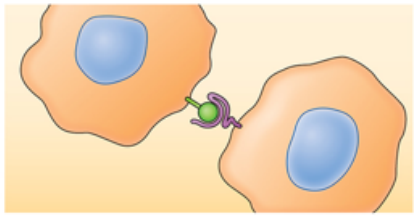
Lecture 8: Cell signaling

Prof. Mamoun Ahram
School of Medicine
Second year, Second semester, 2025-2026

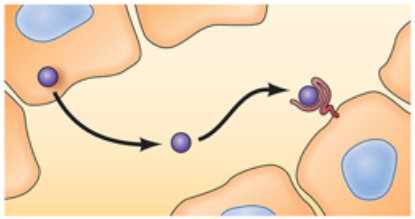
Modes of cell signaling

(A) Direct cell–cell signaling



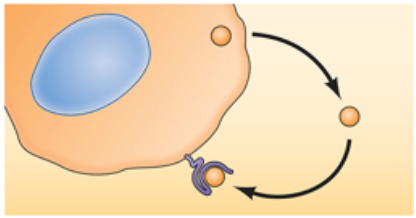
Cell-cell interaction
Direct interaction of a cell with its neighbor

Paracrine signaling



Paracrine signaling
A molecule released by one cell acts on neighboring target cells.

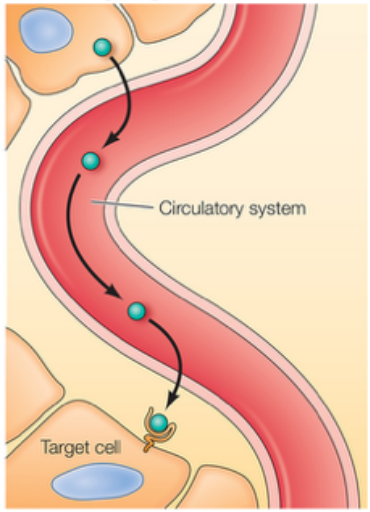
Autocrine signaling



Autocrine signaling
Cells respond to signaling molecules that they themselves produce.

(B) Signaling by secreted molecules

Endocrine signaling

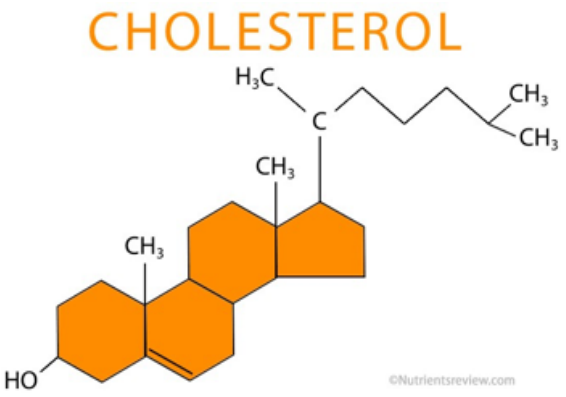


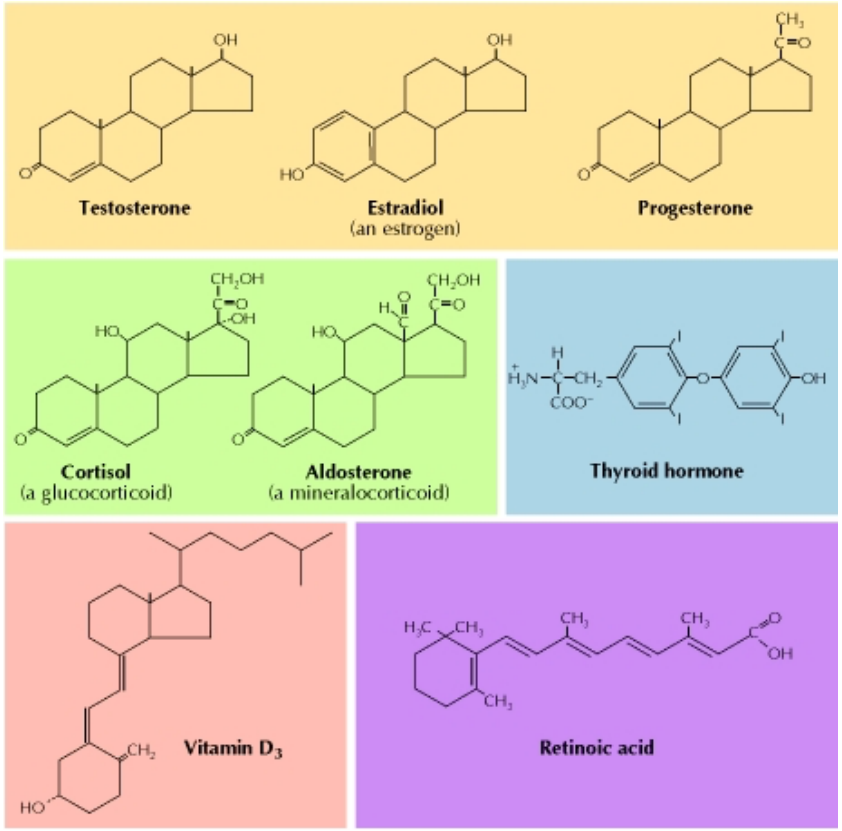
Endocrine signaling
Signaling molecules are secreted by endocrine cells and carried through the circulation to act on target cells at distant body sites.

Classification of signaling molecules

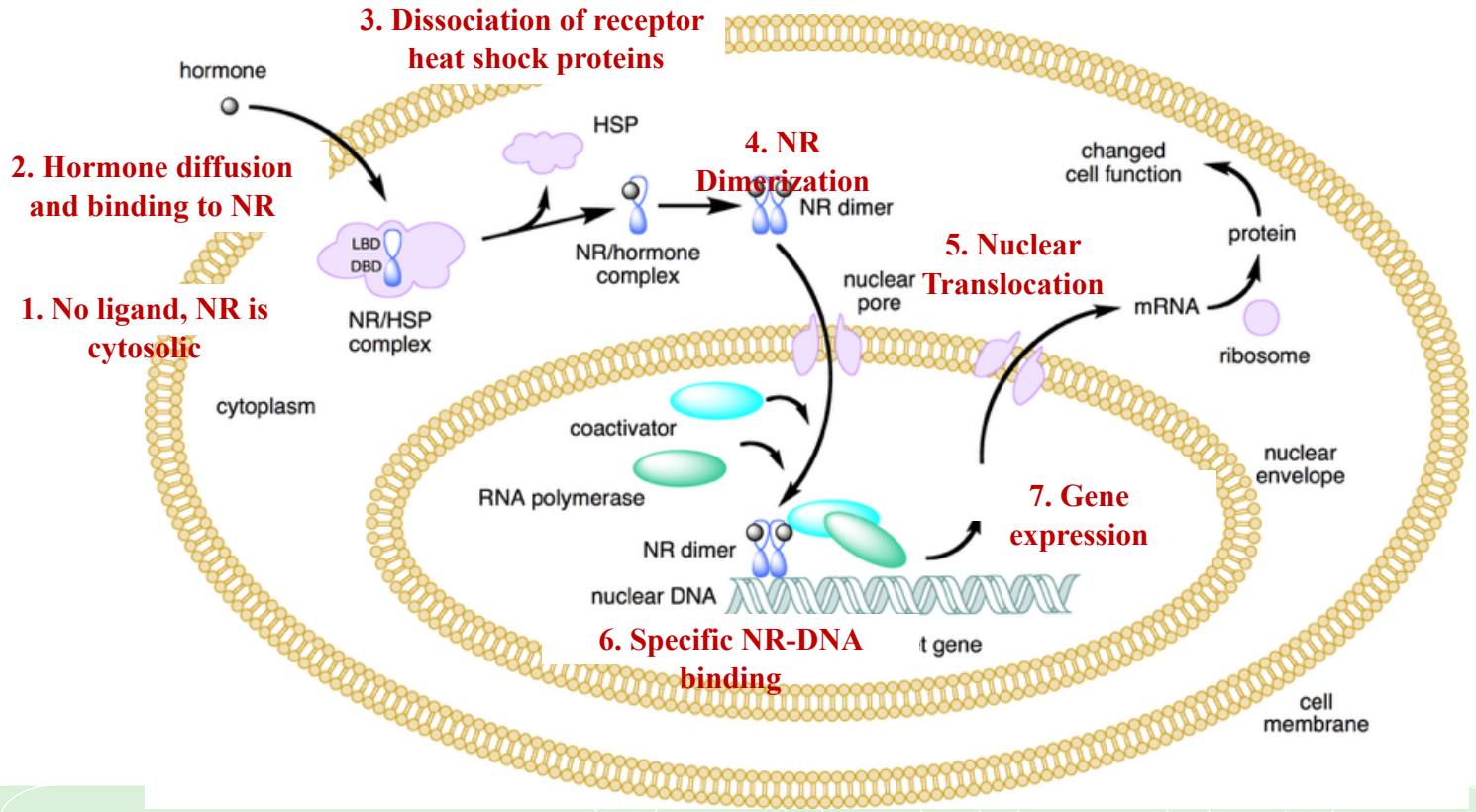
- Peptides: growth factors (EGF), peptide hormones (insulin, glucagon), or neuropeptides (oxytocin, enkephalins)
- Small molecule neurotransmitters: derived from amino acids like Epinephrine and thyroid hormone (tyrosine), serotonin (tryptophan).
- Steroids: derived from cholesterol like estradiol, cortisol, calciferol (Vitamin D), and testosterone.
- Eicosanoids: derivatives of arachidonic acid including prostaglandins, leukotrienes, and thromboxanes B.
- Gasses: Nitric oxide (NO) and carbon monoxide (CO)

Lipophilic hormones





Mechanism of action of steroid nuclear receptors (NR)

