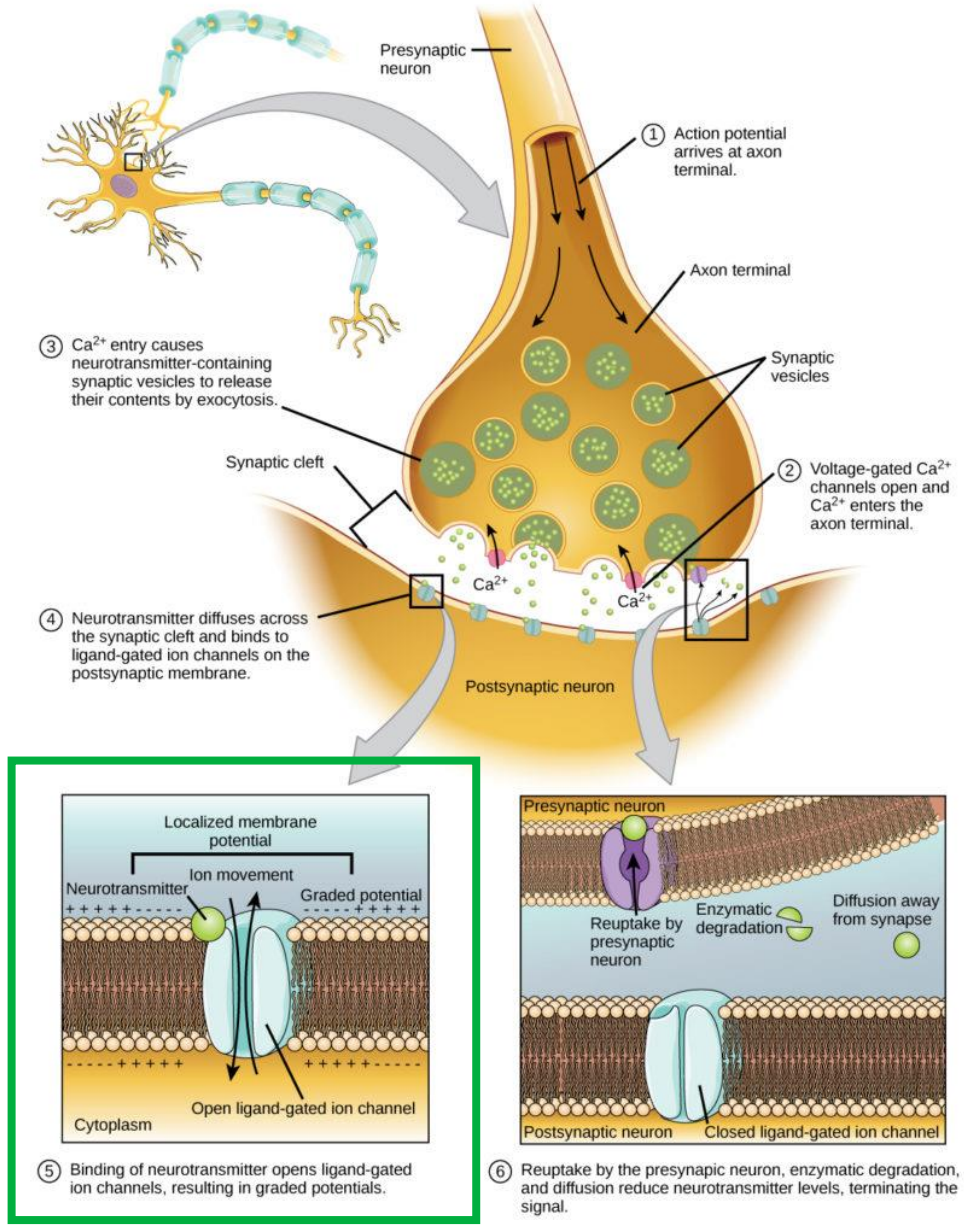
– Catalytic activity

– Most commonly transmembrane proteins with protein kinase activity

Ion-channel coupled receptors

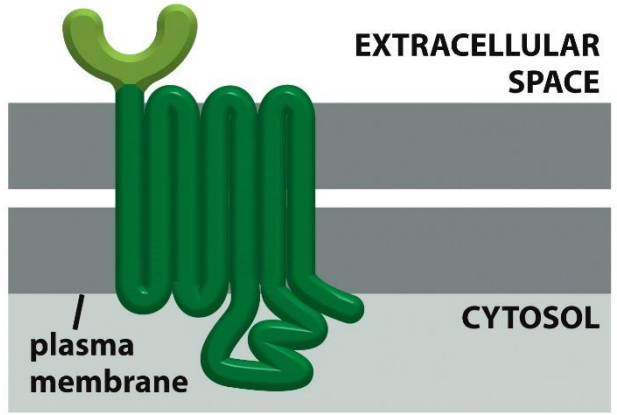
e.g., **Chemical synapse**

- Conversion of chemical signal (neurotransmitter) to electric signal (action potential) at the postsynaptic neuron



G-protein coupled receptors (GPCRs)

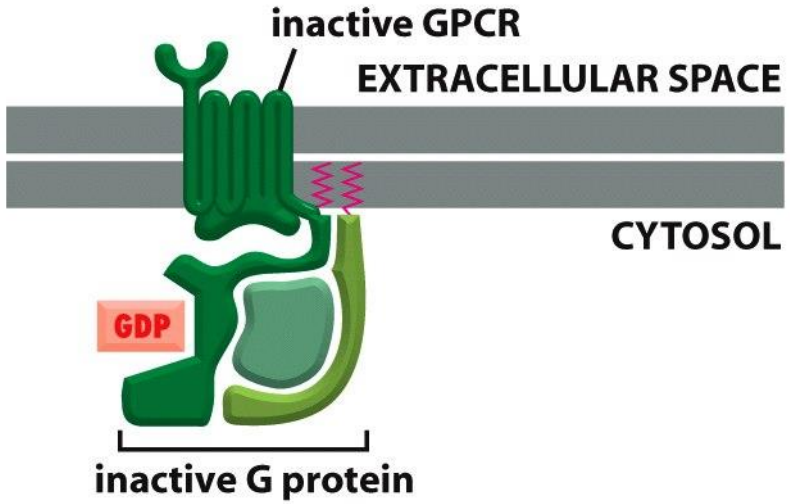
- **Largest family of cell-surface receptors**, hundreds of members
- Various signals: hormones, neurotransmitters, local mediators, light…
- **Heptaspan (7×) transmembrane proteins**
- Evolutionary conservative – sensory receptors across eukaryotic organisms
  - Smell (olfactory receptors) and vision (rhodopsin) in vertebrates
- Intracellular domains **binding G protein**



Heterotrimeric structure of G proteins

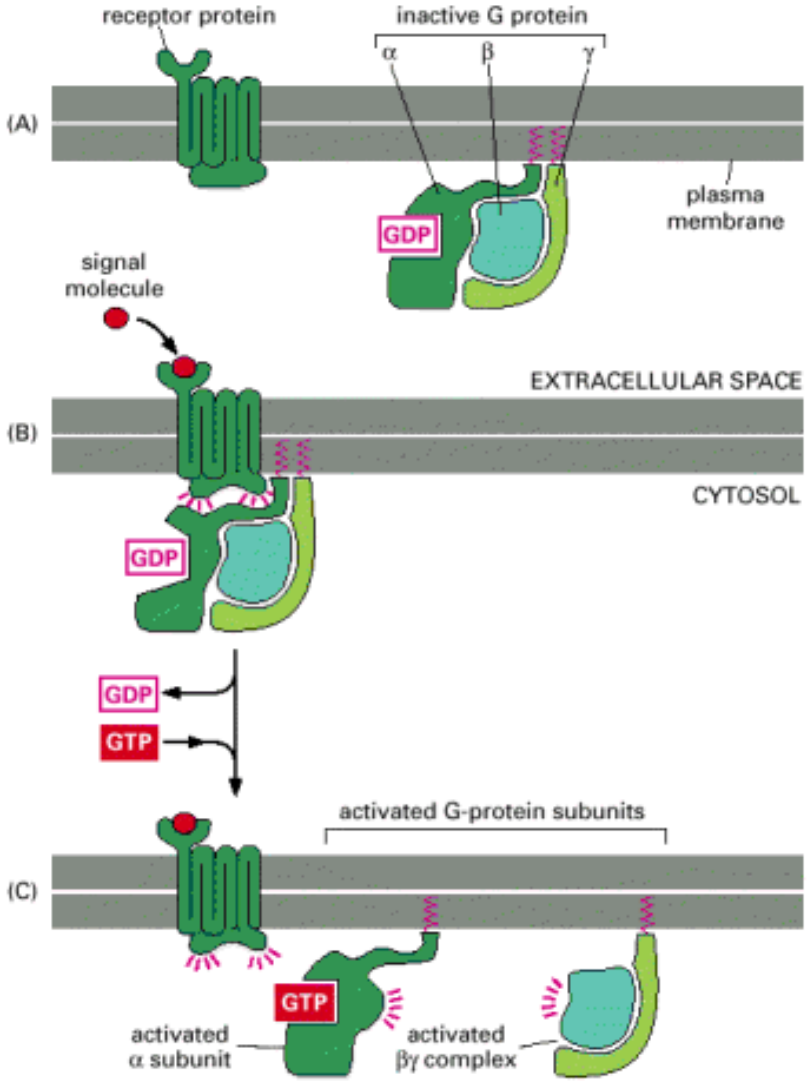
- **GTP-binding protein** (G protein) **– three protein subunits**
- **α (GDP/GTP binding), β, γ**

