بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Cytology & Molecular Biology | Lecture 12

Cell Signaling pt.1

Written by : DST

Reviewed by : NST

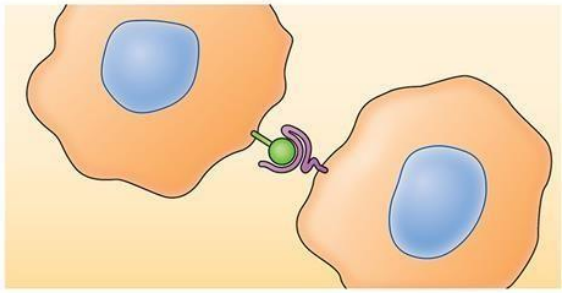
Short Quiz regarding last lecture:

CLICK HERE

Modes of cell signaling

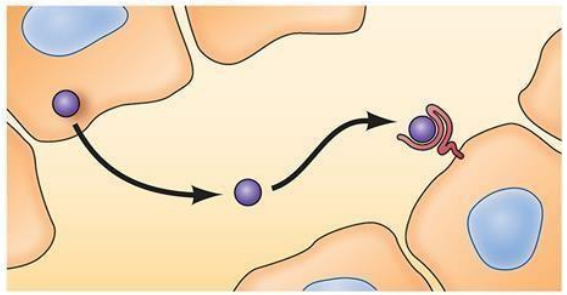
We well study secreted molecule signaling

(A) Direct cell–cell signaling



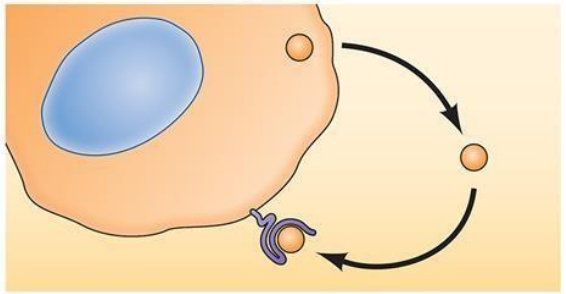
Cell-cell interaction
Direct interaction of a cell with its neighbor

Paracrine signaling



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

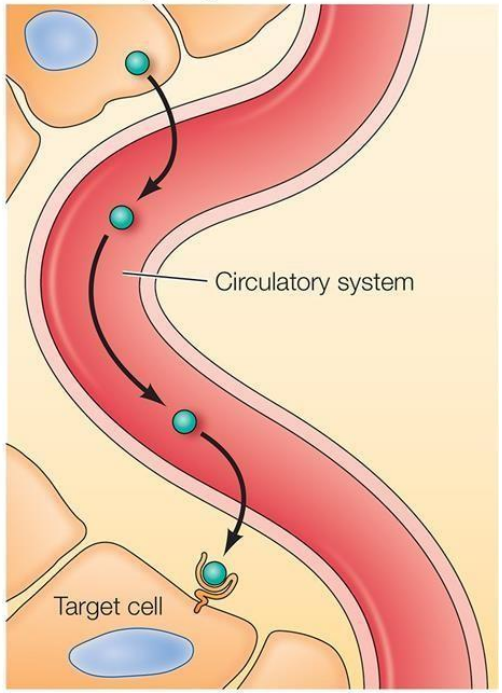
Autocrine signaling



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 blood circulation to act on target cells at distant body sites.

Endocrine → long-distance (through blood)
Paracrine → short-distance (neighboring cells)
Autocrine → self-targeting (same cell)
Cell-cell interaction → direct physical contact and communication between neighboring cells.

Classification of signaling molecules

Ligands: small molecules that act on a receptors and can be of different chemical natures as shown in this slide

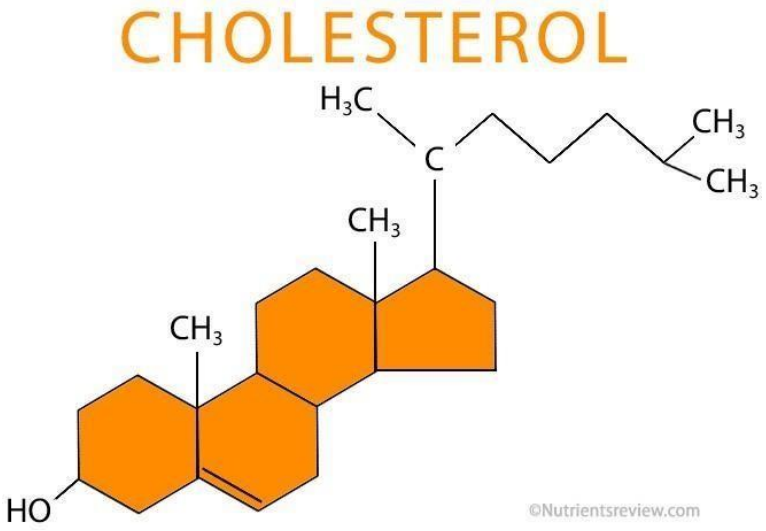
- Peptides: growth factors (EGF- Epidermal growth factor), peptide hormones (insulin, glucagon), or neuropeptides-acting on nerve cells-(oxytocin, enkephalins) Could be Large (Polypeptide or Protein)
- Small molecule neurotransmitters: derived from amino acids like Epinephrine (adrenaline) and thyroid hormone (tyrosine), serotonin (tryptophan).
- Steroids: derived from cholesterol like estradiol, cortisol, calciferol (Vitamin D), and testosterone (Androgens). They are Lipophilic molecules (Hydrophobic)
- Eicosanoids: derivatives of arachidonic acid (Arachidonic acid: polyunsaturated fatty acid with a 20-carbon chain) including prostaglandins, leukotrienes, and thromboxanes B.
- Gasses: Nitric oxide (NO) and carbon monoxide (CO)