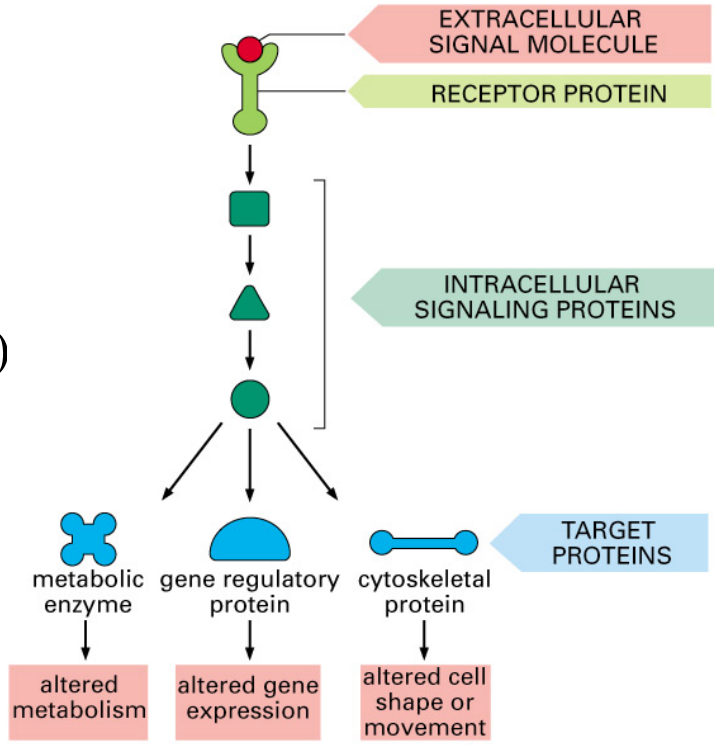


Cell surface receptors

Signal transduction

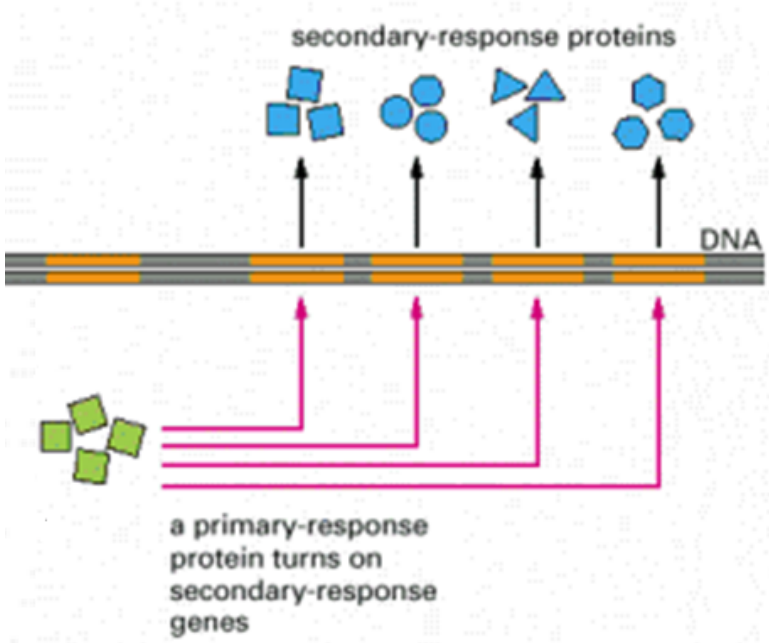
A chain of reactions that transmits chemical signals from the cell surface to their intracellular targets.

- Ligand (hormone, growth factor)
- Receptor (GPCR, RTK)
- Transducers (G protein, Ras)
- Effector molecules (adenylate cyclase, MAPK)
- Secondary messengers (cAMP, cGMP, Ca2+)
- Final target molecules (e.g., DNA, protein, enzyme, channel)
- Response (gene expression, cell behavior)



Types of response

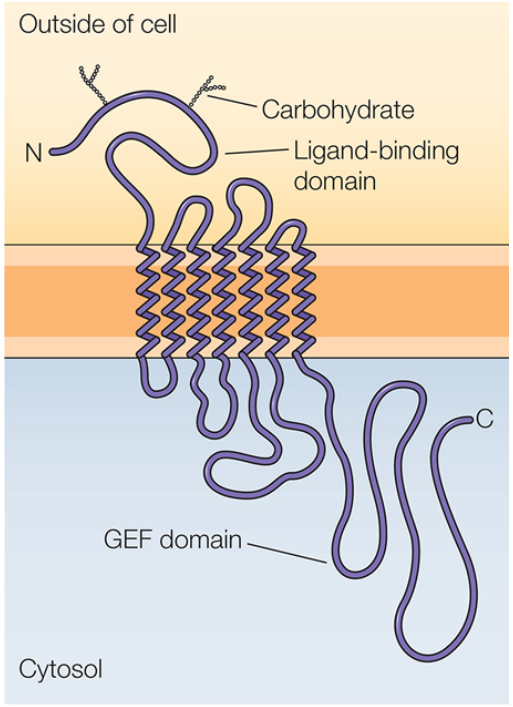
- Primary response entails direct activation of a small number of specific genes (hours), some of which are transcription factors.
- Secondary response entails the transcription factors generated from the primary response activate other genes.
- Tertiary response…



G protein-coupled receptors, G Proteins, and Cyclic AMP

G protein-coupled receptors

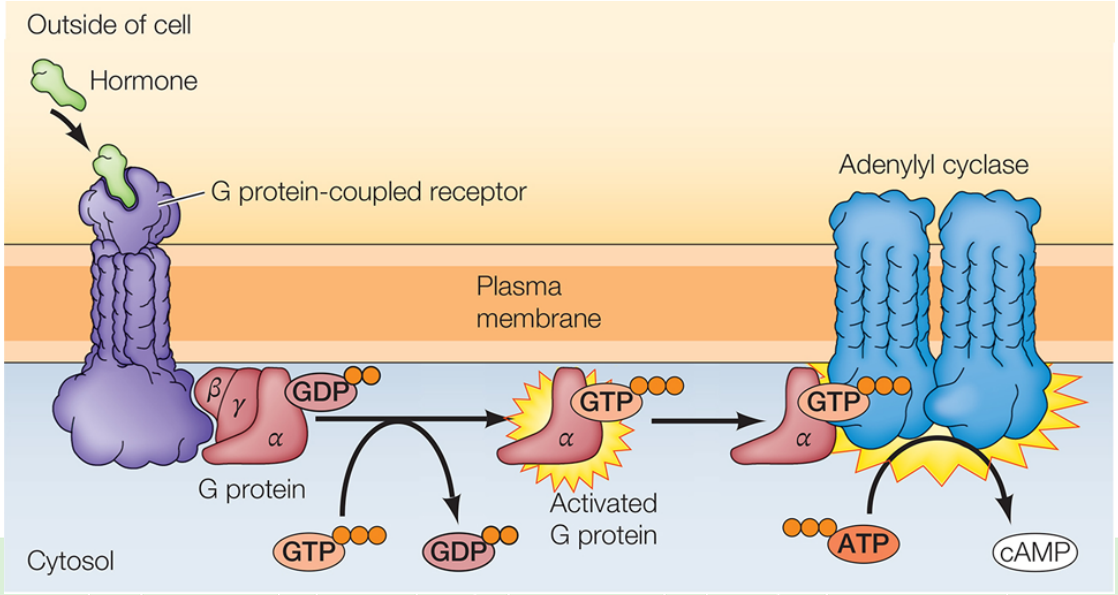
- A family of receptors composed of seven membrane-spanning α helices.
- The binding of ligands to the extracellular domain of these receptors induces a conformational change that is transmitted to the cytosolic domain of the receptor to bind to a G protein.

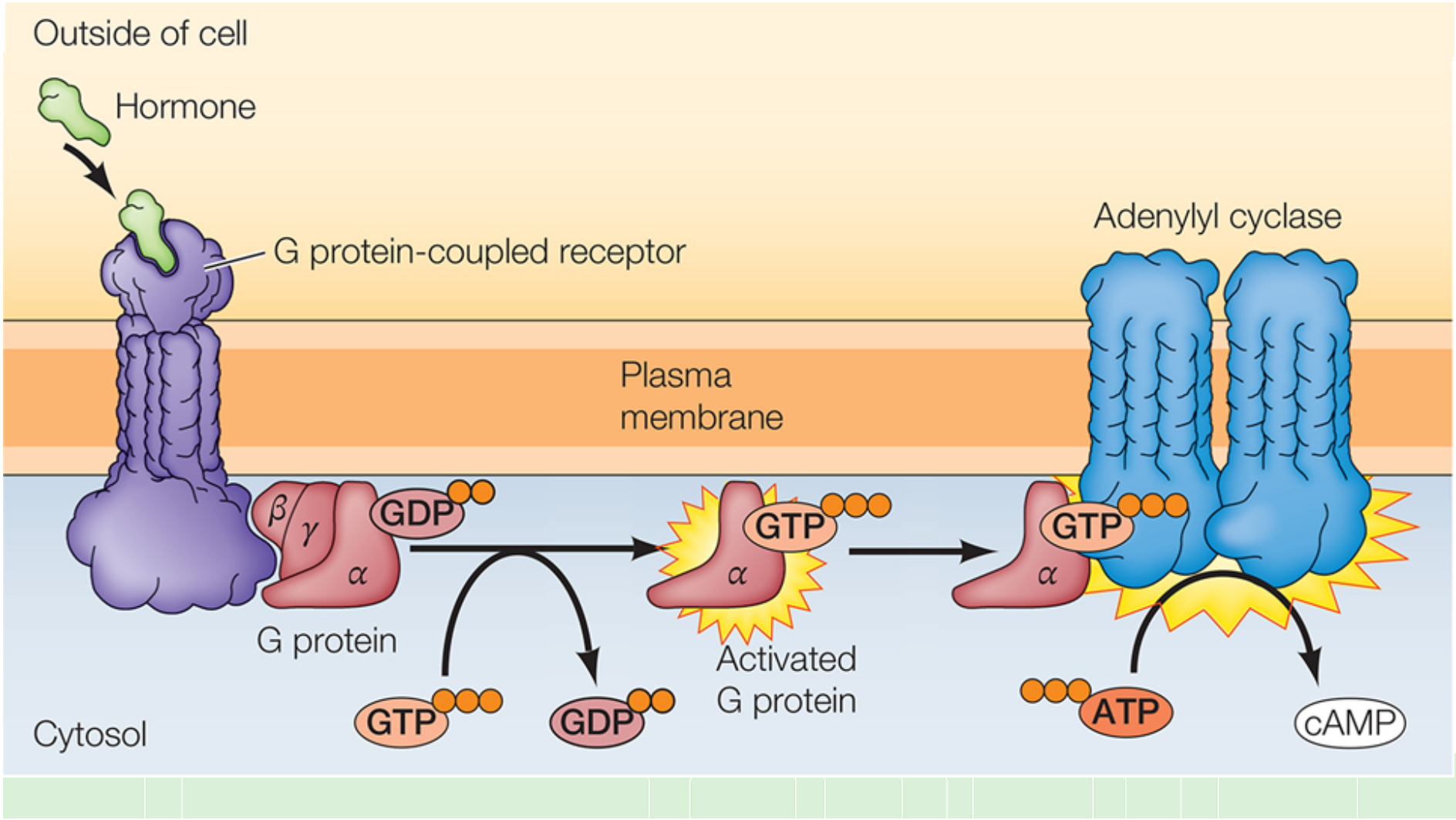


Heterotrimeric G proteins

- G proteins are composed of three protein subunits—α, β, and γ.
- In the unstimulated state, the α subunit has GDP bound and the G protein is inactive.
- When stimulated, the α subunit releases its bound GDP, allowing GTP to bind in its place.
- This causes the trimer to dissociate into an α subunit and a βγ complex.