- Inactive state: all subunits bound together, GDP at α subunit



G protein activation by GPCR

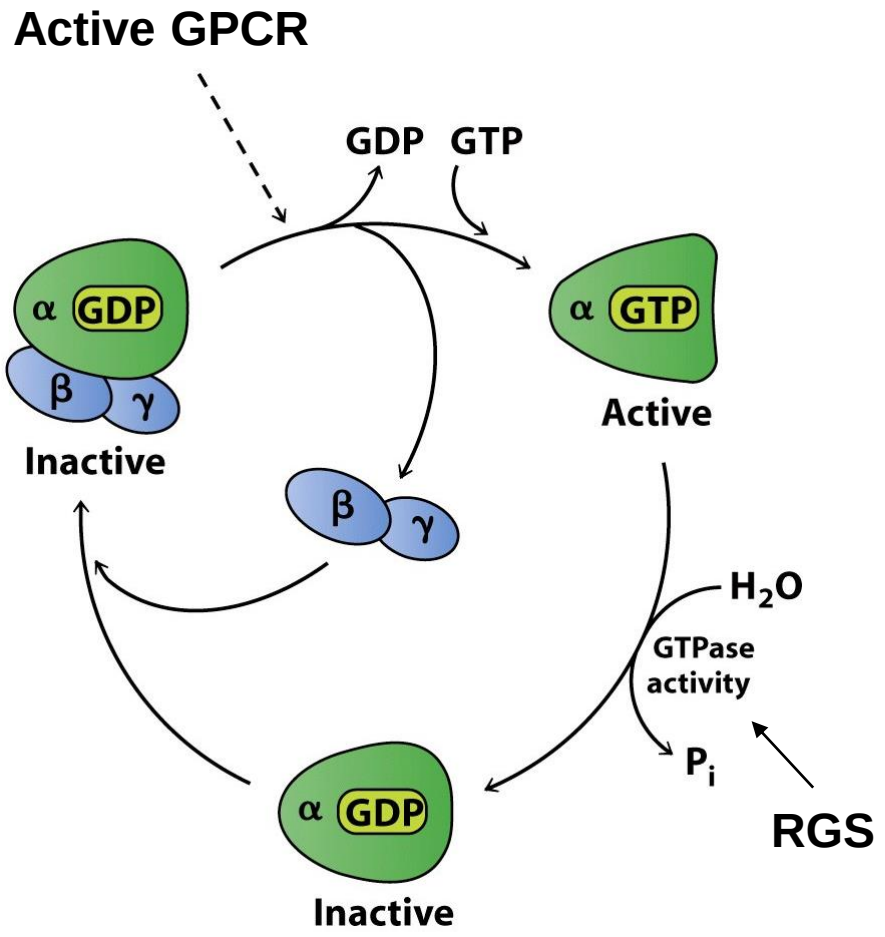
- **Ligand binds to GPCR**
- Conformation change and interaction with G protein
- **GTP replaces GDP and binds to α subunit**
- G protein separates into **α subunit** and **βγ complex**: both freely diffuse in plasma membrane and relay signal to target molecules



Short-term action of G proteins

- **α subunit exhibits GTPase activity: GTP hydrolyzed to GDP**
- α subunit and βγ complex **re-form inactive trimeric G protein**

- α subunit GTPase activity regulates the signaling window
  - Regulators of G protein signaling (RGS) promote GTPase activity = reduce the time available for activation of target molecules



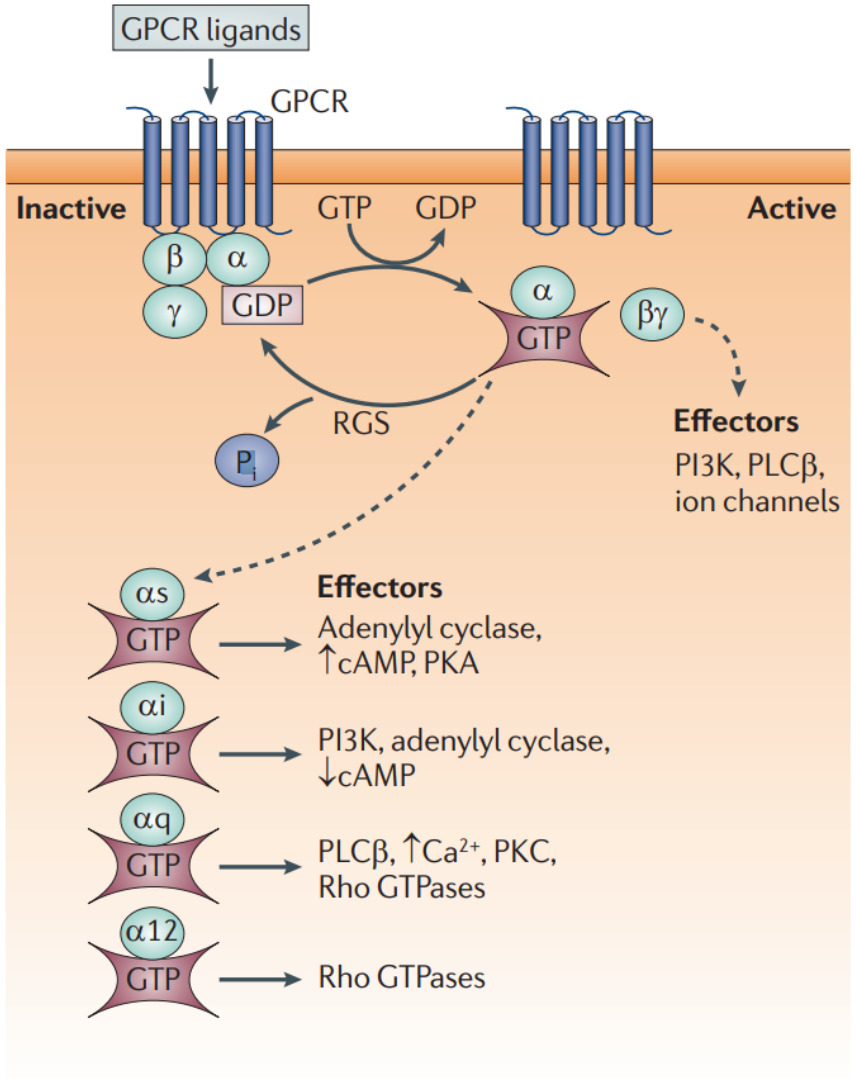
G protein targets

1. **Ion channels**
2. Enzymes responsible for the **production of second messengers:**

– **Adenyl cyclase:** **cyclic AMP (cAMP)**

– **Phospholipase C:** hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into **inositol triphosphate (IP_3)** and **diacylglycerol (DAG)**

3. **Rho GEFs:** activate Rho GTPase

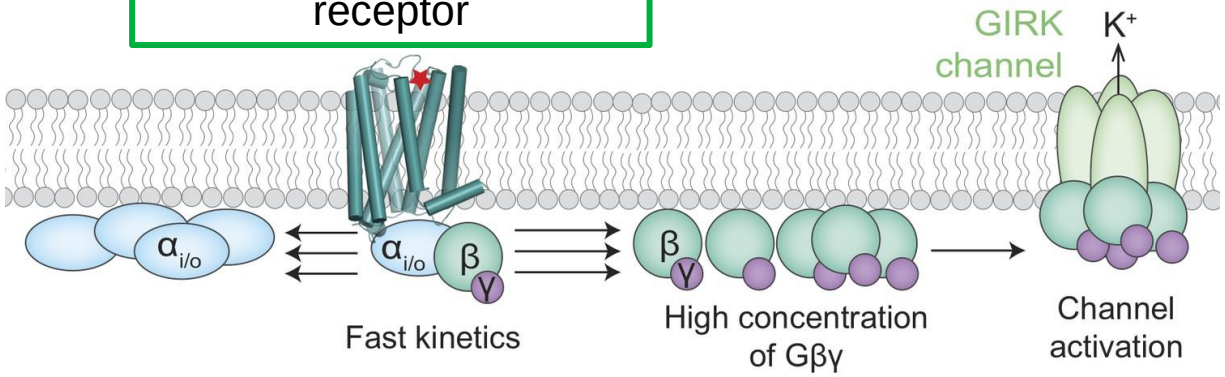


G protein-gated ion channels

Slow depolarization = decreased heartbeat

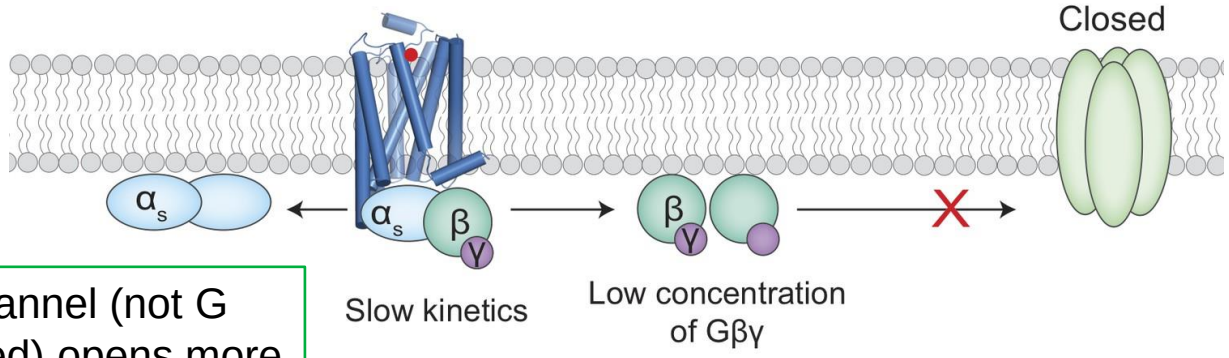
– e.g., **muscarinic acetylcholine receptors** regulating potassium ion channels in heart muscle cells