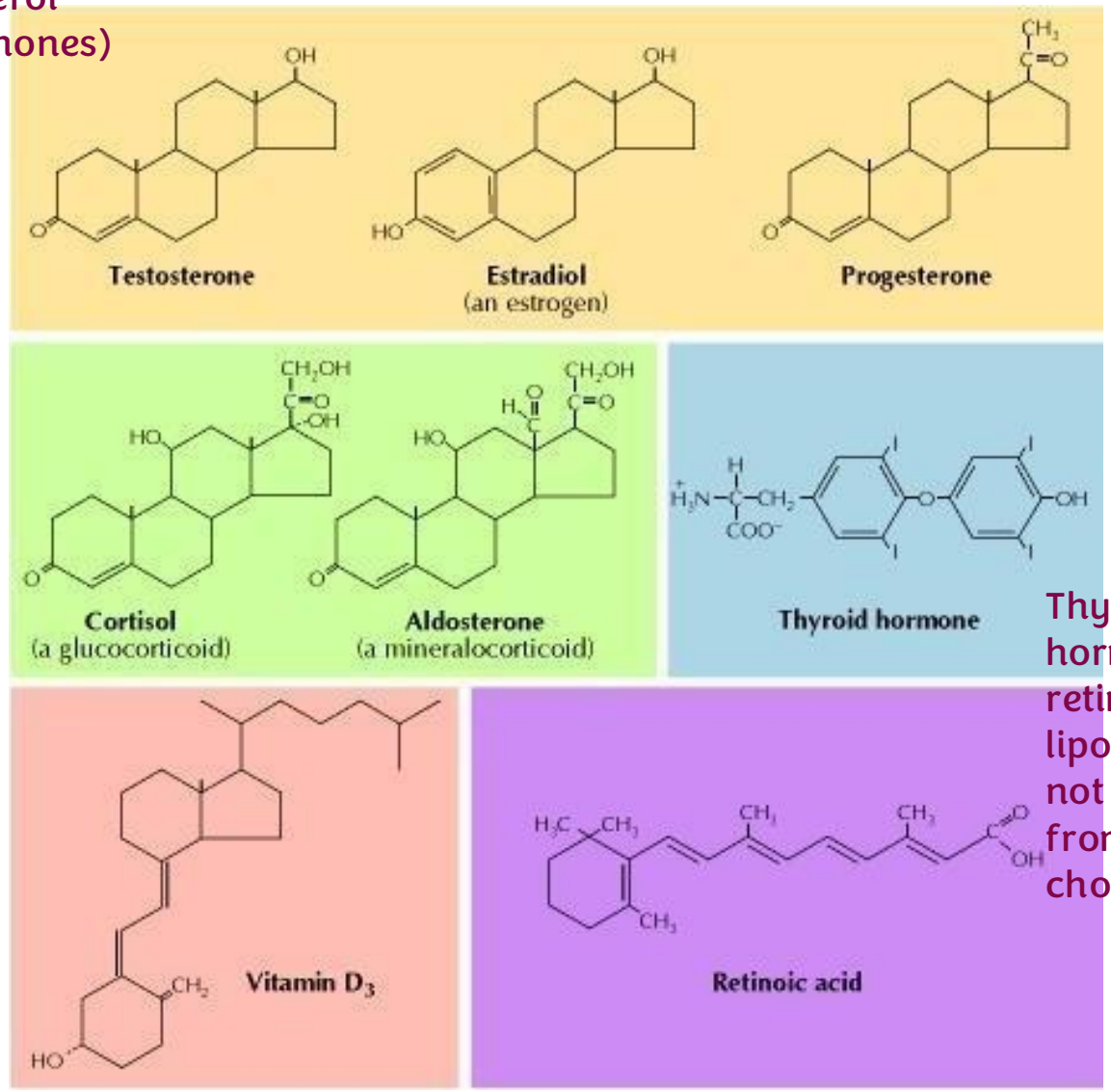
Lipophilic hormones

How do they function (send signal)?
- They are small and hydrophobic, have the ability to diffuse through the membrane, so they mainly function Intracellularly.

They are small , specially steroids that are derived from cholesterol (cholesterol can be modified and converted into a steroid hormones)

Mainly derived from Cholesterol, (you can note that looking to groups added to the structure).





Thyroid hormones and retinoic acid lipophilic but not derived from cholesterol

all (signal molecules) send signals by binding to a receptor, and once (the ligand) binds, the receptor sends a signal.