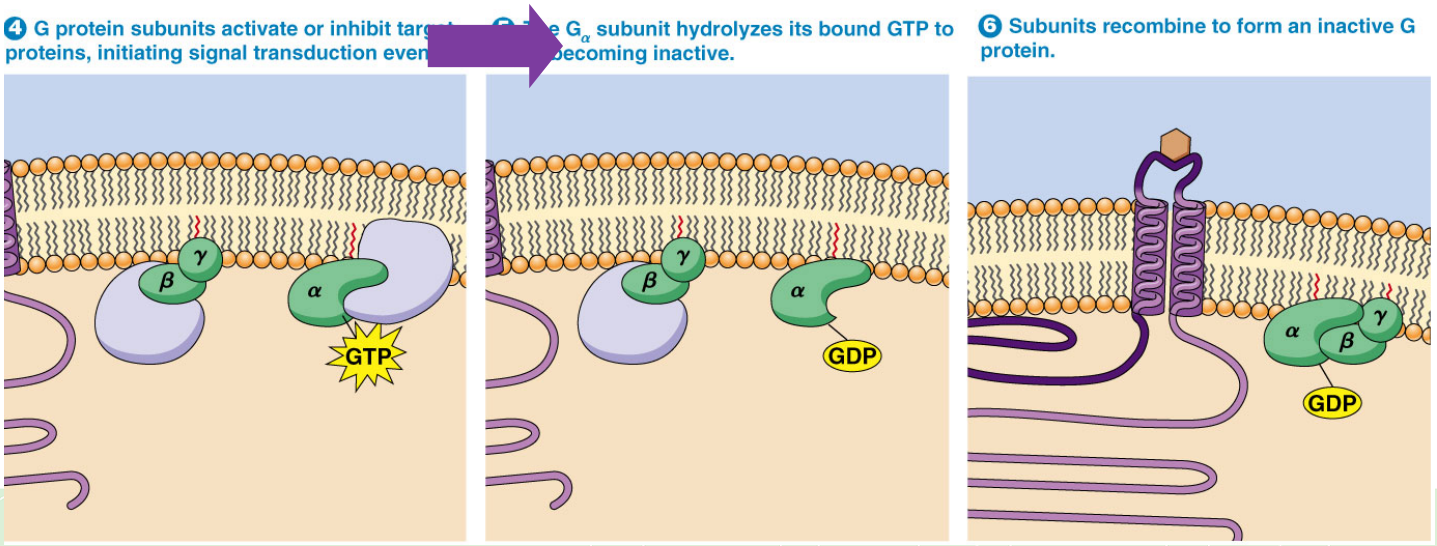
- Both the active GTP-bound α subunit and the βγ complex then interact with their targets to elicit an intracellular response.
- For example, the α subunit, which is now activated, binds to adenylyl cyclase activating it.
- The enzyme catalyzes the conversion of ATP to cAMP.



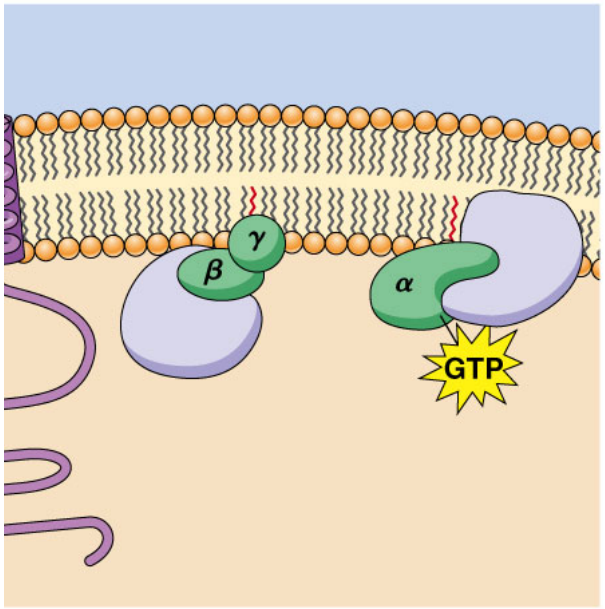

Outside of cell
Hormone
G protein-coupled receptor
Adenylyl cyclase
Plasma membrane
β
γ
α
GDP
G protein
GTP
α
Activated G protein
GTP
α
ATP
cAMP
GTP
GDP
Cytosol

G protein inactivation

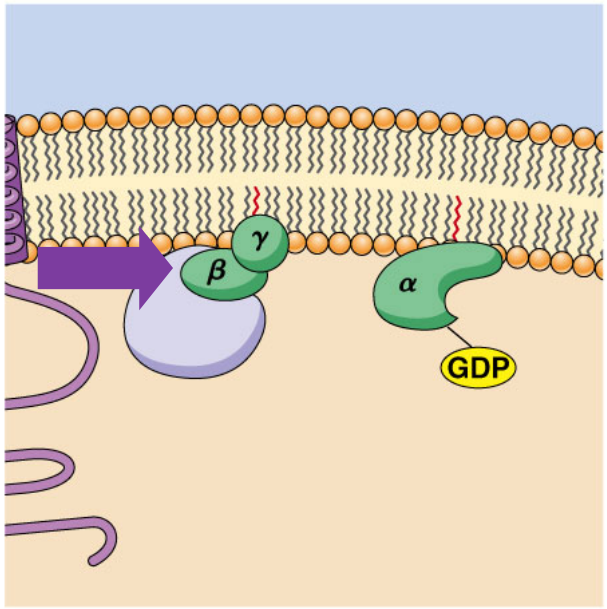
- The activity of the α subunit is terminated by hydrolysis of the bound GTP by an intrinsic GTPase activity, and the inactive α subunit (now with GDP bound) then re-associates with the βγ complex.
- The intrinsic GTPase activity is stimulated by RGS (regulator of G protein signaling) proteins, which act as GTPase-activating proteins (GAPs) for the α subunit.



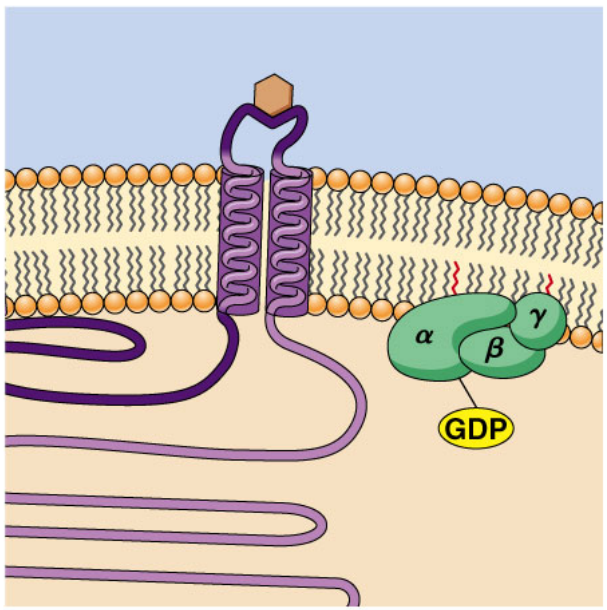
4 G protein subunits activate or inhibit target proteins, initiating signal transduction events.



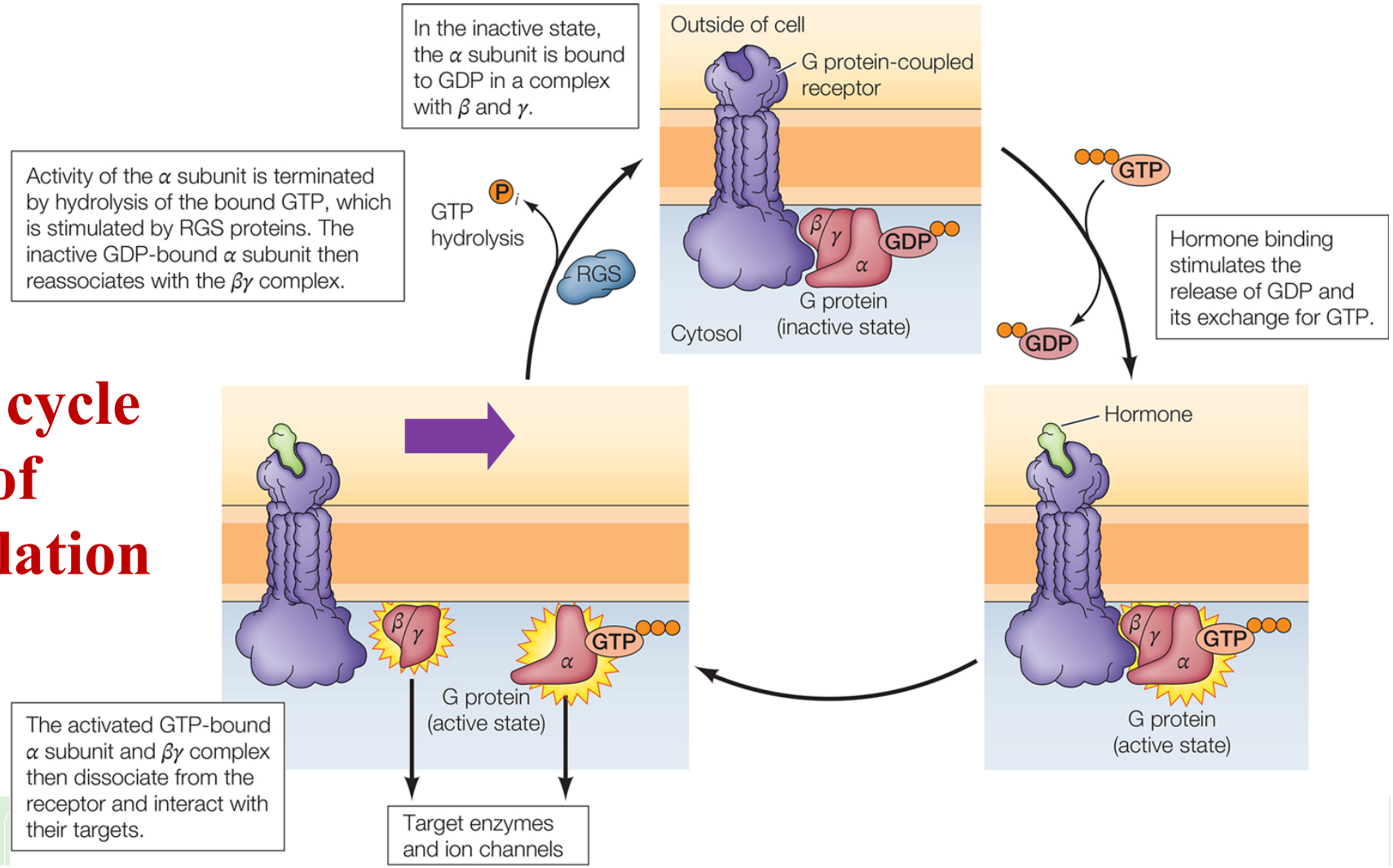
5 The G_α subunit hydrolyzes its bound GTP to GDP, becoming inactive.