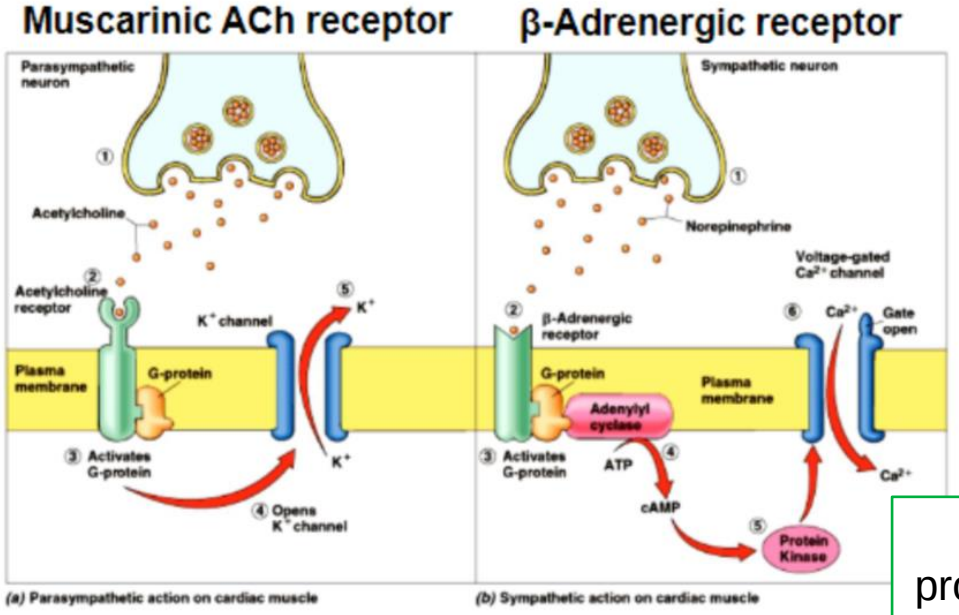
Muscarinic acetylcholine receptor



vs.

β-adrenergic receptor





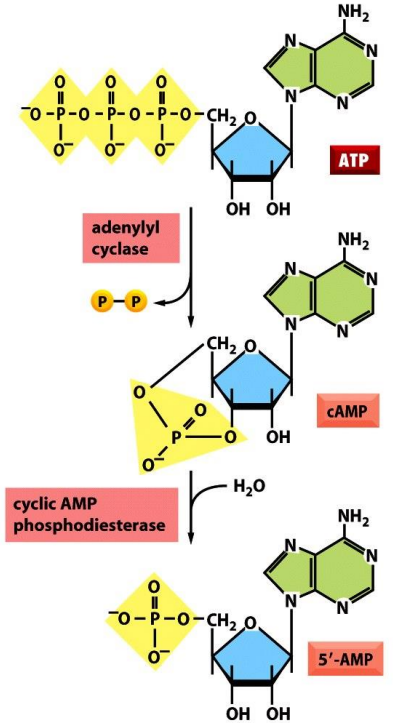
Ca2+ channel (not G protein-gated) opens more readily = heart rate increases

G protein-regulated enzymes

- Complex response: production of second messengers
  - Small molecules that easily diffuse in the cytosol, and initiate and coordinate intracellular signaling pathways

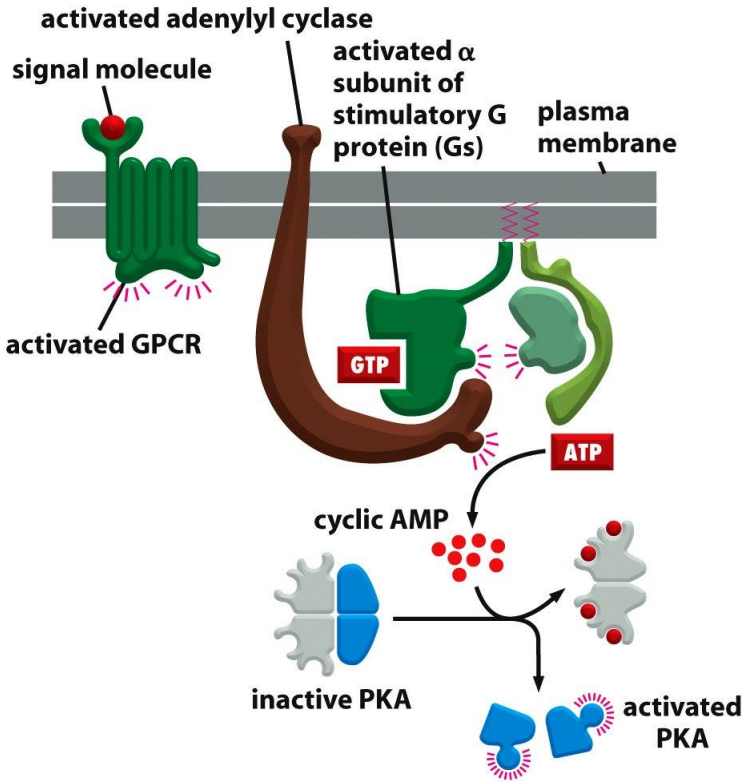
Adenyl cyclase

- Activated by **stimulatory G protein** (Gs/αs), inhibited by **inhibitory G protein** (Gi/αi)
- **cAMP from ATP** (rapid synthesis)
- cAMP removed by phosphodiesterase with constitutive activity (rapid removal)



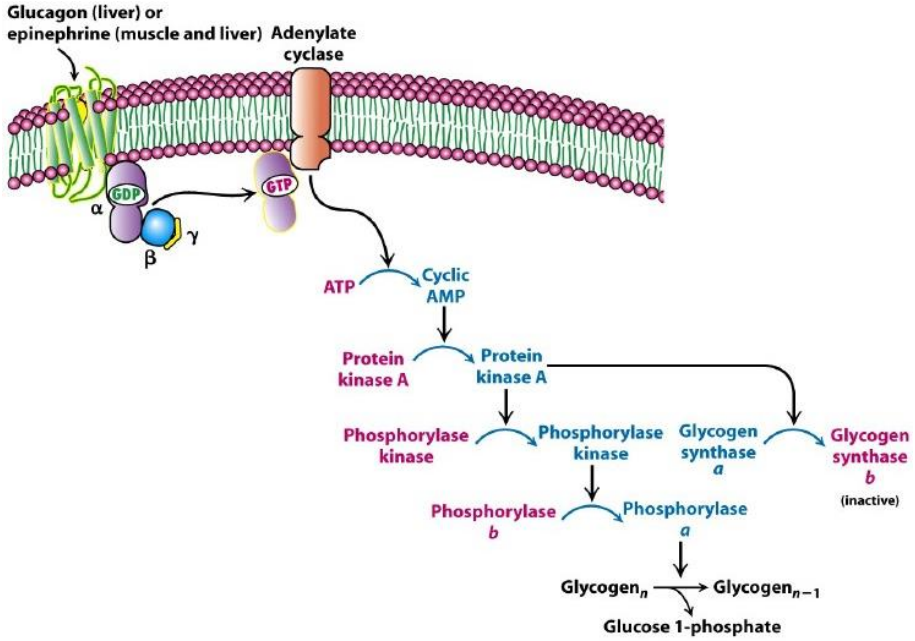
cAMP activates cAMP-dependent protein kinase (PKA)