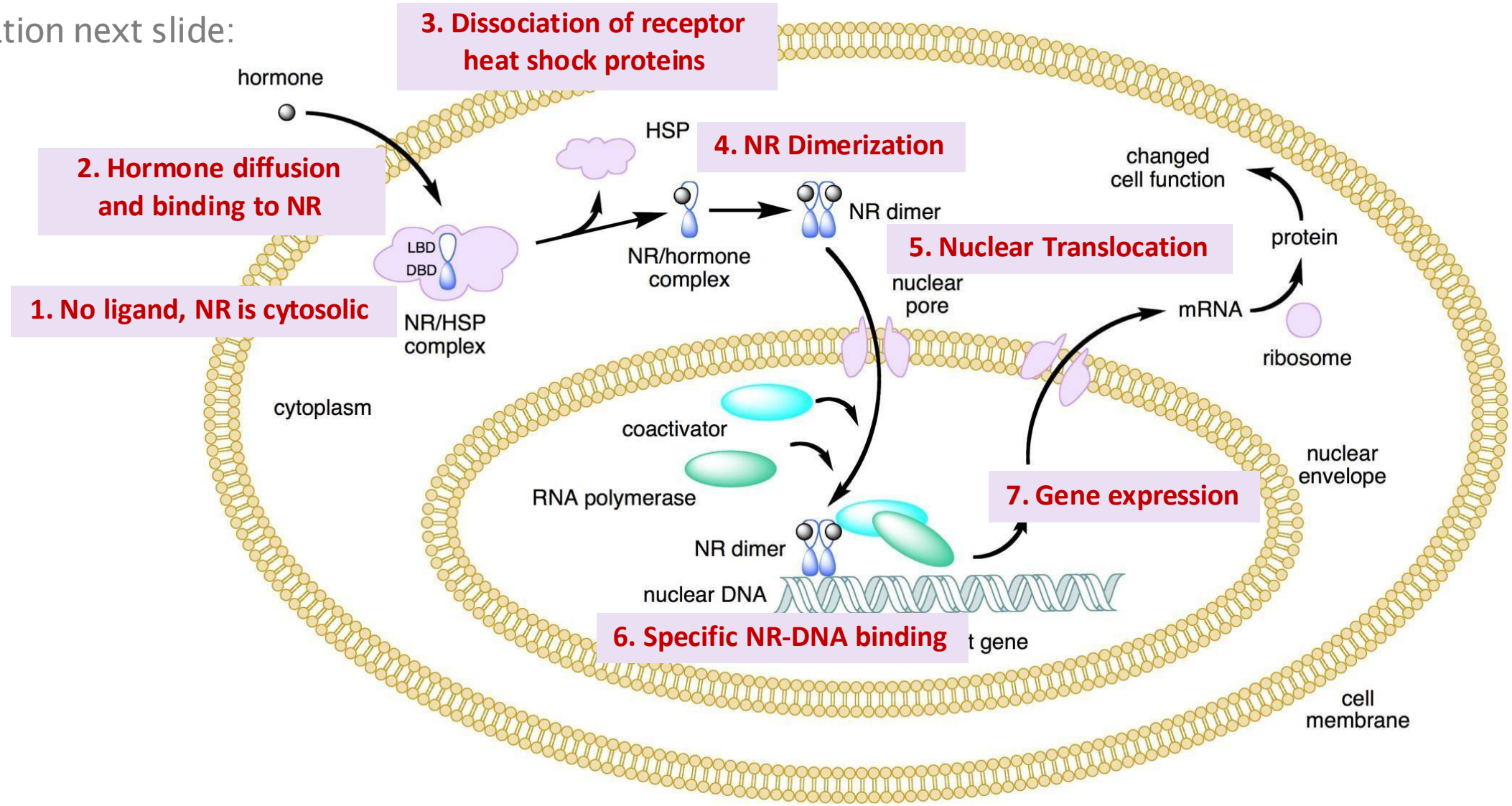
Androgen is a general term for male sex hormones, such as testosterone and dihydrotestosterone. Although these hormones are predominantly found in males, females also produce androgens in smaller amounts. Similarly, males produce estrogen, but in much lower quantities than females.

Don't memorize structures, you will come to it in next semester

Mechanism of action of steroid nuclear receptors (NR)

This is how lipophilic hormones function (Send signals)

Explanation next slide:



Mechanism of action of steroid nuclear receptors (NR)

1. Because they are small and lipophilic: they diffuse into the cytoplasm through the membrane, without needing a channel / carrier.

2. They bind to intracellular receptor which is cytosolic (some receptors can be nuclear) and bound to Heat Shock Protein (HSP); which prevents the receptor being active.

Once hormone binds to receptor:
-HSP is released.
-Receptor dimerizes.
-Receptor gets translocated to Nucleus.
-Receptor binds to DNA (in a specific place) and controls gene expression.
-It changes cell behaviour.

The possible outcomes or effects that happen after the hormone-receptor complex activates gene expression:

(It can die, live, develop, differentiates, carry out cortisol metabolism, bone resorption, and other reactions).

Recent research on lipophilic hormones such as estrogen and androgen has revealed that some of these hormones can bind to cell surface receptors, triggering rapid intracellular signaling. Additionally, they can diffuse into the cell and initiate signaling pathways within the cytoplasm or nucleus

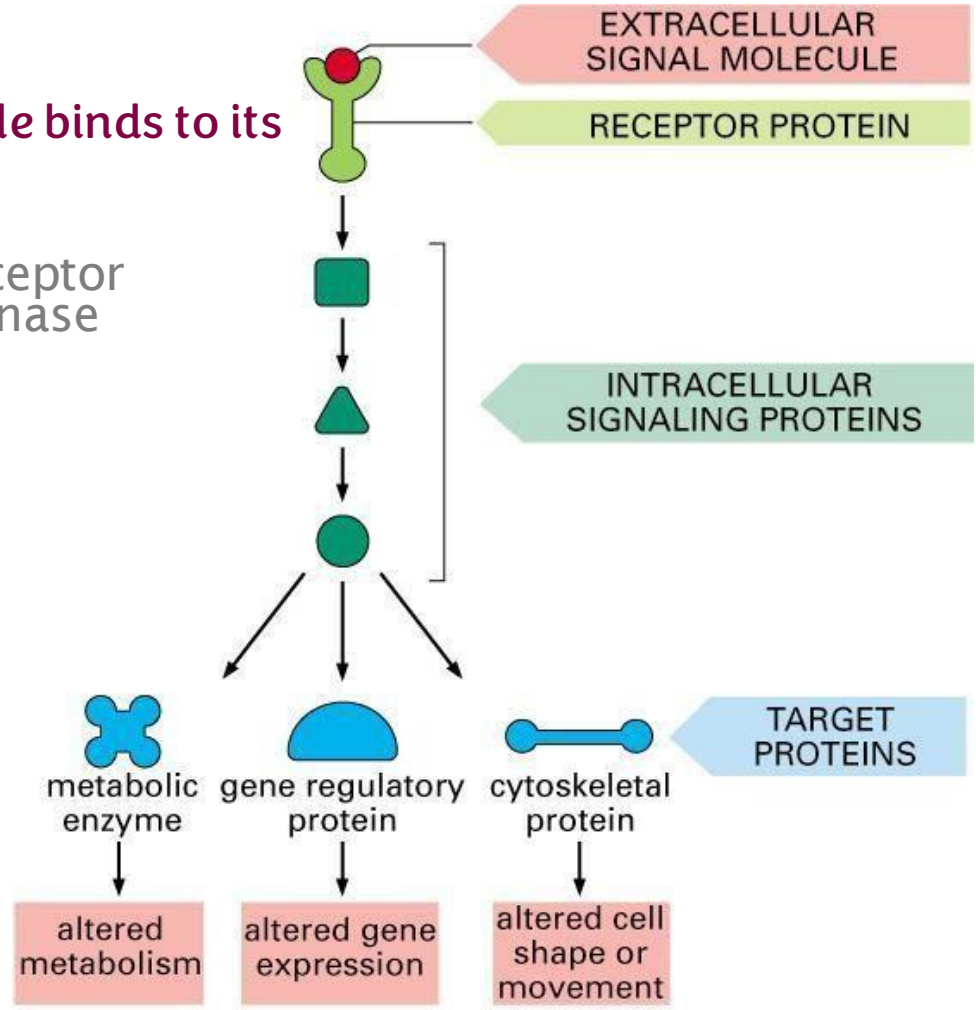
Cell surface receptors

Signal transduction

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

Ligand (hormone, growth factor) signaling molecule binds to its receptor (maybe on the cell surface or inside the cell)