6 Subunits recombine to form an inactive G protein.



The cycle of regulation

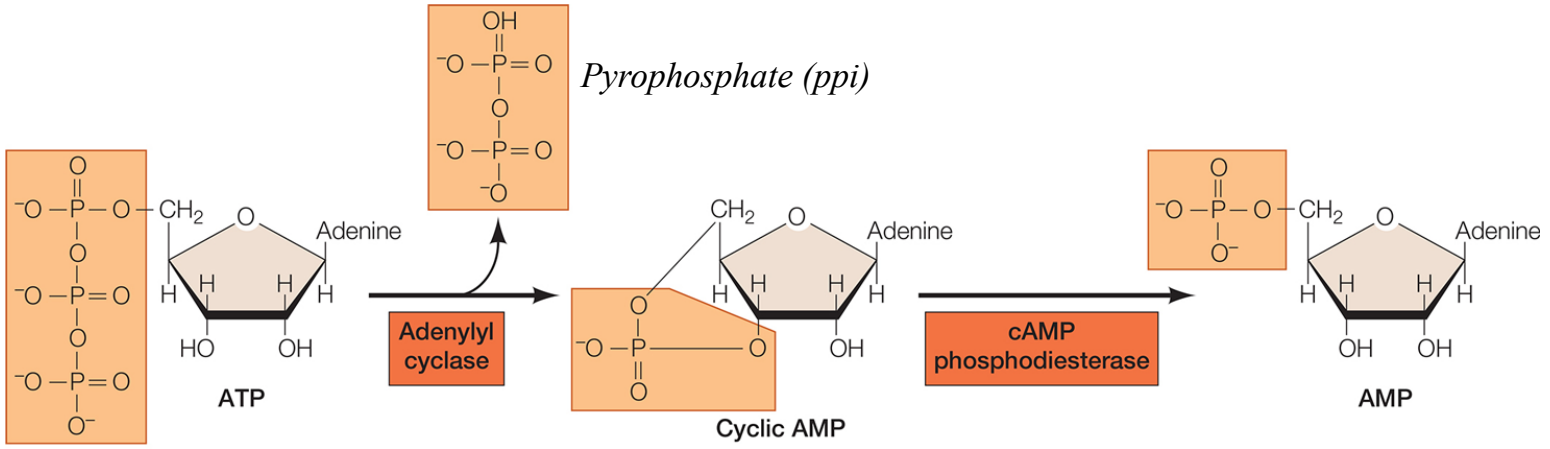


Secondary messengers

A compound whose metabolism is modified as a result of a ligand-receptor interaction; it functions as a signal transducer by regulating other intracellular processes.

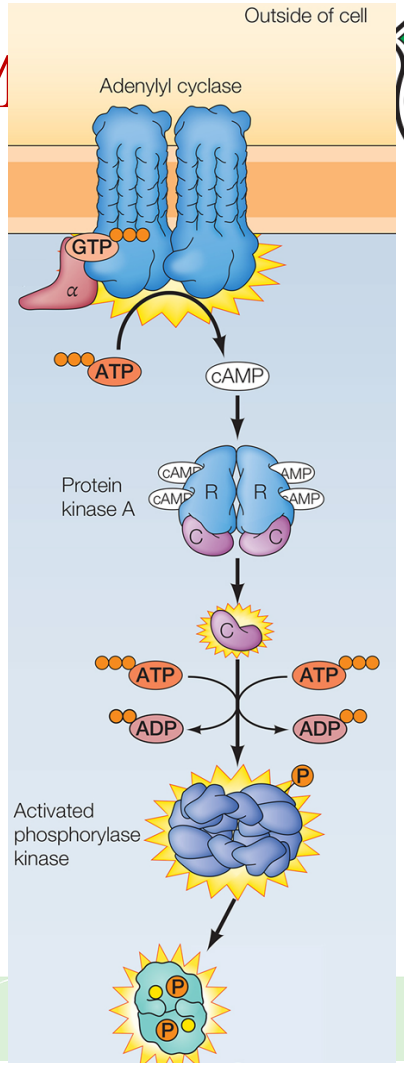
Note: The first messenger is the hormone itself

Synthesis and degradation of cAMP

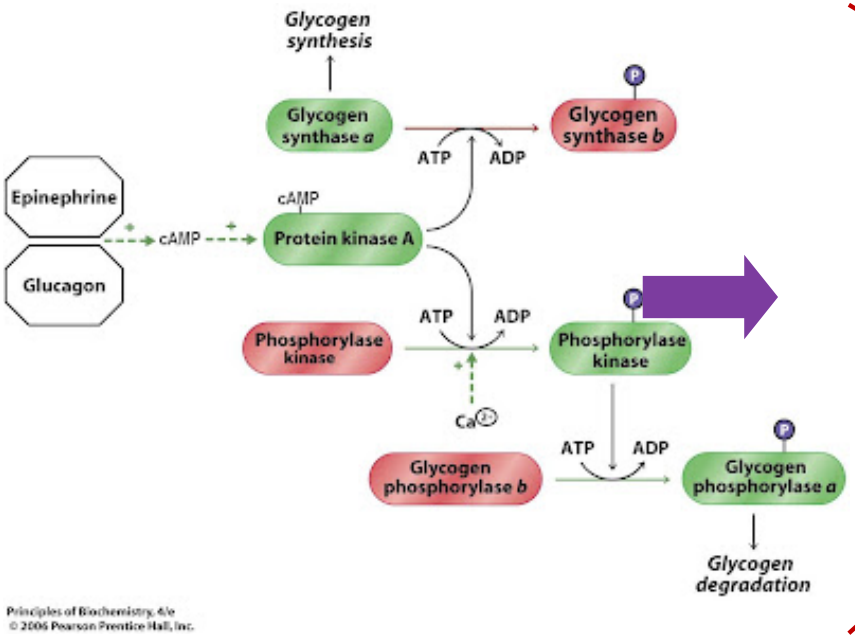


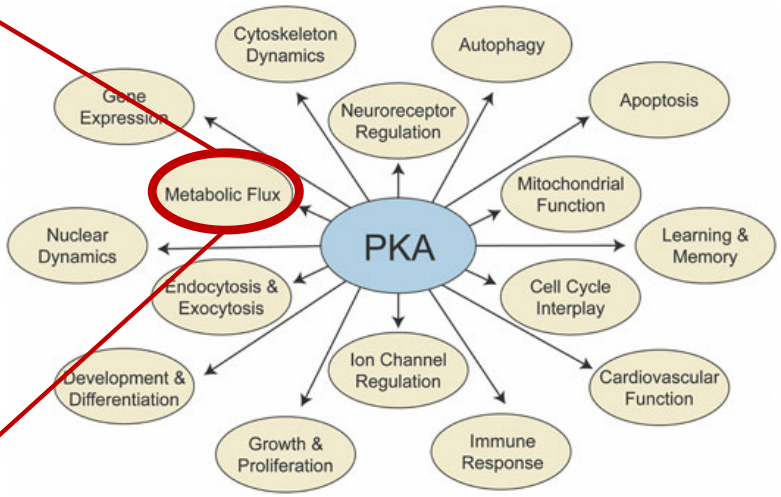
Regulation of protein kinase A by cAM

- cAMP activates protein kinase A, which consists of two regulatory (R) and two catalytic (C) subunits in its inactive form.
- Binding of cAMP to the regulatory subunits induces a conformational change that causes dissociation of the catalytic subunits, which are then enzymatically active.
- Protein kinase A is a serine/threonine kinase that has many targets in numerous cells and tissues.



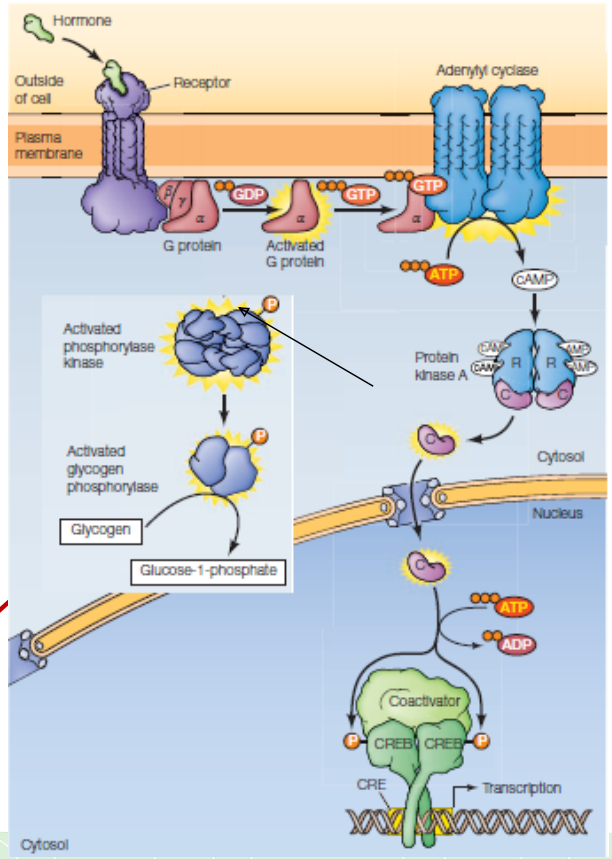
The many targets of PKA





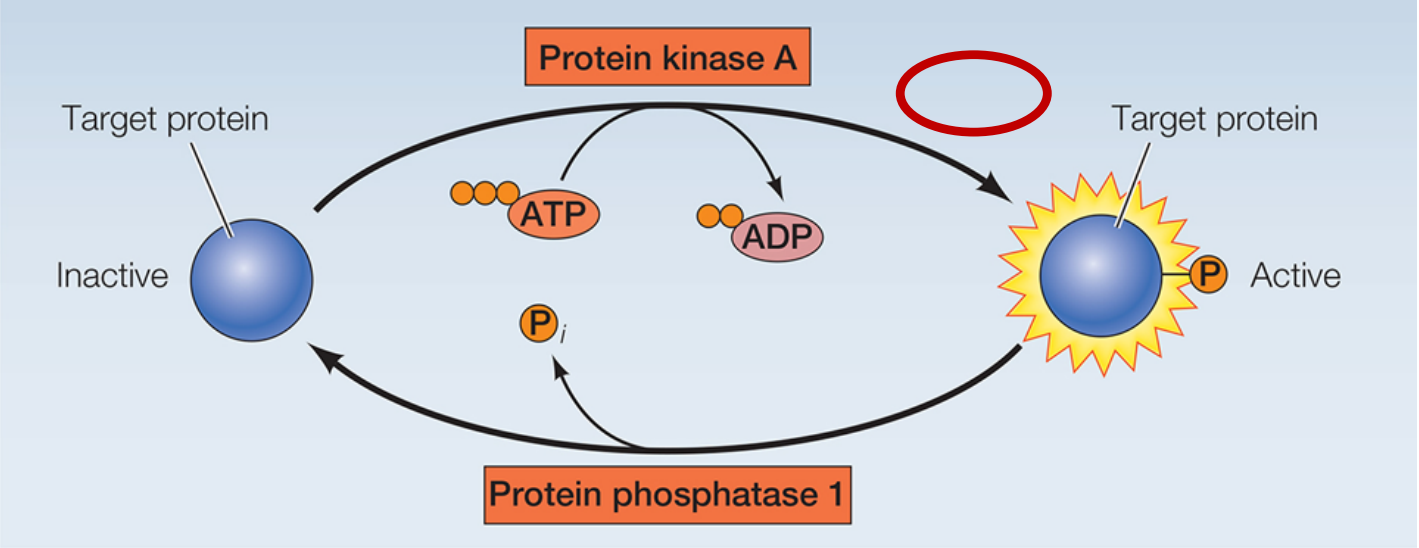
Example: cAMP-inducible gene expression

- The free catalytic subunit of protein kinase A can translocate into the nucleus and phosphorylates transcription factors like CREB (CRE-binding protein), leading to the expression of cAMP-inducible genes.