– GPCR → Gs → adenyl cyclase → cAMP → PKA

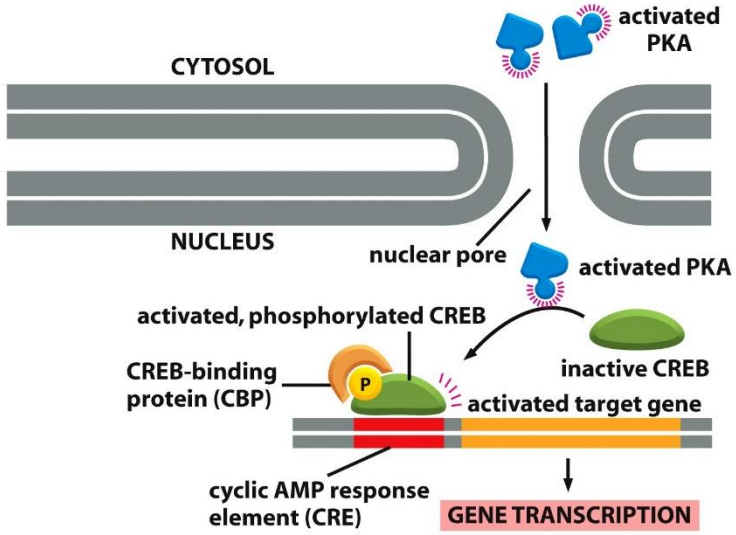


cAMP: fast response vs. slow response

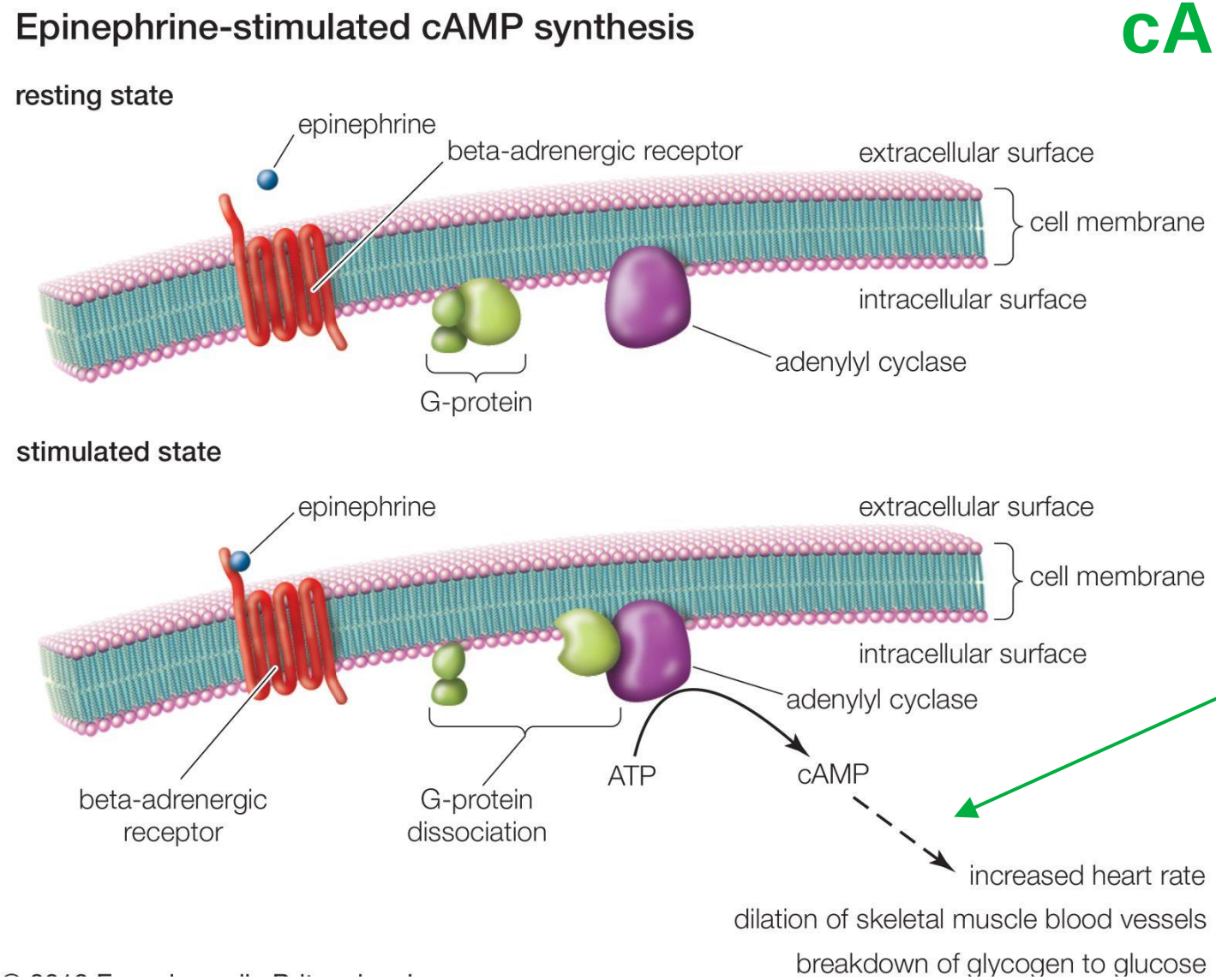
- Adrenalin → PKA: **metabolic enzymes** (e.g., promotes glycogenolysis), **ion channels** (e.g., increases heart rate)

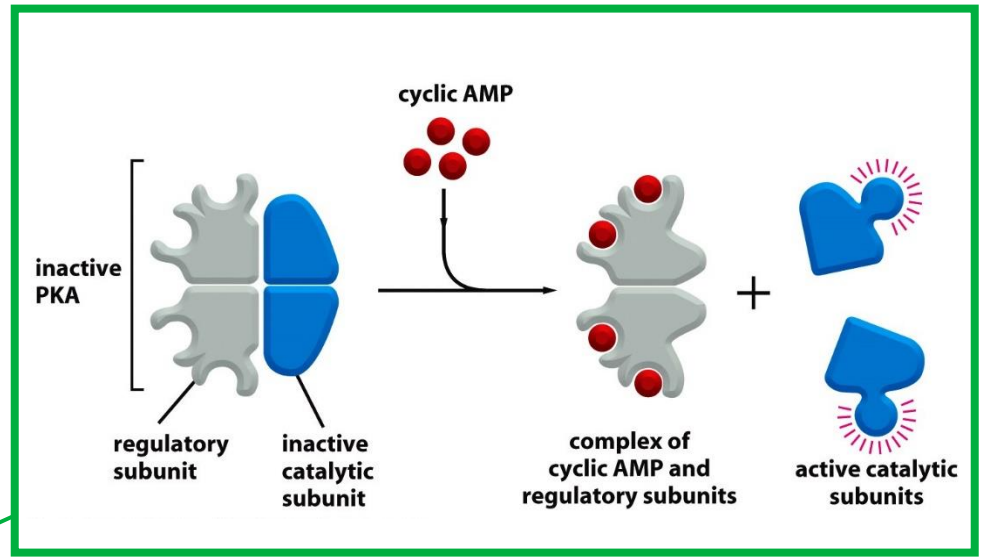


PKA phosphorylates cAMP responsive element-binding (CREB) protein → **altered gene transcription**