- Receptor (GPCR, RTK) → G-protein coupled receptor / Receptor Tyrosine Kinase
- Transducers (G protein, Ras)
- Effector molecules (adenylate cyclase, MAPK)
- Secondary messengers (cAMP, cGMP, Ca^{2+})
- Final target molecules (e.g., DNA, protein, enzyme, channel, actin binding protein).
- Response (gene expression, cell behavior)

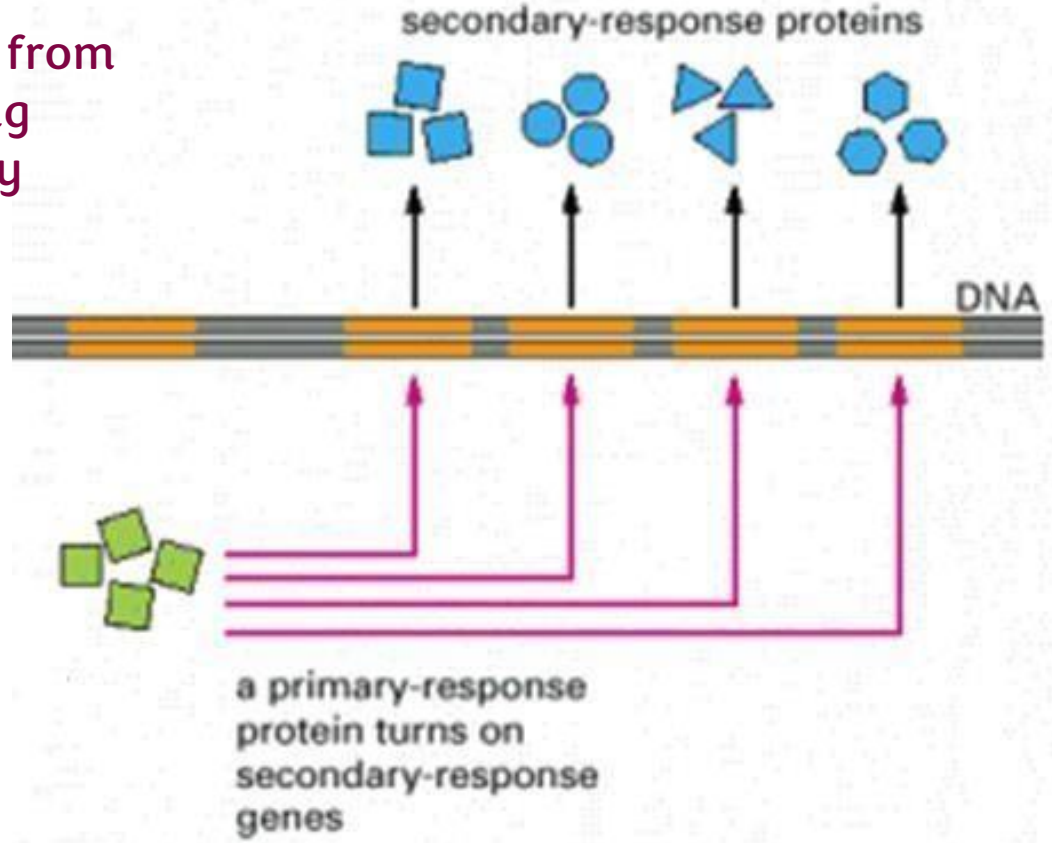


- Transducers: protein transmits a signal
- Effectors molecules: are enzymes (mainly) which can act on other pathways
- Response: release a hormone , cell movement , cell death etc....

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…

Directly from signaling pathway



Explanation: The result (primary response) of the signaling pathway it could activate another pathway/action

For e.g: the expression of a gene (primary response) can activate another gene (secondary response)
Sometimes these other genes can activate another set of genes (tertiary response).

Receptor

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

Transducer / transmitter

Secondary messenger

Outlines:

- Definitions
- Mechanism of G-protein,
- Regulation of G-protein (cycle)
- cAMP

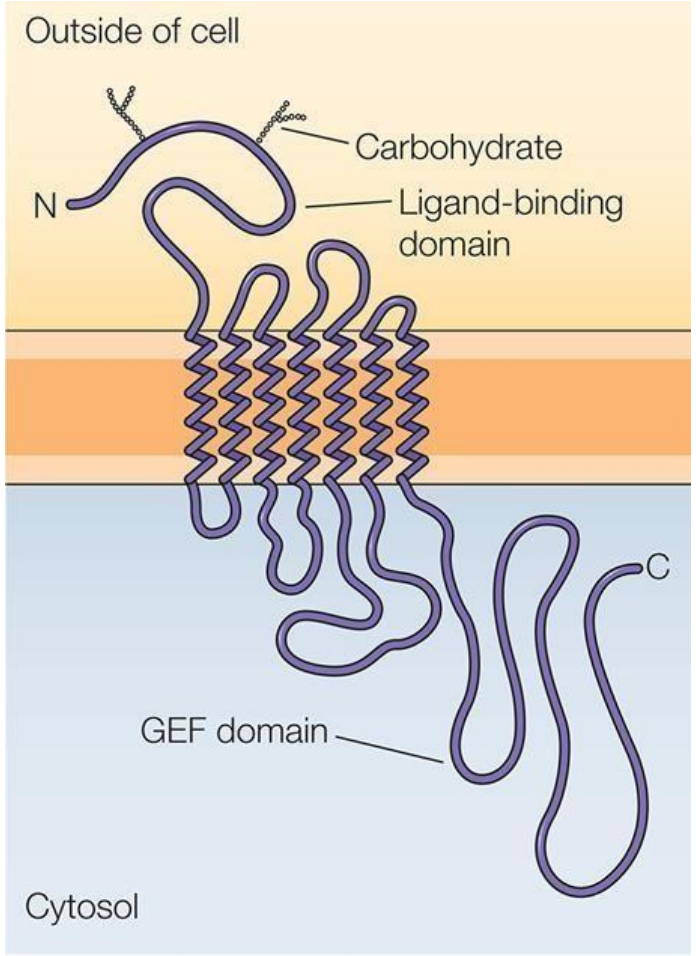
G protein-coupled receptors

- A family of receptors composed of seven membrane-spanning α helices (**Transmembrane domains**).
- 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.

Coupled to G Proteins (transducers)

45% of drugs target membrane receptors and 25% GPCRs

These receptors are very important, since they represent large portion of receptors in body, in addition to their functions in controlling crucial processes such as: hearing, taste, vision, cell proliferation, etc. Making them a target for many drugs.



Heterotrimeric G proteins

Made of three (trimeric) different (hetero) polypeptide chains, regulated by binding to GTP (G-protein). This is the transducer.

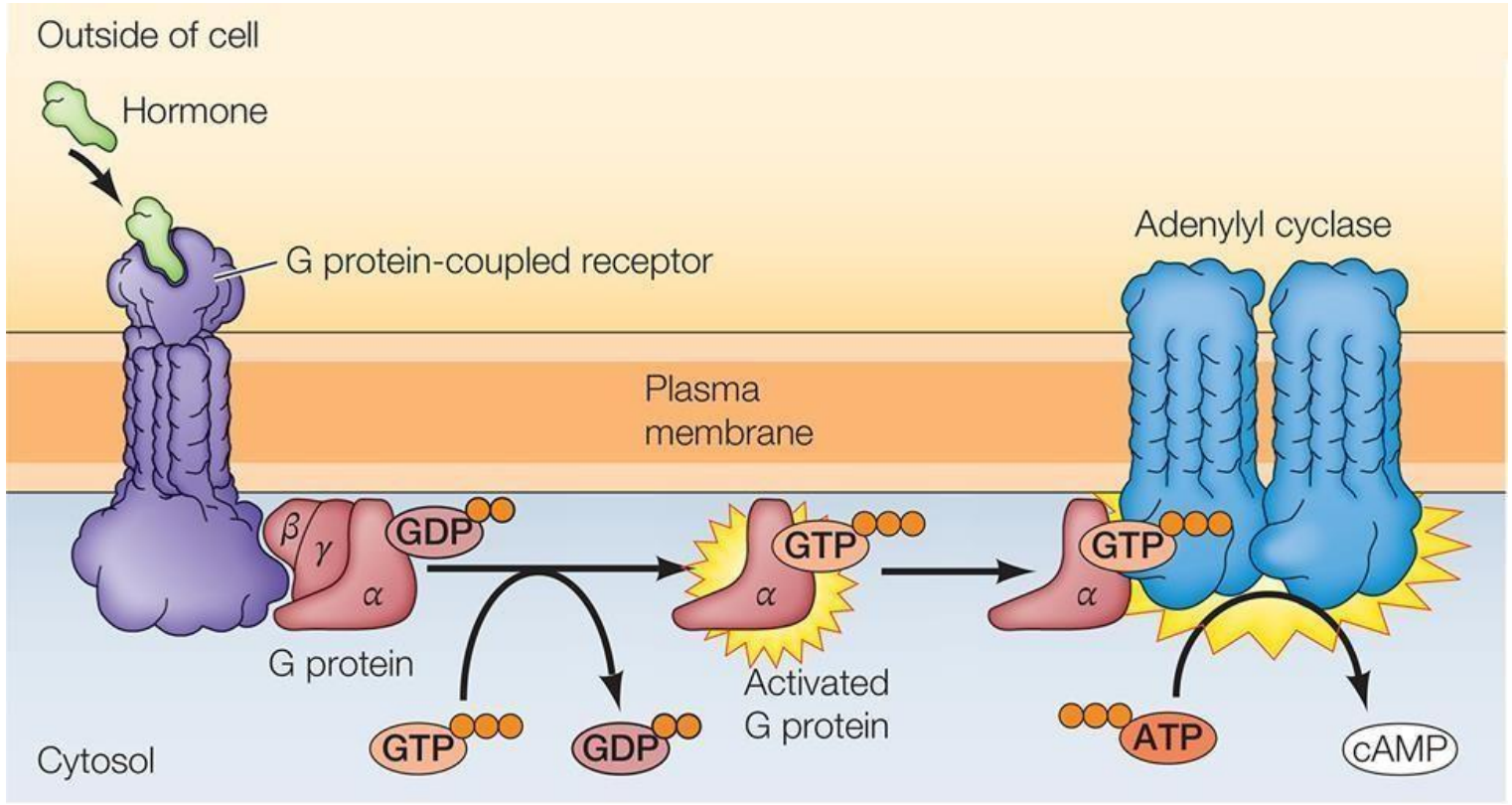
- G proteins are composed of three protein subunits—α, β, and γ.
- In the unstimulated state, the α subunit has GDP bound and the G protein is inactive.
- When stimulated, the α subunit releases its bound GDP, allowing GTP to bind in its place.
- This causes the trimer to dissociate into an α subunit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