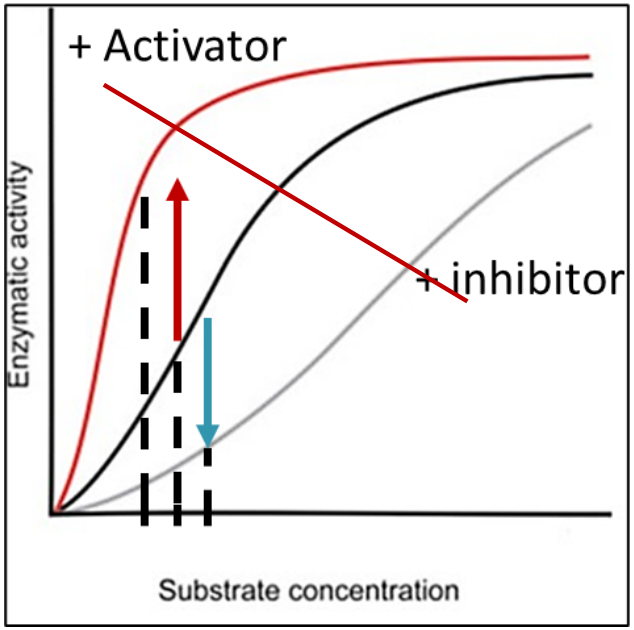
Regulation by dephosphorylation

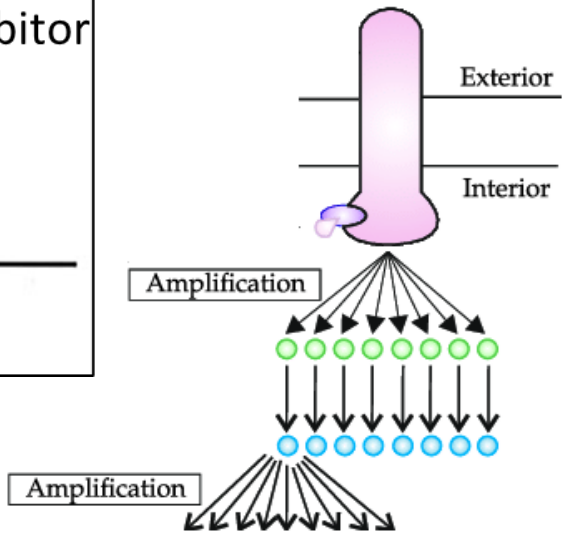
- The phosphorylation of target proteins by protein kinase A is reversed by the action of a phosphatase called protein phosphatase 1.



Why are effectors enzymes?

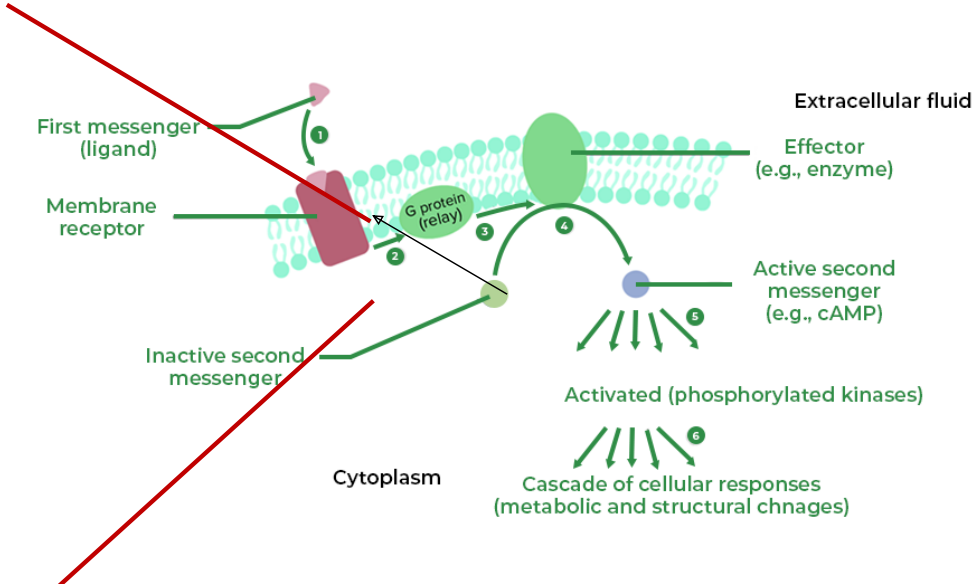
- Easy and quick regulation
  - Reversible covalent modification (e.g., phosphorylation)
  - Binding to small molecules (e.g., cAMP)
- Sensitive
  - Allostery
- Amplification





Why are secondary messengers good?

- Secondary messengers can be stored and can diffuse freely from one cell compartment to another.
  - Calcium ions (ER to cytosol)
  - Diacylglycerol and phosphatidylinositol-3-phosphate (from plasma membrane to cytosol)
- The signal can be amplified.
- Different signaling pathways can crosstalk by using a common secondary messenger.



Crosstalk: A regulatory mechanism in which one signaling pathway controls the activity of another.

Signaling pathways involving enzyme-linked receptors

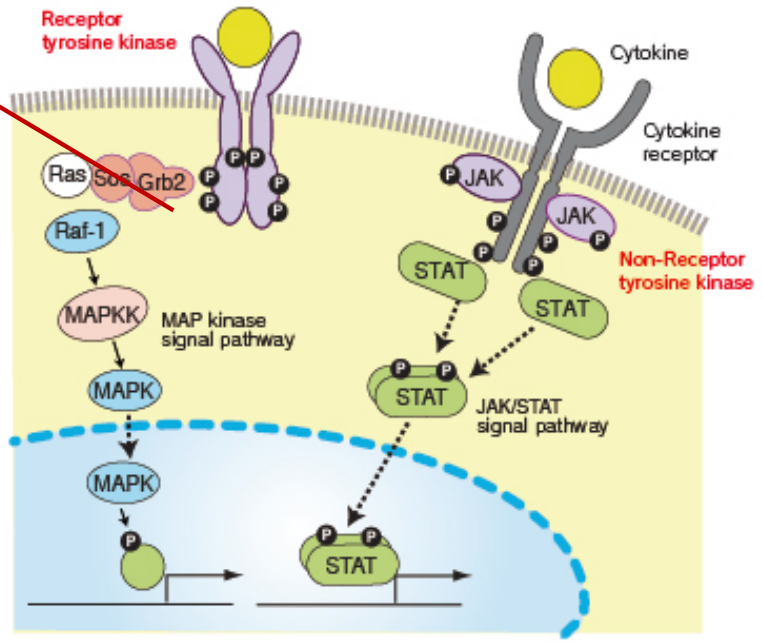
Receptor + tyrosine kinases (RTK)

- Some receptors either
- Receptor tyrosine kinases (have an intrinsic tyrosine kinase activity)

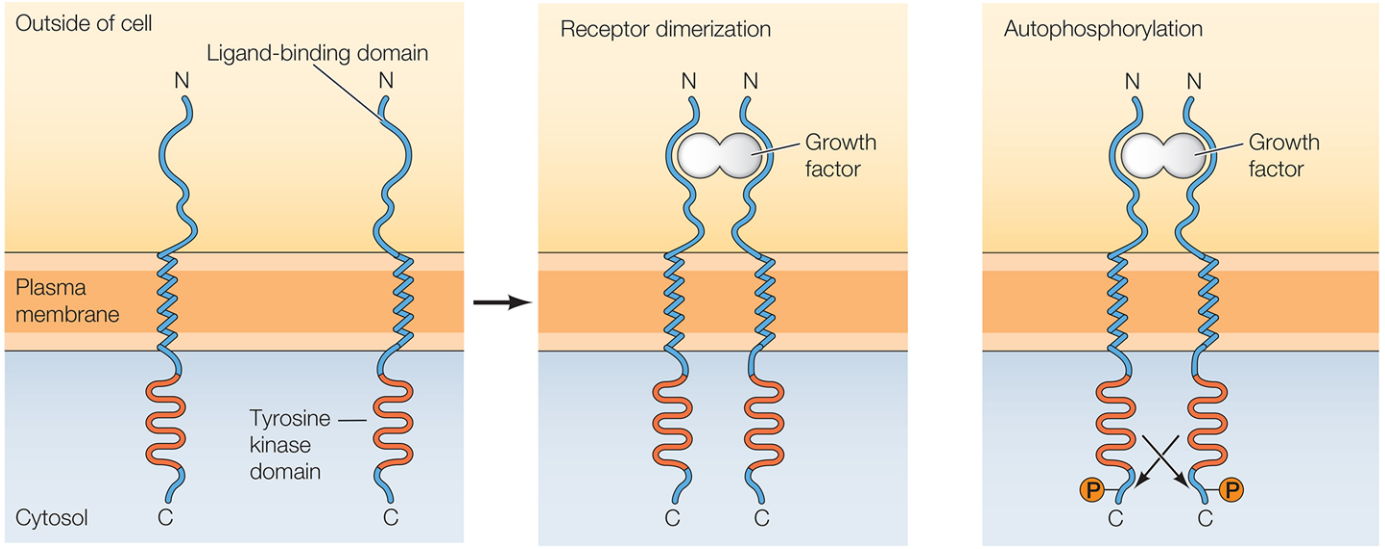
OR

- Nonreceptor tyrosine kinases (directly and noncovalently associated with tyrosine kinases.

Binding of ligands extracellularly activates the kinase activity resulting in a phosphorylation cascade.



Mechanism of action of receptor tyrosine kinases



1

Each receptor consists of an extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic domain with tyrosine kinase activity.

Growth factor binding induces receptor dimerization,

2

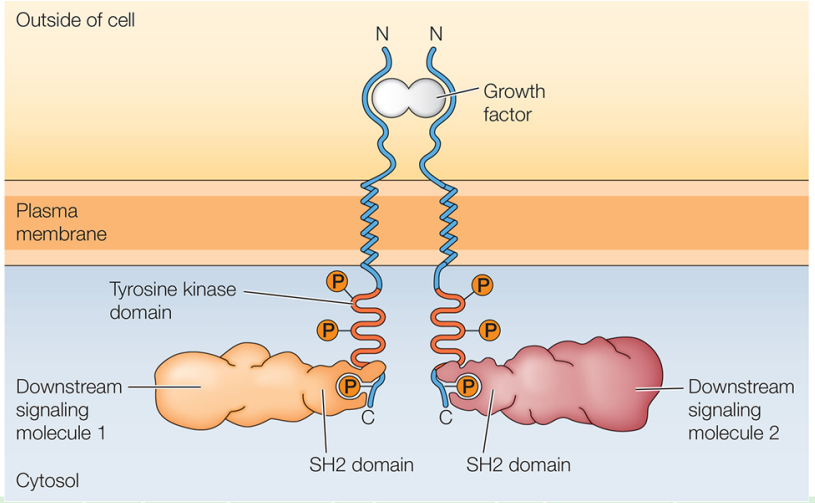
3

Dimerization results in receptor autophosphorylation as the two polypeptide chains cross-phosphorylate one another. The receptor is now active.