cAMP = second messenger

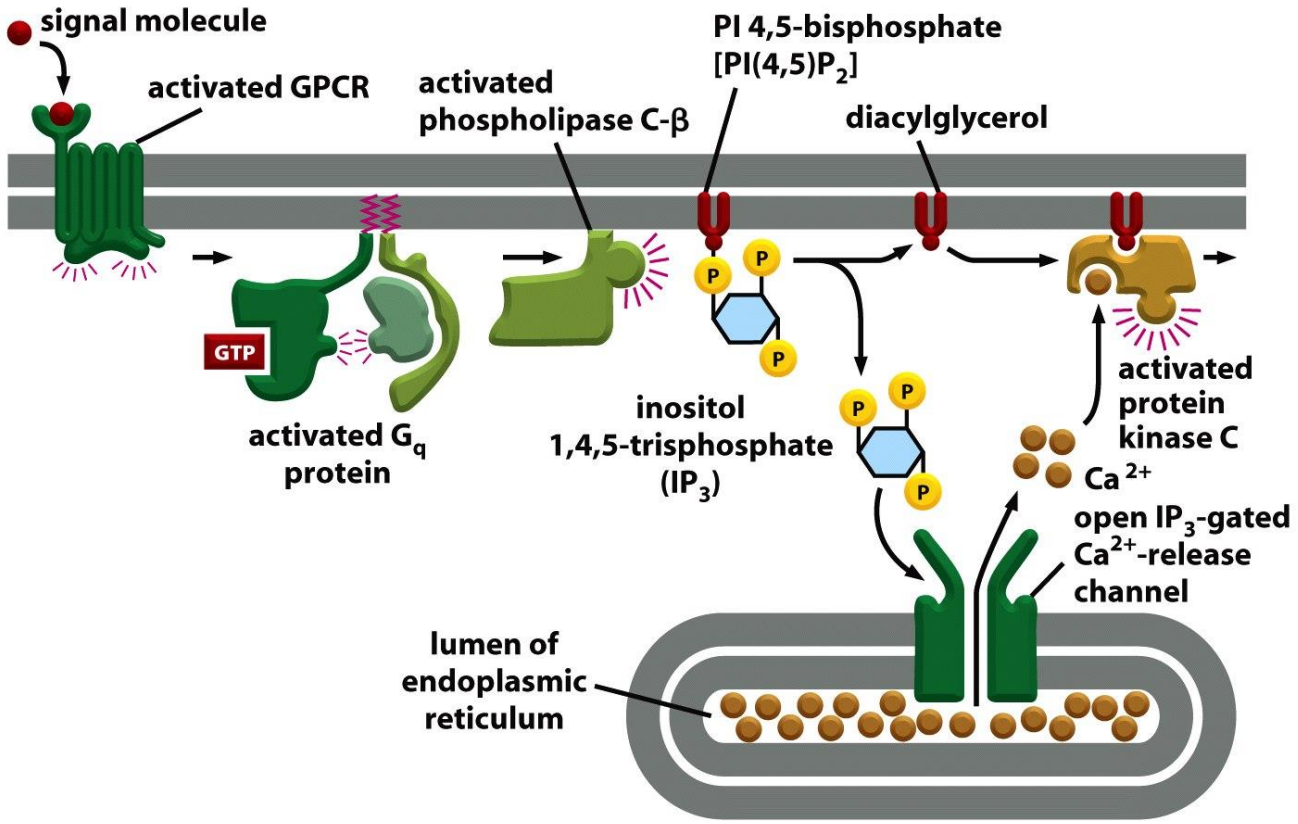




G protein-regulated enzymes

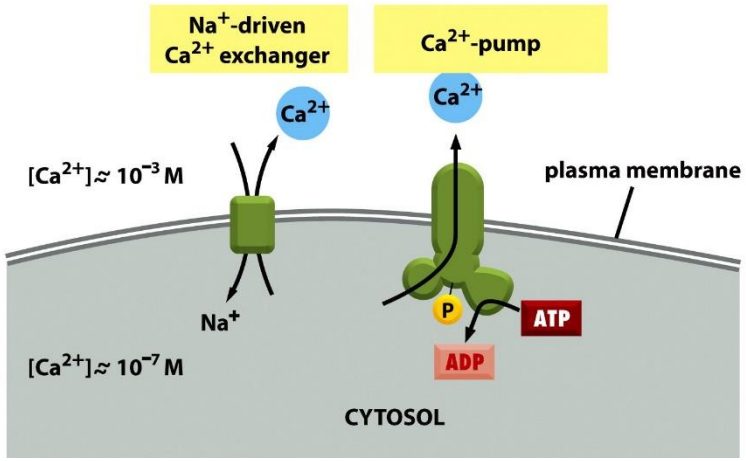
Phospholipase C

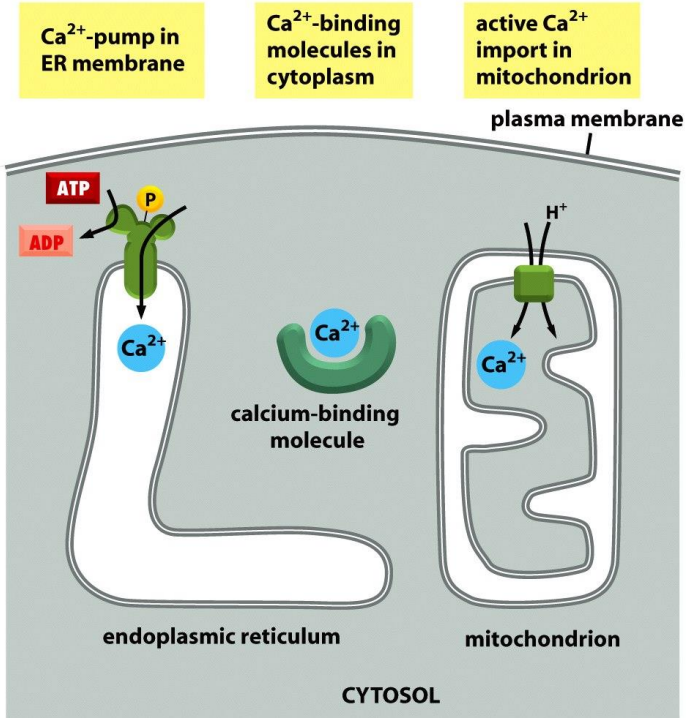
- PIP_2 into **second messengers: DAG and IP_3**
- DAG recruits **PKC**
- IP_3 diffuses in the cytosol to open ER **Ca^{2+} channels**
- **Ca^{2+}** serves as **another second messenger: activation of PKC – downstream signaling**



Ca^{2+} as a signaling molecule

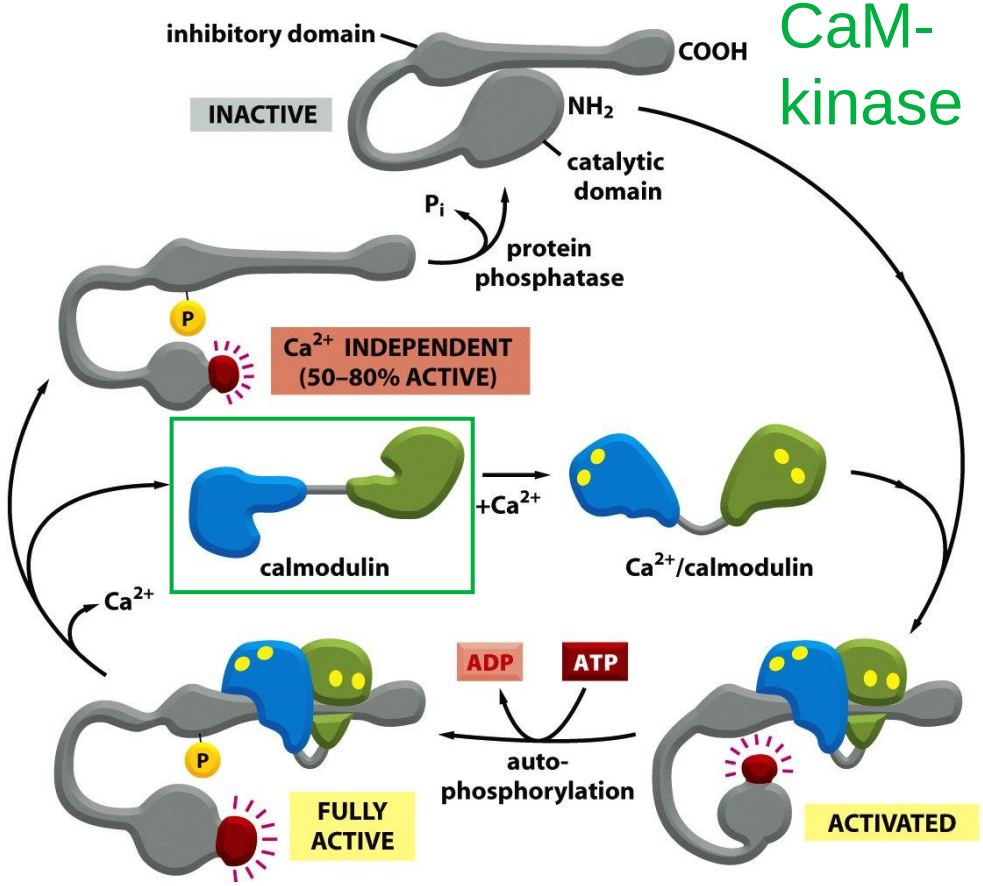
- **Concentration in the cytosol very low** vs. extracellular space and some cellular compartments (mainly ER, mitochondria)
- **Concentration gradient** maintained by specific membrane pumps & transporters
- Opening of the Ca2+ ion channels: **fast influx** (extracellular) or release (ER) into the cytosol





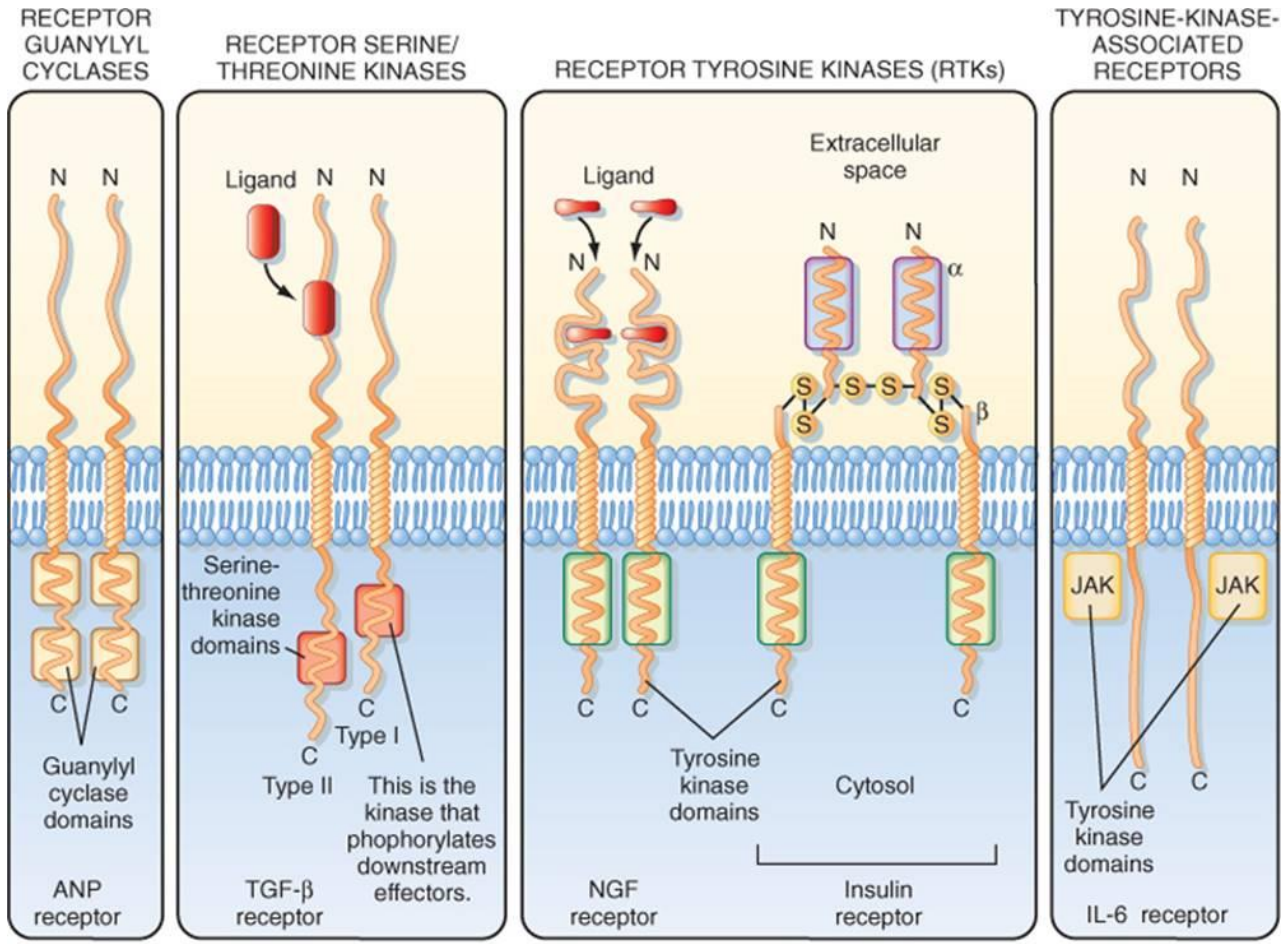
CaM-kinases (Ca^{2+}/calmodulin-dependent kinases)

- **Calmodulin** serves as an **intracellular Ca^{2+} receptor**
- Binding of Ca^{2+} (≥2 ions; 4 sites in the structure) → active conformation → calmodulin binds to other proteins and affects their activity: serine/threonine **CaM-kinases**
- Active CaM-kinases phosphorylate **gene regulatory proteins**