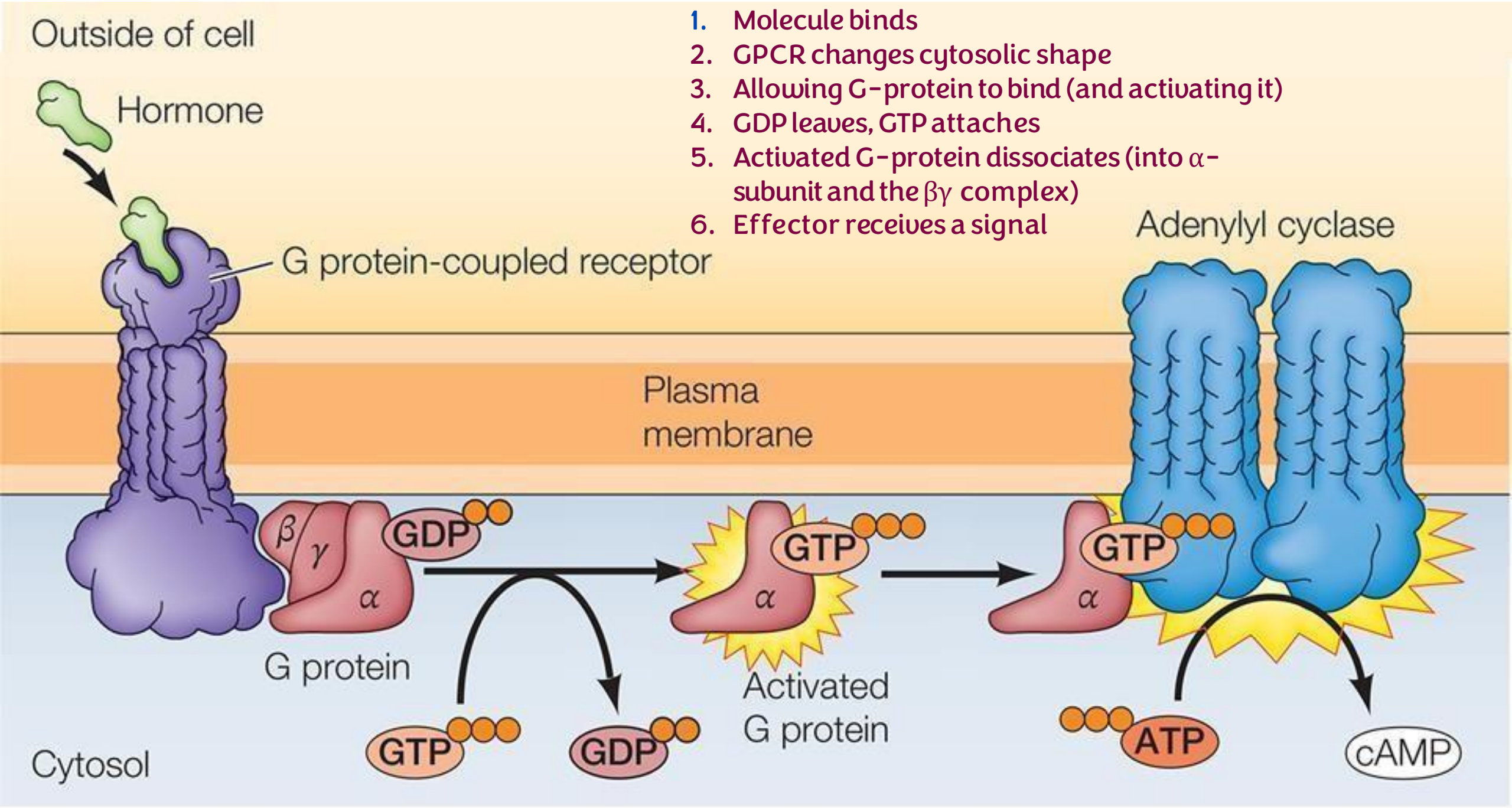
Before binding of ligand

After binding of ligand

- 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.

βγ complex can perform functions; it doesn't just inhibit alpha subunit.





1. Molecule binds
2. GPCR changes cytosolic shape
3. Allowing G-protein to bind (and activating it)
4. GDP leaves, GTP attaches
5. Activated G-protein dissociates (into α-subunit and the βγ complex)
6. Effector receives a signal

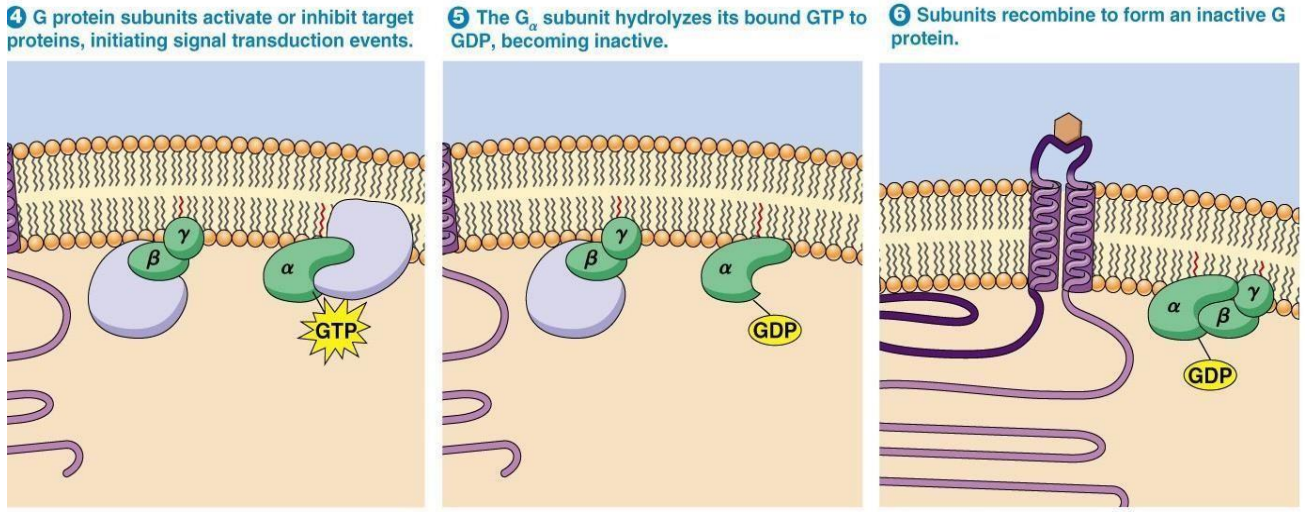
G protein inactivation

The alpha subunit must be inactivated, otherwise signal is always on

- 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.