What is the effect of autophosphorylation?

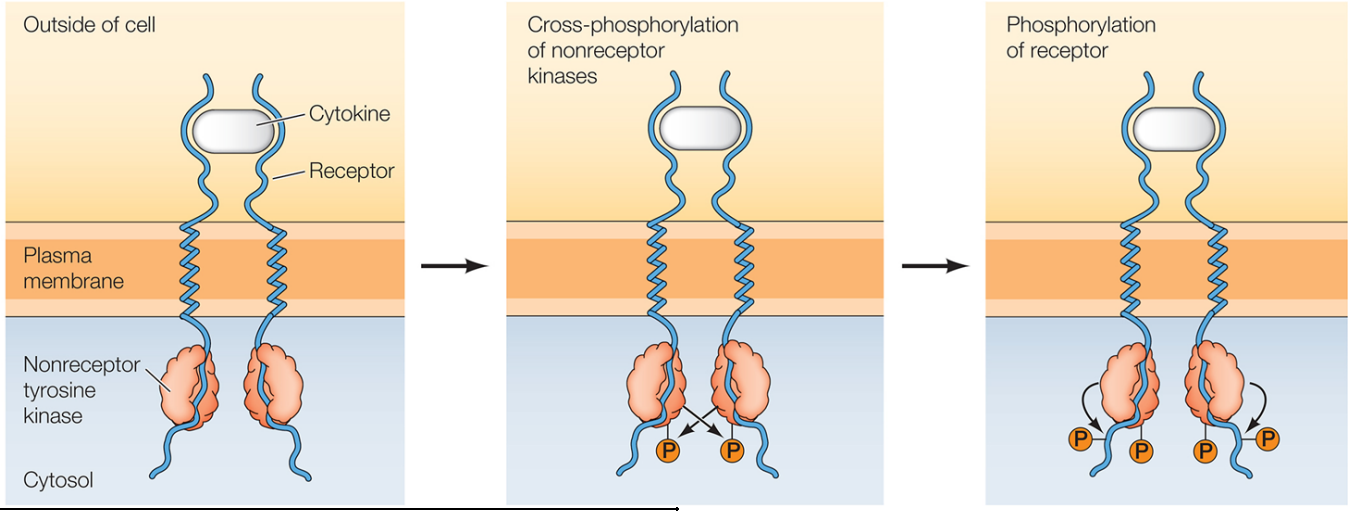
- Autophosphorylation of the tyrosine residues has two effects.
  - It increases the protein kinase activity.
  - It creates specific binding sites for additional proteins that transmit intracellular signals downstream of the activated receptors.

The consequence of protein association with activated receptor tyrosine kinases:
They localize to the plasma membrane they associate with other proteins this promotes the phosphorylation of further proteins this stimulates their enzymatic activities.



Nonreceptor protein tyrosine kinases

Cytokine receptor superfamily



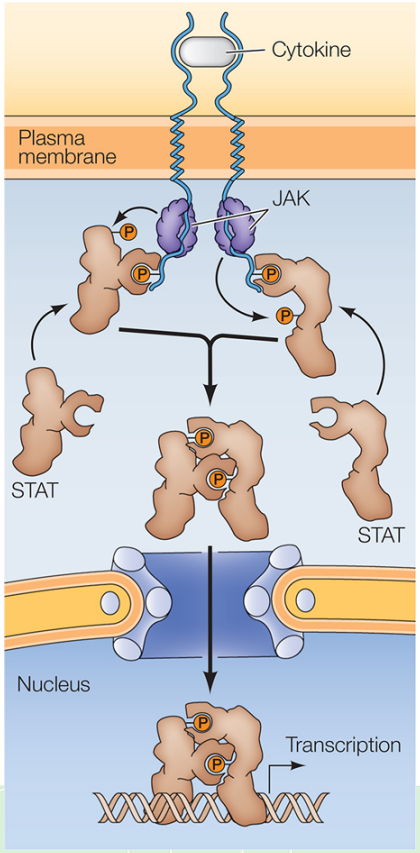
Ligand binding induces receptor dimerization and leads to the activation of associated nonreceptor tyrosine kinases as a result of cross-phosphorylation.

The activated kinases then phosphorylate tyrosine residues of the receptor, creating phosphotyrosine-binding sites for downstream signaling molecules.

The JAK/STAT pathway

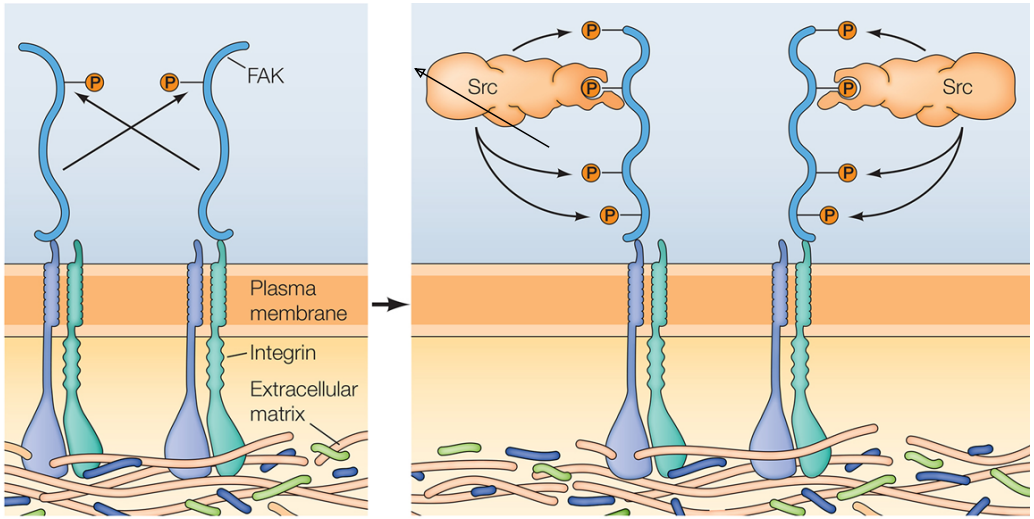
Cytokines are small proteins that signal for the control of the growth and activity of immune cells and blood cells.

- Stimulation of cytokine receptors leads to the binding of the transcription factor, STAT, to phosphotyrosine-binding sites on the cytokine receptor
- The STAT proteins are phosphorylated by the receptor-associated JAK tyrosine kinases.
- The phosphorylated STAT proteins then dimerize and translocate to the nucleus, where they activate the transcription of target genes.



Integrin signaling

- Binding of integrins to the extracellular matrix leads to integrin clustering and activation of the nonreceptor tyrosine kinase FAK (focal adhesion kinase) by autophosphorylation.
- The nonreceptor tyrosine kinase, Src, then binds to the FAK and phosphorylates FAK on additional tyrosine residues, which serve as binding sites for downstream signaling molecules (e.g., Ras).
- *Other like-receptors: members of the Ig superfamily and cadherins*