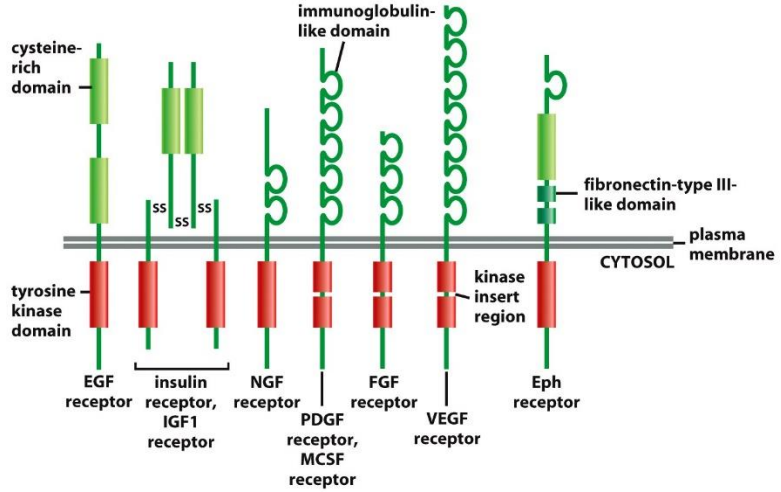
Enzyme-coupled receptors

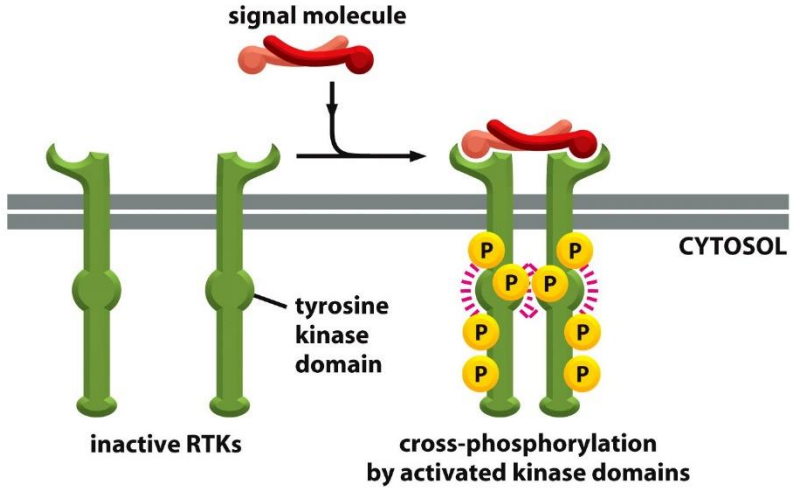
- Transmembrane proteins
- Upon ligand binding:
  Cytosolic domain → activation of catalytic activity or association with enzymes
- RTKs represent the major group of these receptors



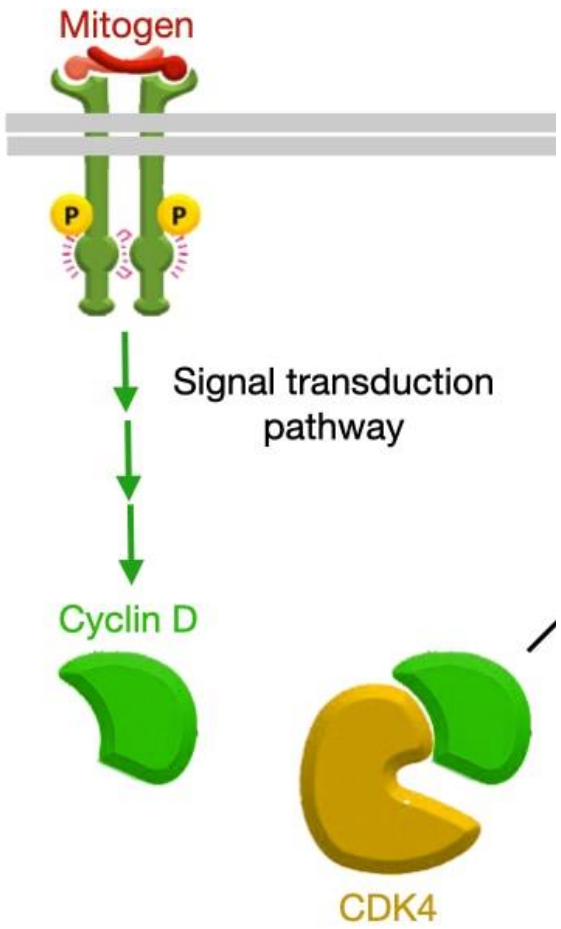
Receptor tyrosine kinases (RTKs)

- Transmembrane glycoproteins
  - Extracellular ligand binding domain
  - Single transmembrane domain
  - Cytosolic **tyrosine kinase domain**
- **Ligands: growth factors = mitogenic signaling (cell cycle)**
- Ligand binding → **dimerization** → **autocatalytic cross-phosphorylation**

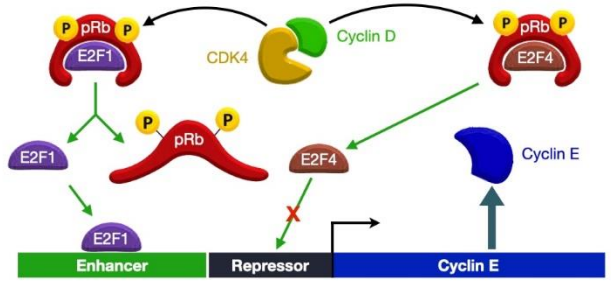


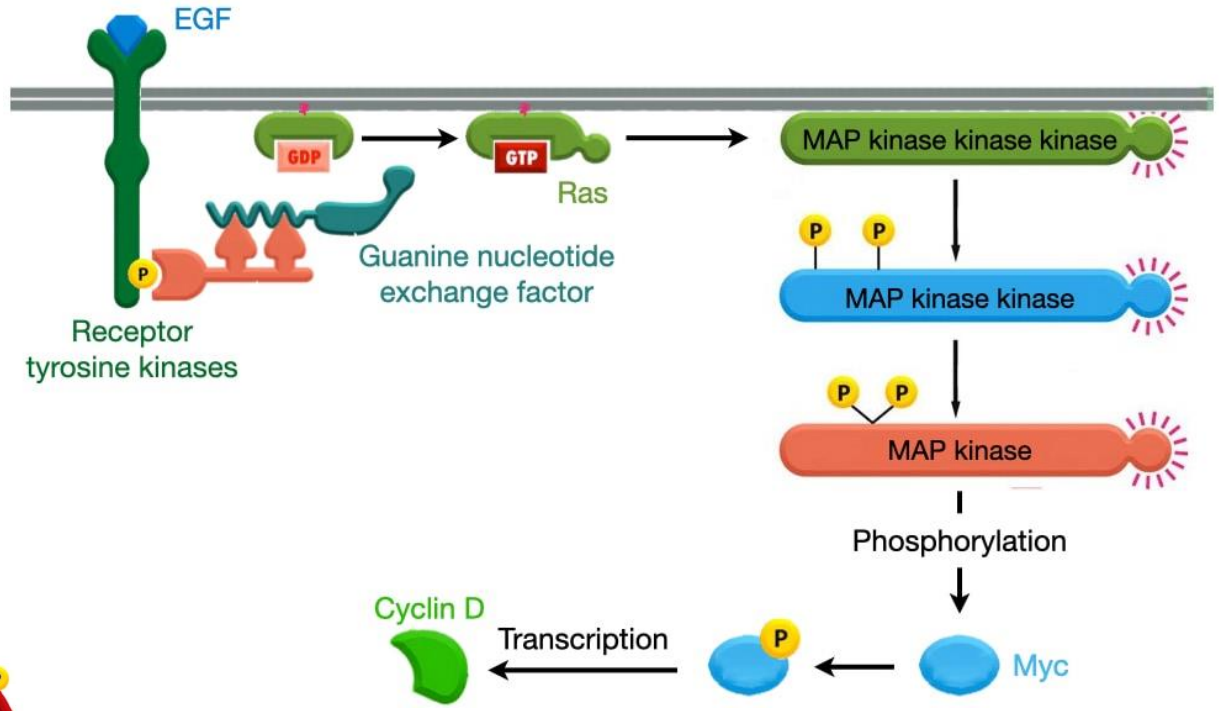


Reminder: mitogenic signaling (overcoming the restriction point)