Intrinsic: Internal enzymatic activity that acts on itself only, not on other proteins

1. GTP is hydrolyzed to GDP on α chain (intrinsically)
2. α Becomes inactive
3. α reassociate with βγ complex (waiting for another signal)



G protein inactivation

G-protein activity is regulated (by RGS):

Usually are activators (GEFs)

They can be Inhibitors:

GTPase activity is regulated by:

GAPs (GTPase activating proteins), they are regulators that speed up intrinsic GTPase activity.

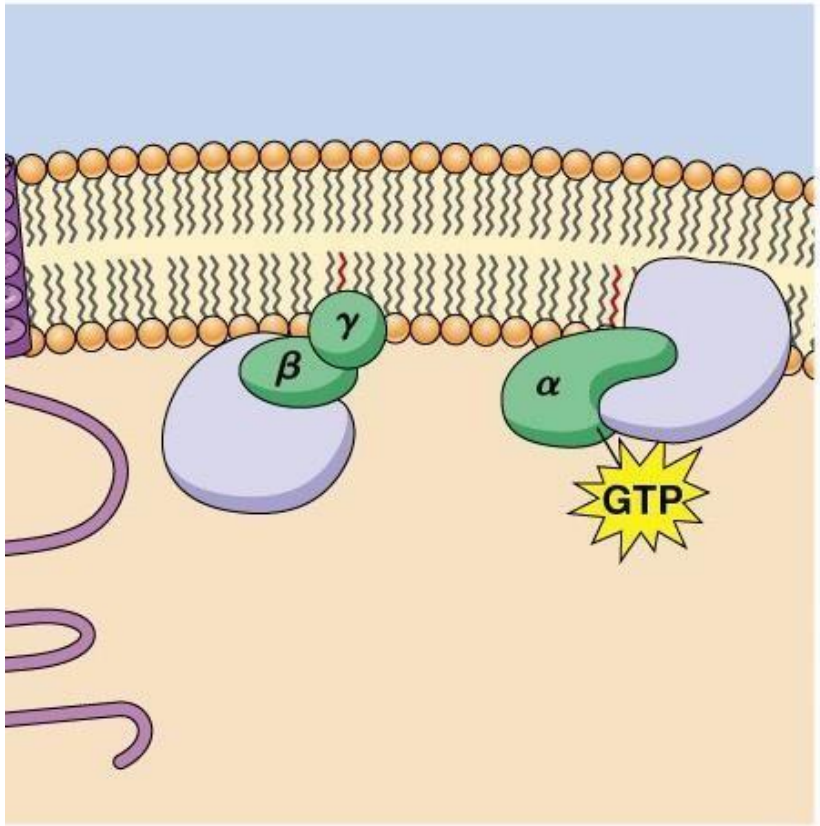
Decreasing length of activity

Or Activators:

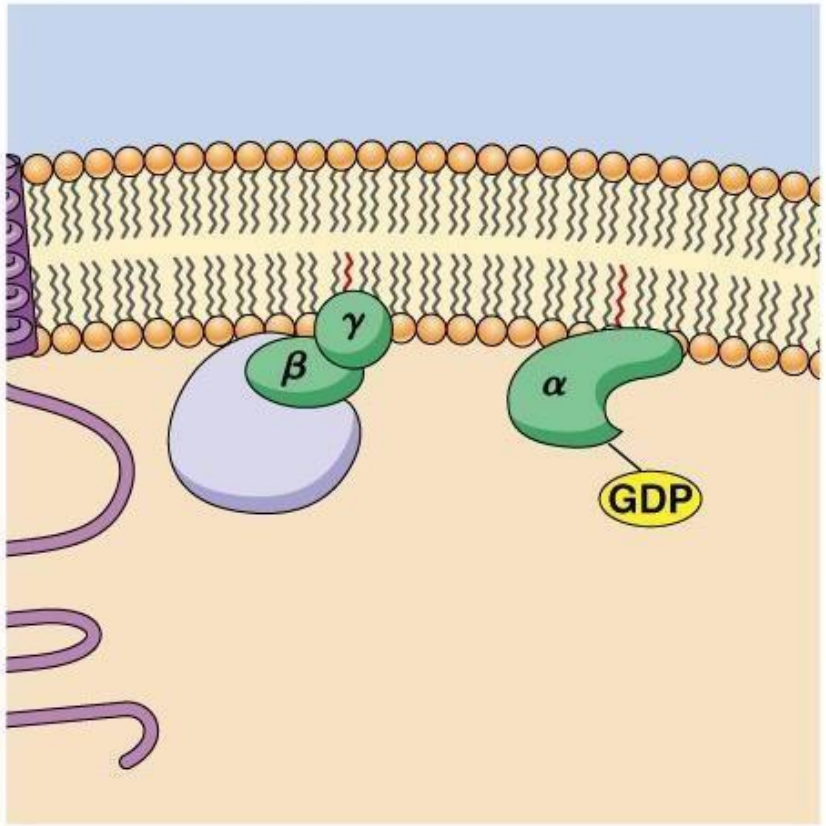
There are also regulators that regulate dissociation of GDP and binding of GTP on G proteins

Guanine nucleotide **E**xchange **F**actors (**GEF**s): they facilitate the release of GDP and the binding of GTP.