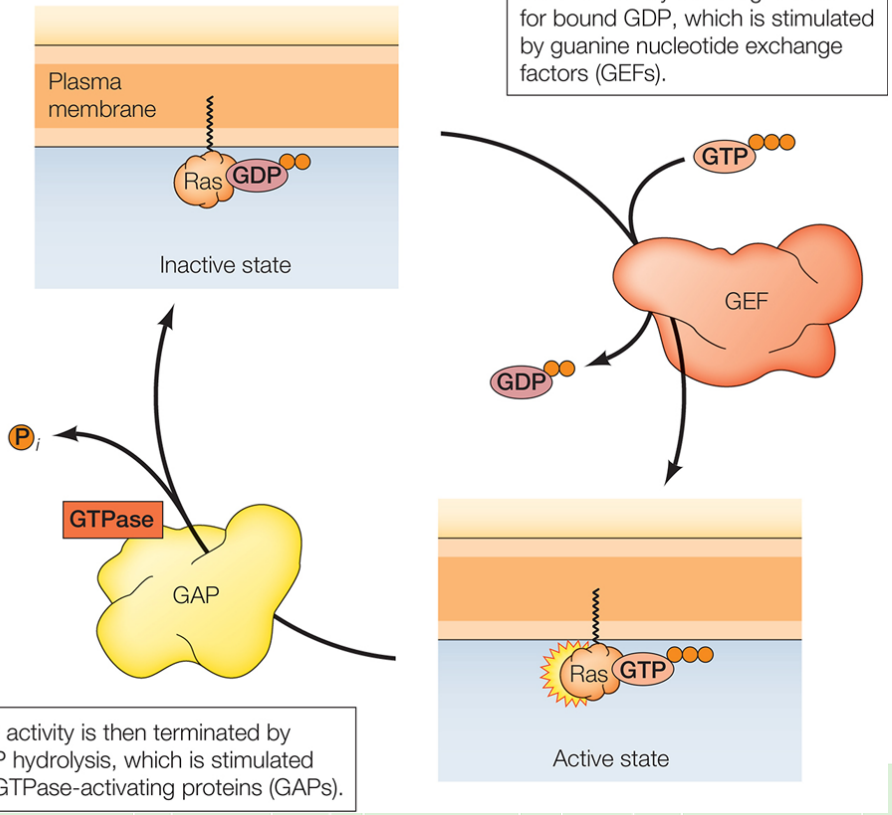
The MAP kinase pathways

MAP: mitogen-activated pathway

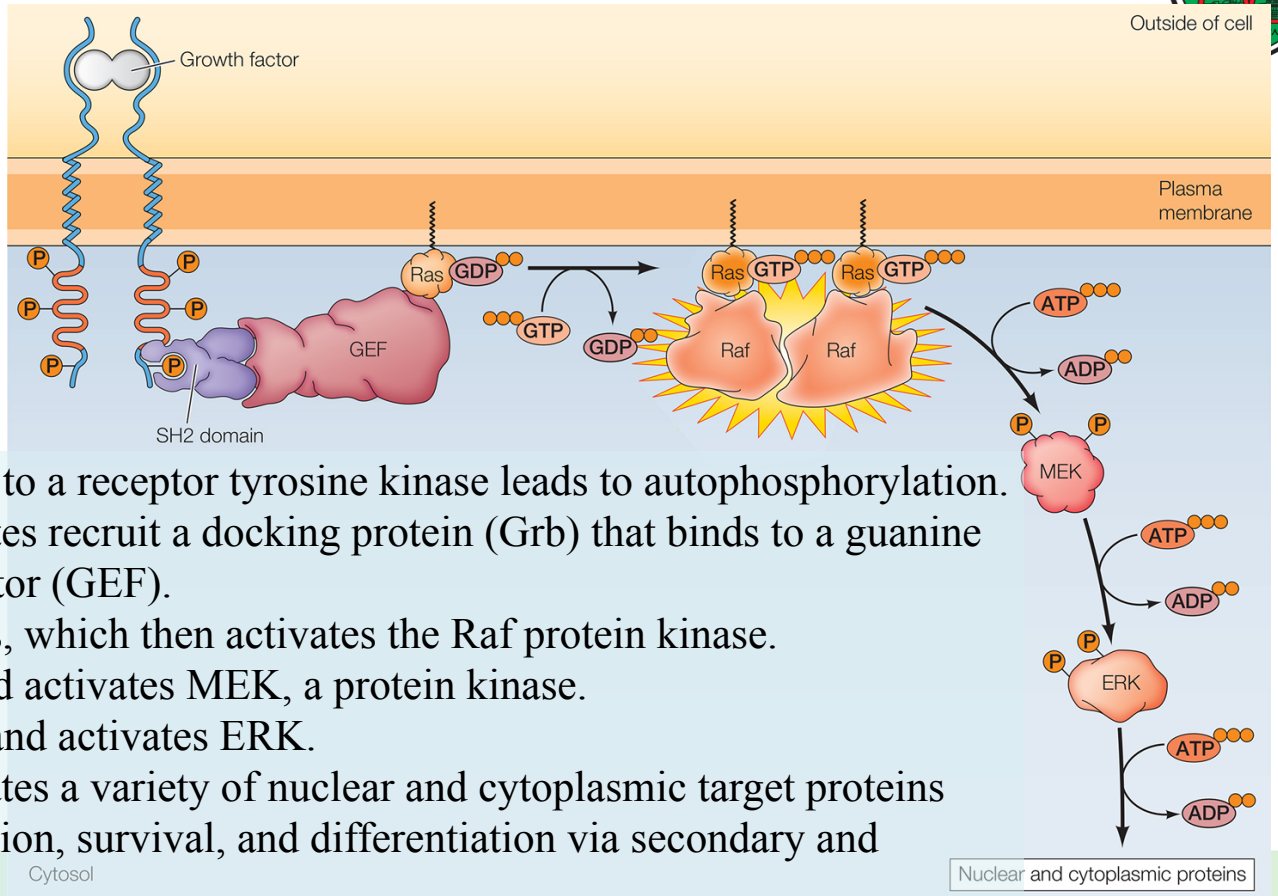
A mitogen is a small bioactive protein or peptide that induces a cell to begin cell division or enhances the rate of division (mitosis).

Let's start with Ras (*a small monomeric G protein*)

- Ras is stimulated by guanine nucleotide exchange factors (GEFs).
- Ras is inhibited by GTPase activating proteins (GAPs).
- Ras is a proto-oncogene that becomes an oncogene when mutated.
- Ras is associated with colorectal cancer.
- *Onco = tumor*



The Ras/Raf/MAPK signaling pathway



Growth factor binding to a receptor tyrosine kinase leads to autophosphorylation.

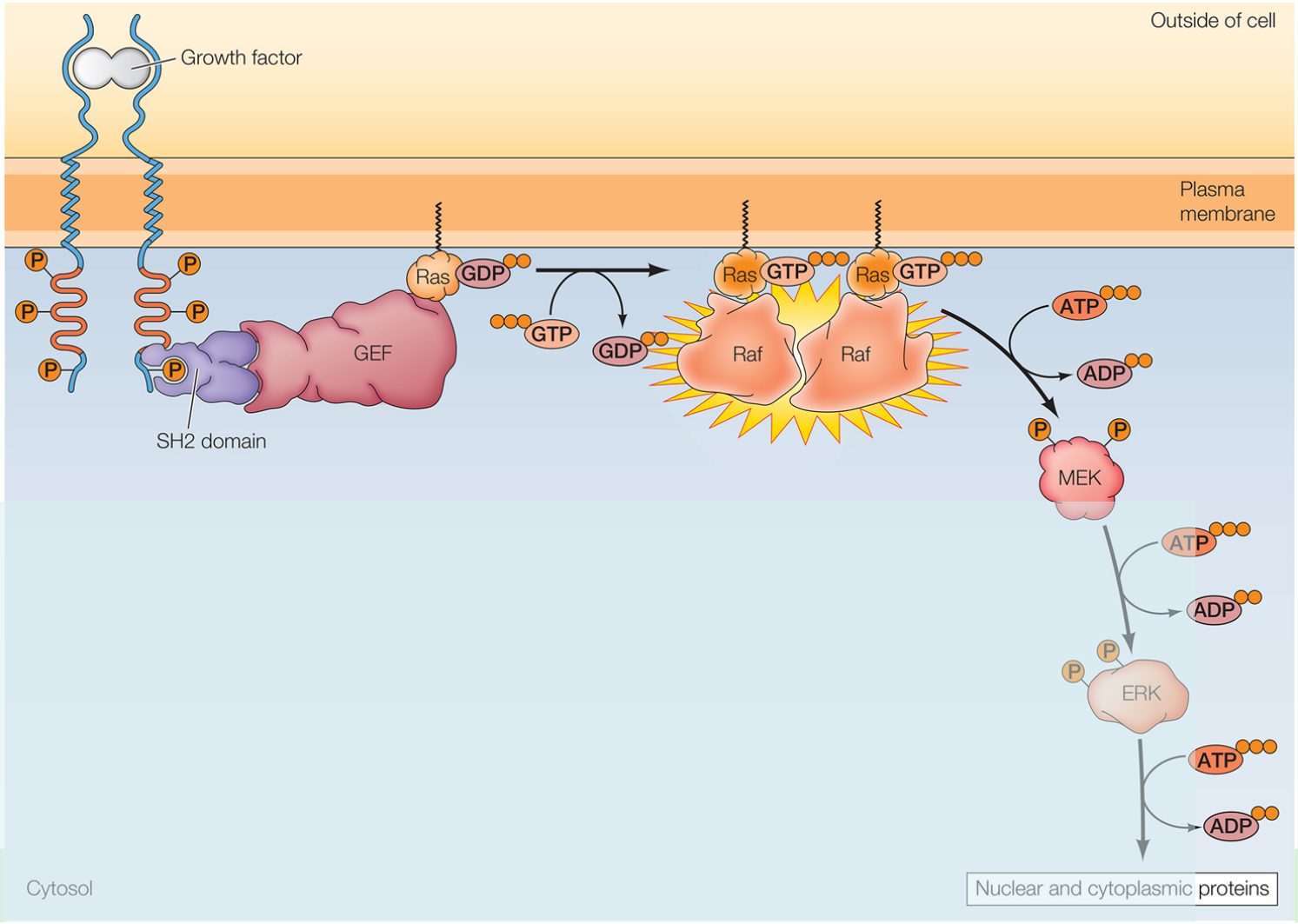
The phosphorylated sites recruit a docking protein (Grb) that binds to a guanine nucleotide exchange factor (GEF).

The GEF activates Ras, which then activates the Raf protein kinase.

Raf phosphorylates and activates MEK, a protein kinase.

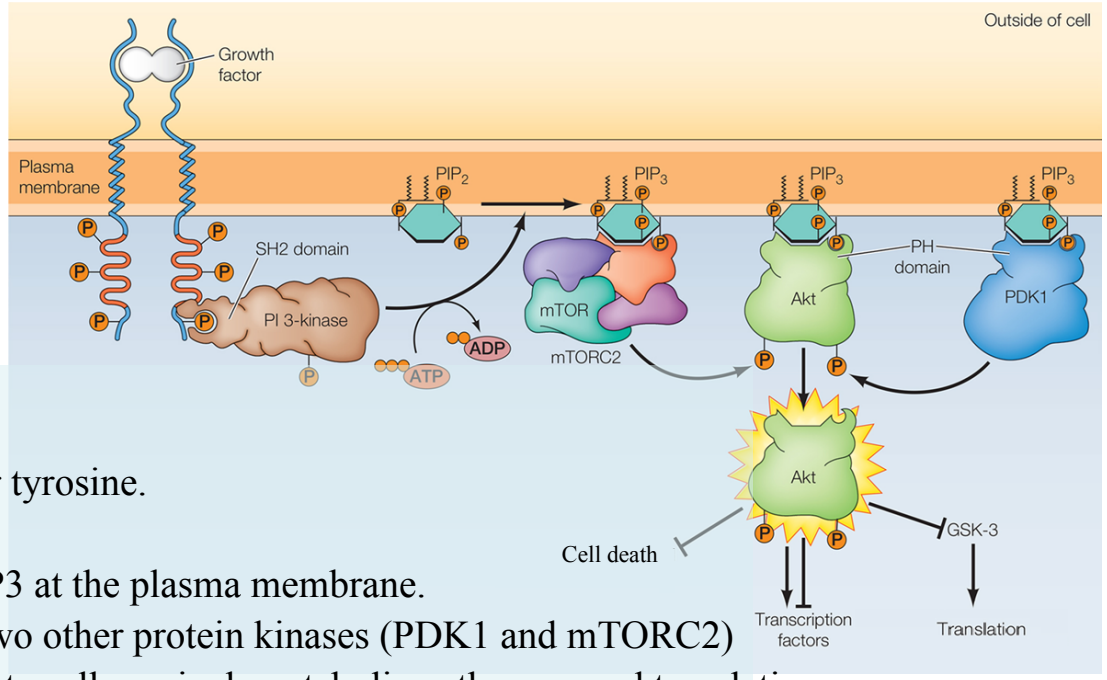
MEK phosphorylates and activates ERK.

ERK then phosphorylates a variety of nuclear and cytoplasmic target proteins promoting cell proliferation, survival, and differentiation via secondary and tertiary responses.