Helps to initiate cell cycle progression



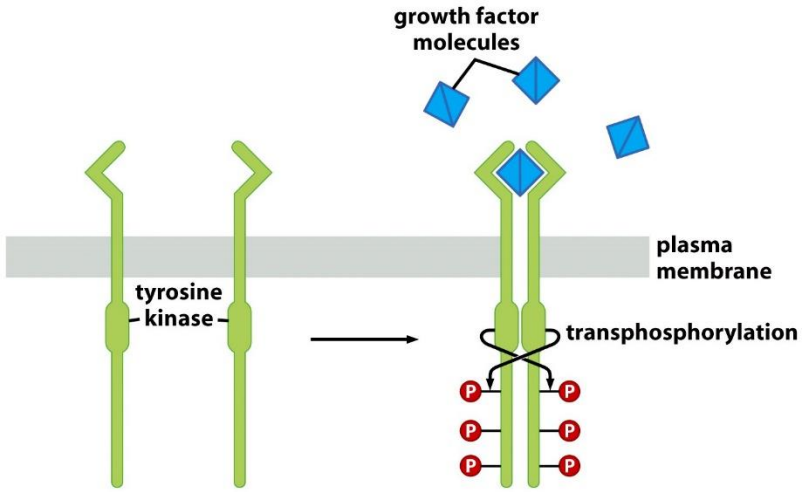


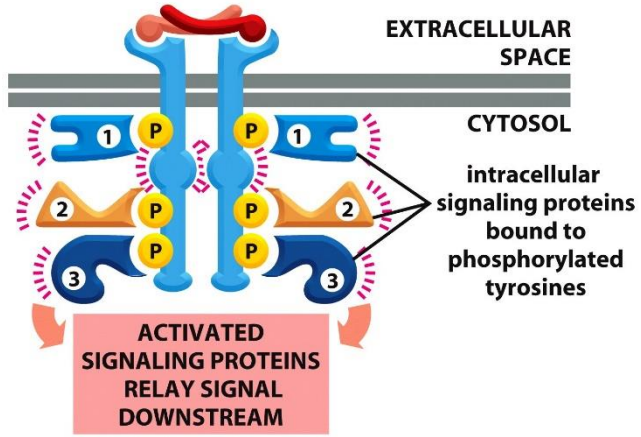
Signal transduction from activated RTKs

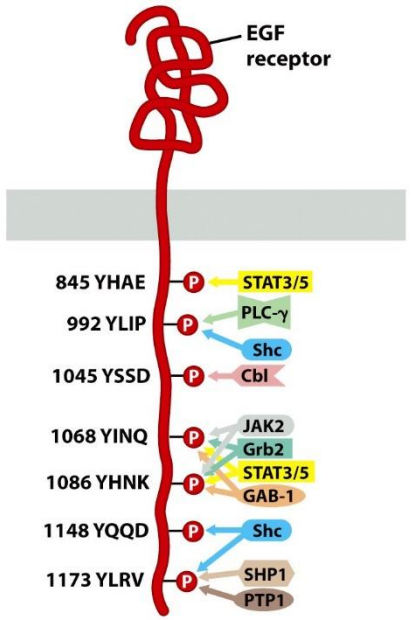
- **Phosphotyrosines serve as docking sites for:**
- **Signaling proteins**: activated by direct binding to the RTK (conformational change or phosphorylation by RTK)
- **Docking/adaptor proteins**: attract other signaling proteins to the membrane where they are then activated

- Binding through **Src homology region 2 (SH2) or phosphotyrosine-binding (PTB) domains**

Signal transduction from activated RTKs



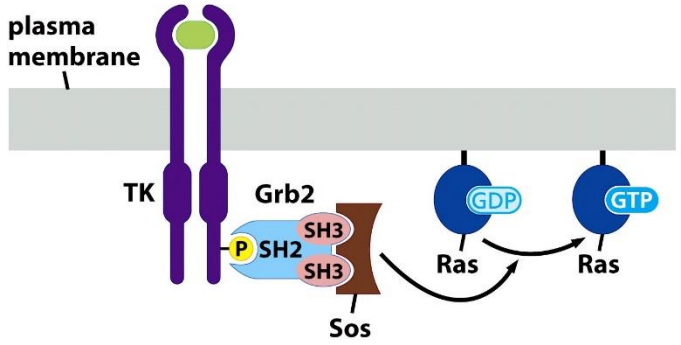




- Each RTK = plethora of docking sites: downstream signaling depends on the cell type and cellular context

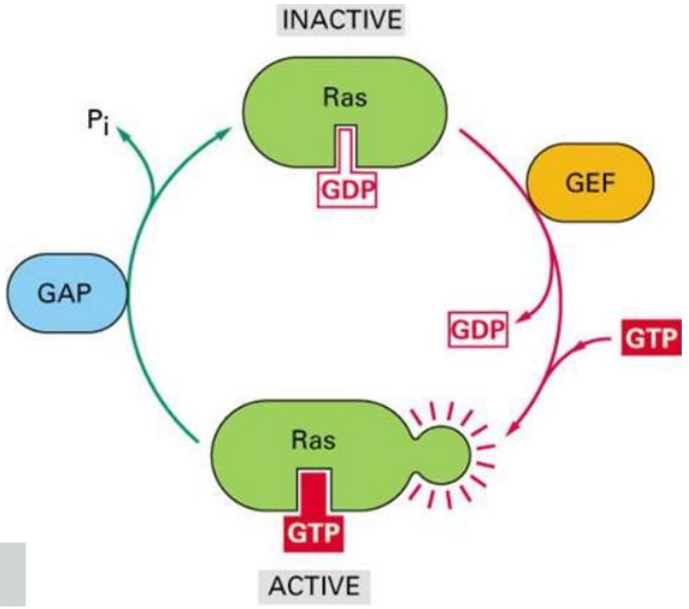
- Signaling proteins directly: e.g., **phospholipase C gamma (cAMP); Src kinase, PI3K**

- Via adaptor proteins: **e.g., SOS (activating Ras)**

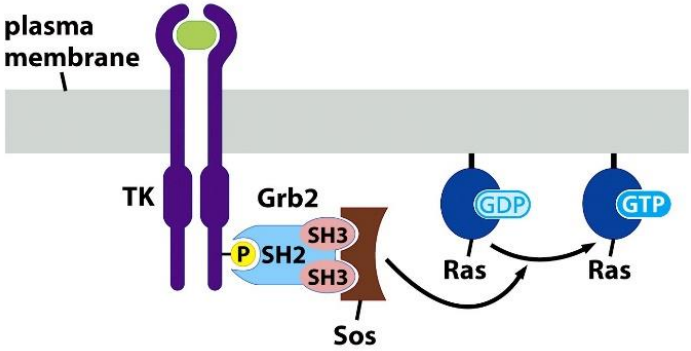


Ras family of proteins

- **Plasma membrane-bound monomeric GTPases** (small G proteins)
- Activity stimulated by Ras guanine nucleotide exchange factors (**GEFs**) and inhibited by Ras GTPase-activating proteins (**GAPs**) promoting GTP hydrolysis

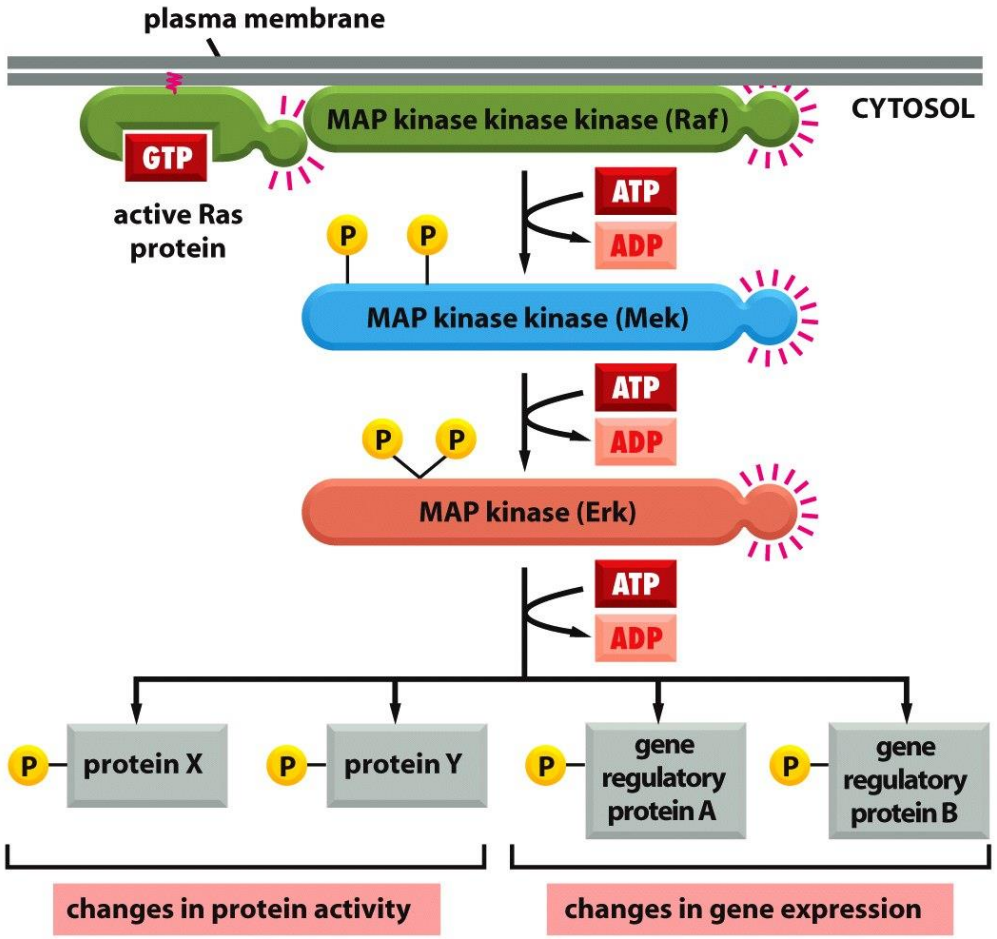


- RTK → adapter proteins → SOS (son of sevenless; = GEF) → active Ras



MAPK cascade activated by Ras

- Canonical **series of phosphorylation of protein kinases**
  - Downstream **mitogen activated protein kinase (MAPK)** phosphorylates target proteins
  - **MAPK kinase (MAPKK)** phosphorylates MAPK
  - **MAPKK kinase (MAPKKK)** activated by Ras, phosphorylates MAPKK
- Signal amplification during signal transduction
- One ligand molecule activates a great number of effector proteins