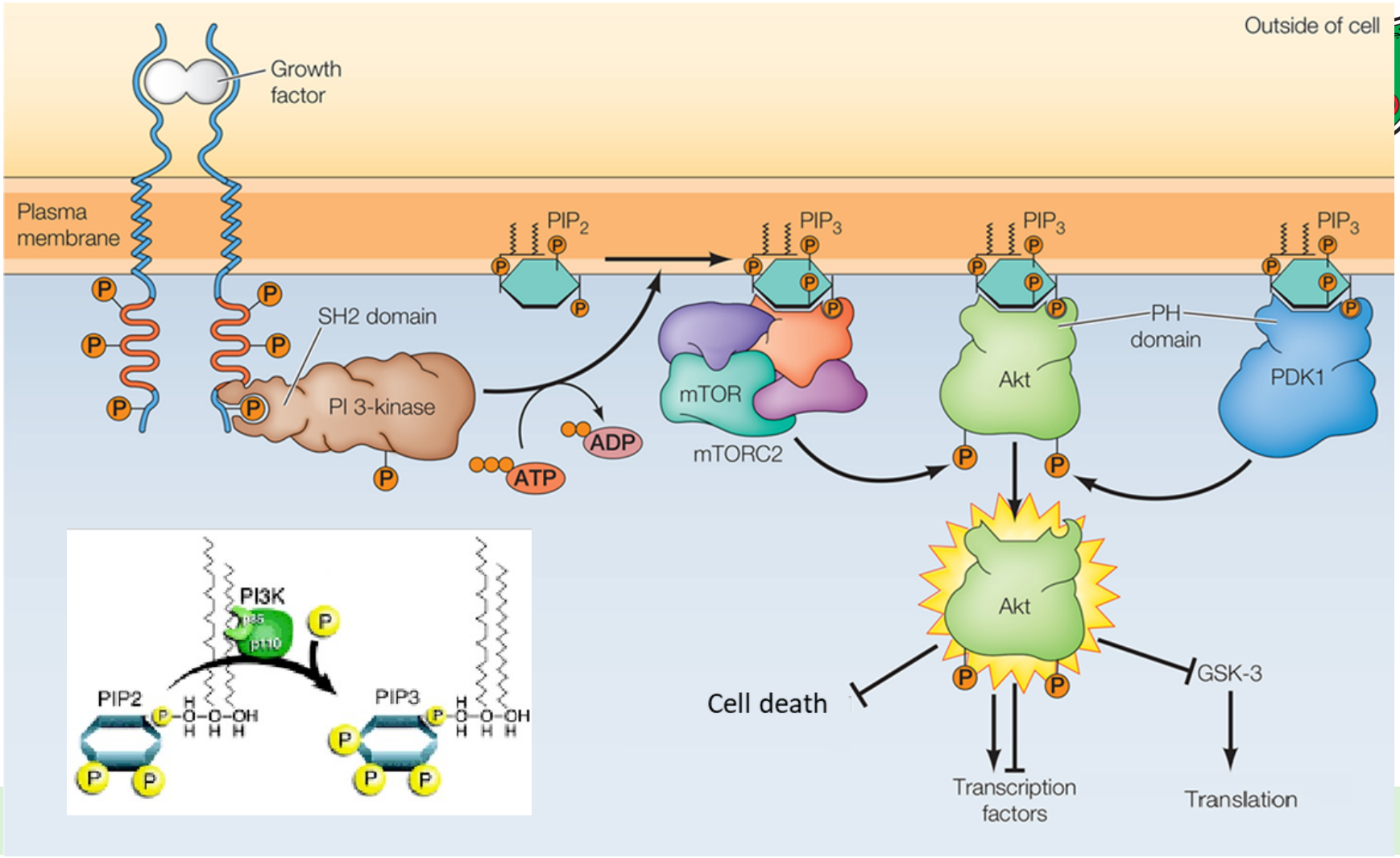

Outside of cell
Growth factor
Plasma membrane
P
P
P
P
P
P
SH2 domain
GEF
Ras
GDP
GTP
GDP
Ras
GTP
Ras
GTP
Raf
Raf
ATP
ADP
P
P
MEK
ATP
ADP
P
P
ERK
ATP
ADP
Cytosol
Nuclear and cytoplasmic proteins

The PI 3-kinase/Akt pathway

PI 3-kinase (phosphatidylinositide (PI) 3-kinase) phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3).



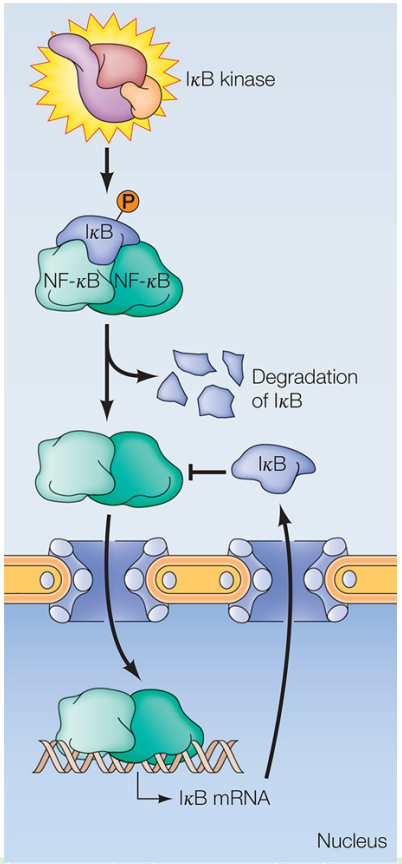
- PI 3-kinase is recruited to activated receptor tyrosine.
- PI 3-kinase phosphorylates PIP2 to PIP3.
- Akt (a serine/threonine kinase) binds to PIP3 at the plasma membrane.
- It is then phosphorylated and activated by two other protein kinases (PDK1 and mTORC2)
- Akt then phosphorylates proteins that regulate cell survival, metabolic pathways, and translation (protein translation).


Outside of cell
Growth factor
Plasma membrane
SH2 domain
PI 3-kinase
ATP
ADP
PIP2
PIP3
mTOR
mTORC2
Akt
PH domain
PDK1
PI3K
p85
p110
Cell death
Transcription factors
Translation
GSK-3

Signaling Dynamics and Networks

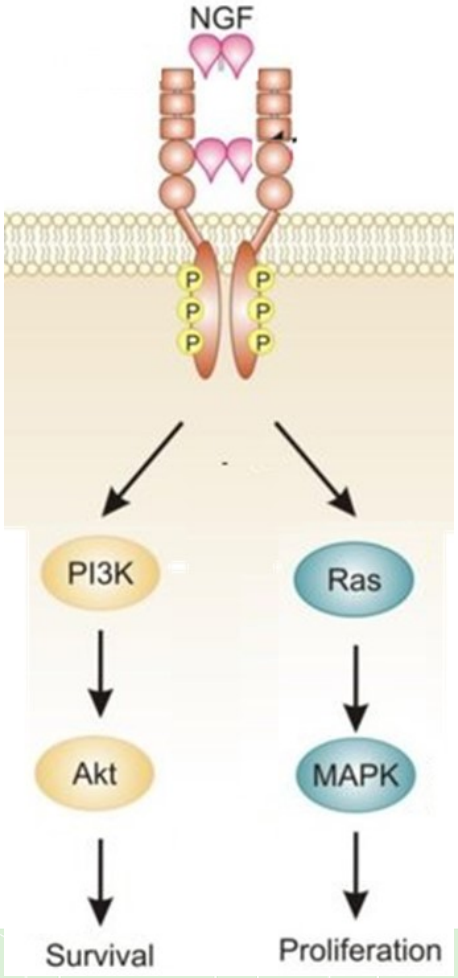
Feedback loops and signaling dynamics

- Activation of certain signaling pathways results in the phosphorylation of IκB, an inhibitor of NF-κB (nuclear factor-kappa B).
- This causes the activation of NF-κB, which translocates into the nucleus and activates transcription of target genes.
- One of the genes activated by NF-κB encodes IκB, generating a feedback loop that inhibits NF-κB activity.



Signaling homeostasis

- In response to nerve growth factor (NGF), ERK signaling can lead either to cell proliferation or to neuronal differentiation depending on the duration of ERK activity.
- Activation of ERK for 30–60 minutes stimulates cell proliferation.
- Sustained activation of ERK for 2–3 hours induces differentiation of the NGF-treated cells into neurons.

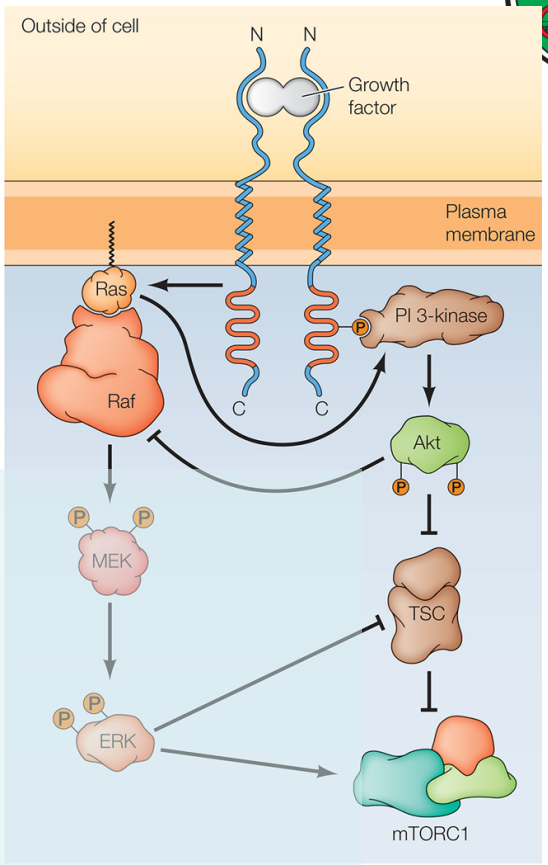


Crosstalk of pathways

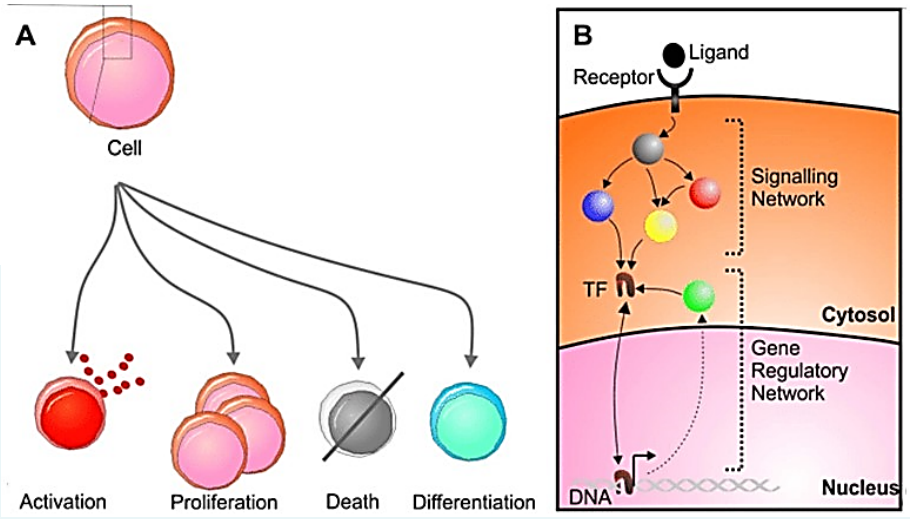
The ERK and PI 3-kinase signaling pathway

- The Ras/Raf/MEK/ERK and PI 3-kinase/Akt/mTORC1 pathways are connected by both positive and negative crosstalk,
  - PI 3-kinase is activated by Ras.
  - Raf is inhibited by Akt.
  - mTORC1 is activated by ERK.

Crosstalk: A regulatory mechanism in which one signaling pathway controls the activity of another.



Cell-specific response. Why?



- Cells have distinct receptors.
- Cells contain a different combination of regulatory molecules (effectors and secondary messengers) that influence the cells' behaviors.
- The final target protein must have access to its target.
  - For example, a transcription factor would bind to the DNA-binding site and activate transcription if the chromatin is packaged loosely (i.e., euchromatin). If the site is packaged tightly (i.e., heterochromatin), the complex would not be able to bind to the DNA.