Ras-Raf-MEK-ERK pathway

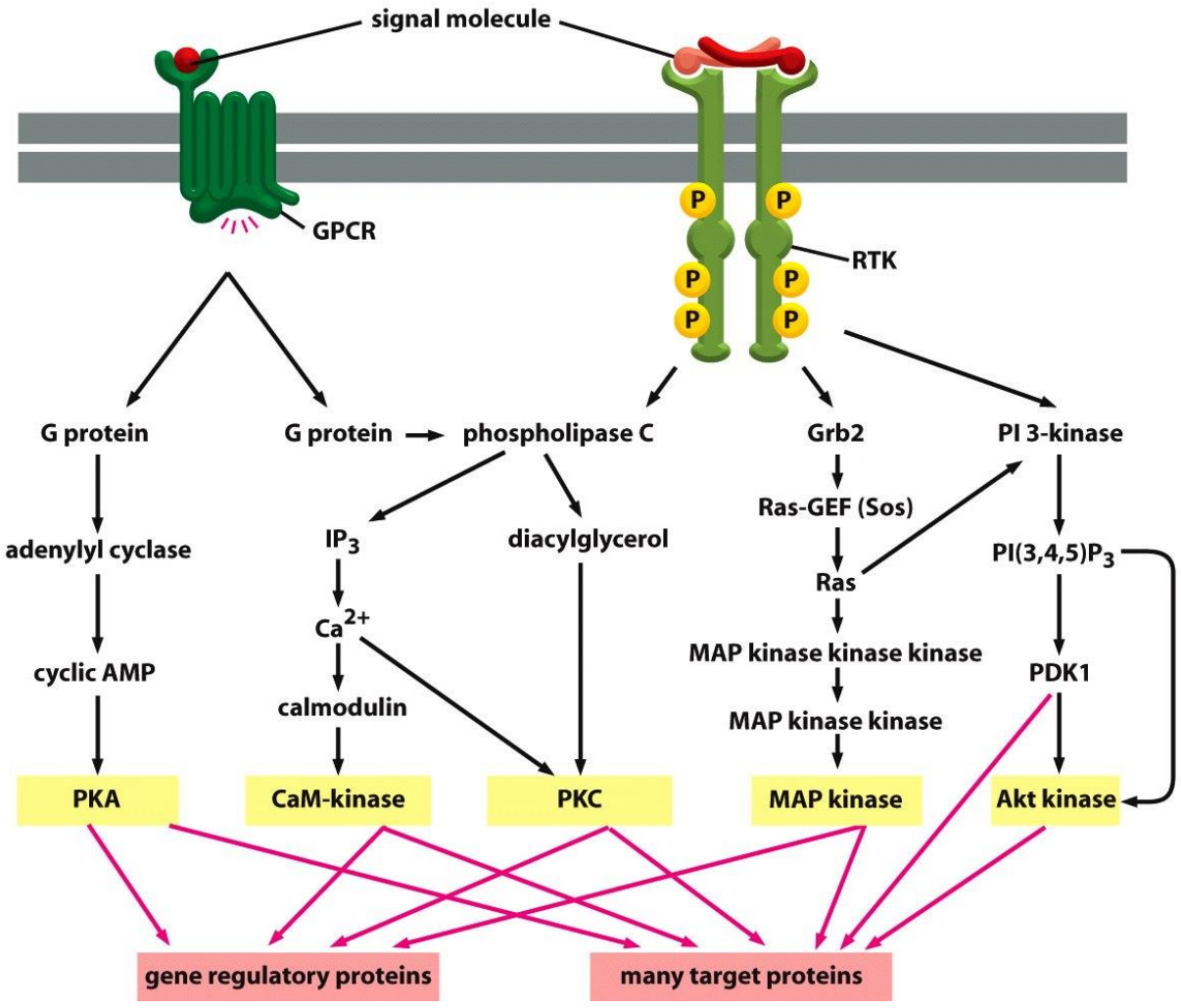
- **Key mitogenic signaling pathway**: cell cycle progression through restriction point – principally by upregulating **cyclin D expression**
- **Promotes cell proliferation, growth and survival**
- Ras-ERK signaling important for differentiation during embryonic development

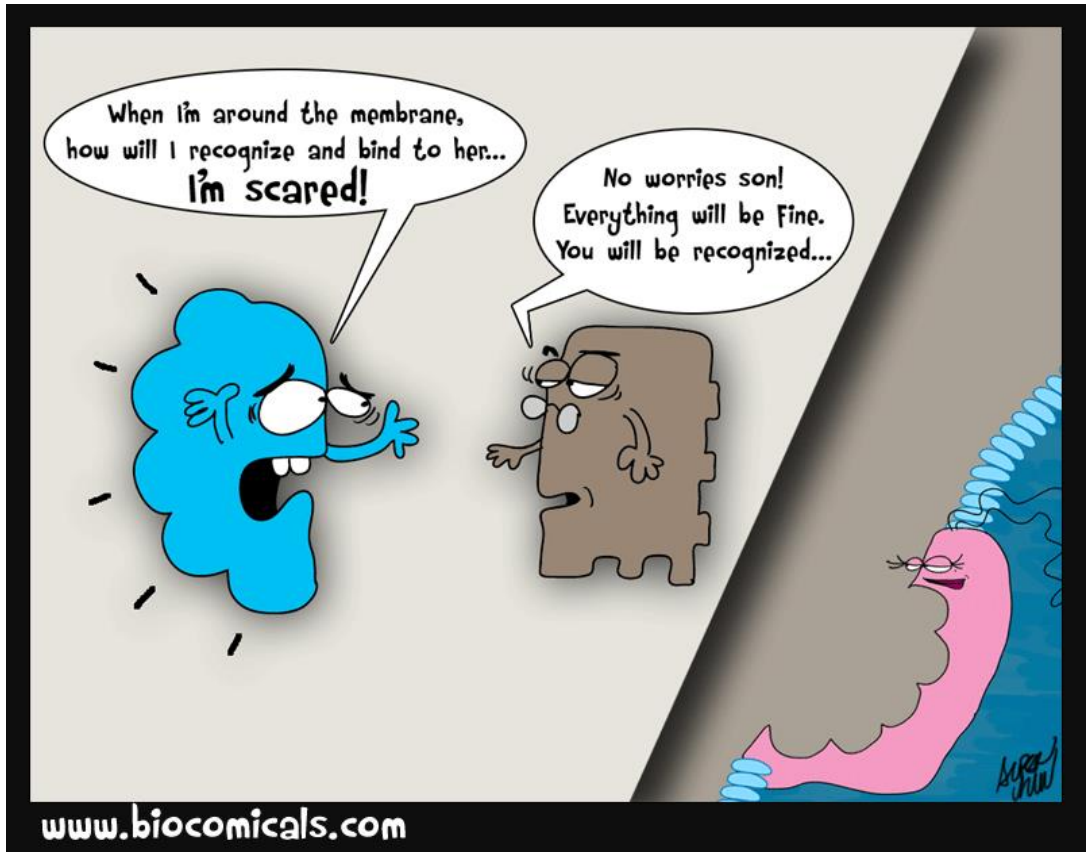
Hyperactive Ras-ERK signaling

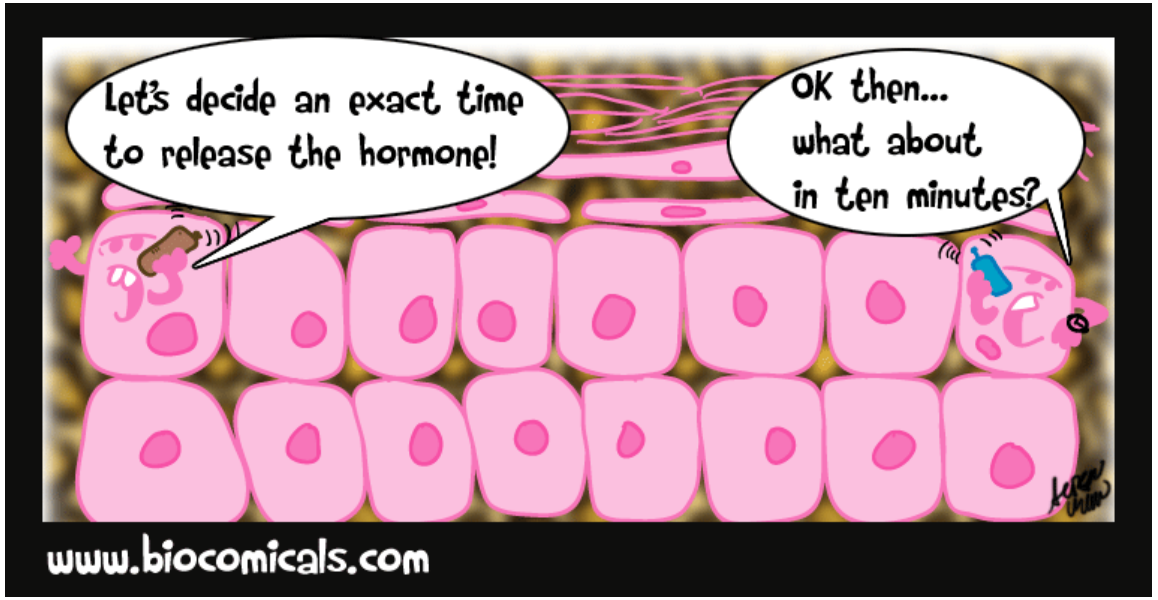
- **RASopathies**
  - hereditary developmental syndromes, germline mutations in genes encoding RAS–ERK pathway proteins, e.g., **Neurofibromatosis type 1 (NF1)**
  - **Associated with increased risk of cancer**
- **Cancer: Ras proto-oncogenes** mutant in more than 30% of cancers

Integration and crosstalk of signaling pathways

- Cytosolic kinases and enzymes are regulated by several pathways
- Crosstalk and integration of signals – **context-dependent processing**
  - Protein activation achieved by additive effects of two (or more) pathways
  - Subunits activated by different pathways




When I'm around the membrane, how will I recognize and bind to her... I'm scared!
No worries son! Everything will be Fine. You will be recognized...
www.biocomicals.com


Let's decide an exact time to release the hormone!
OK then... what about in ten minutes?
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