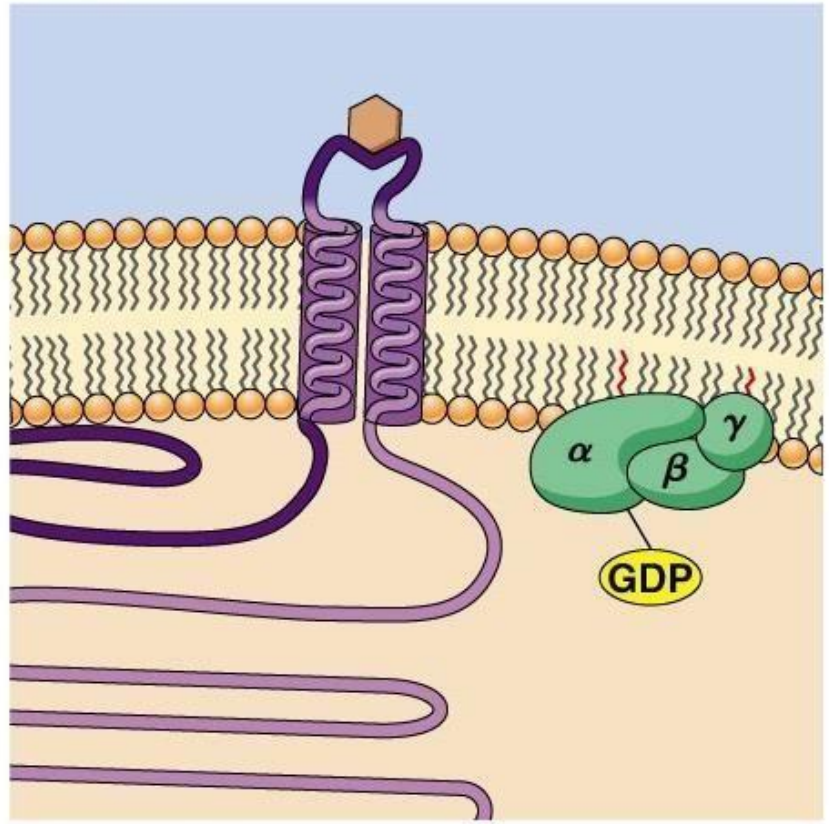
4 G protein subunits activate or inhibit target proteins, initiating signal transduction events.



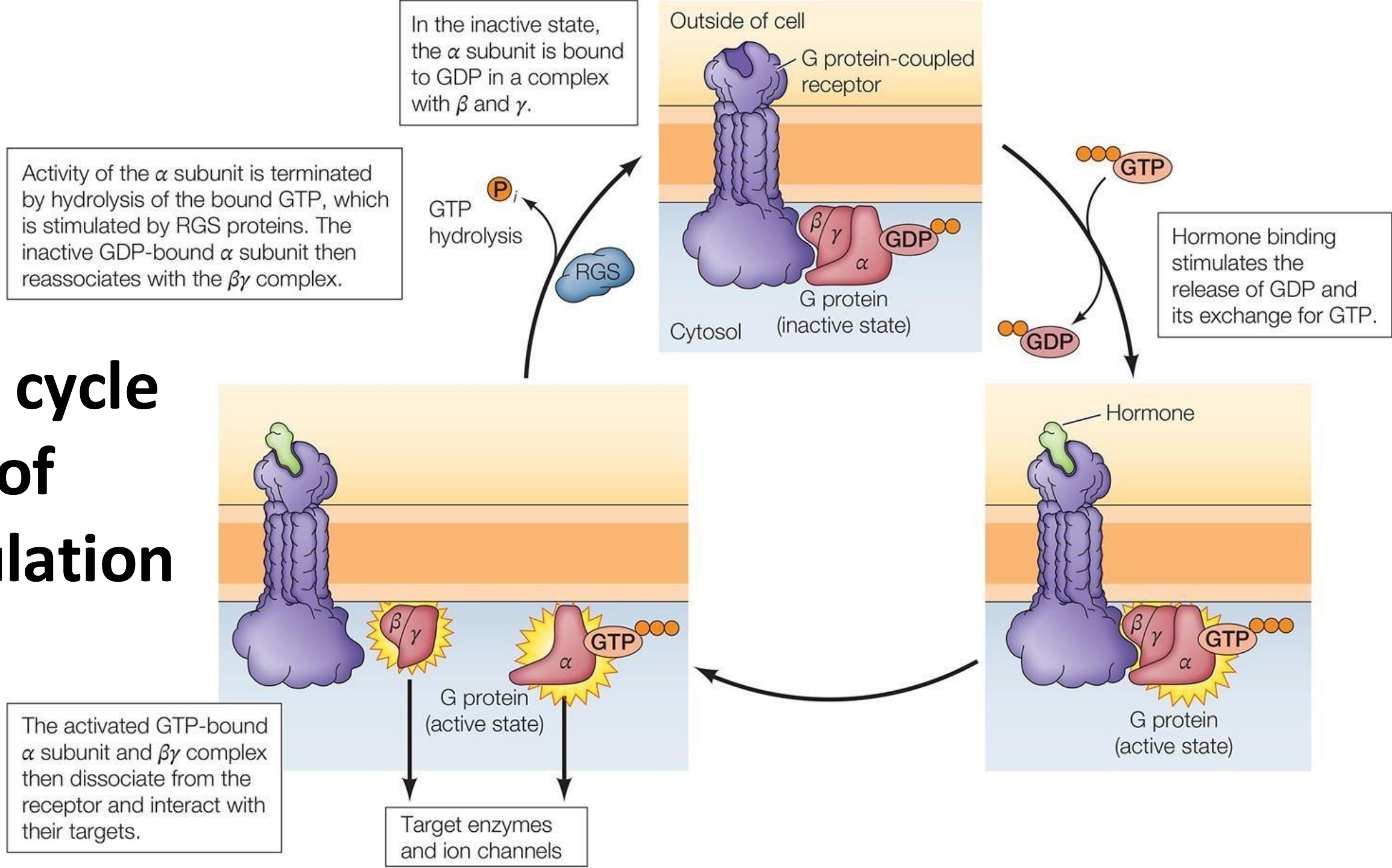
5 The G_α subunit hydrolyzes its bound GTP to GDP, becoming inactive.



6 Subunits recombine to form an inactive G protein.



The cycle of regulation



Secondary messengers

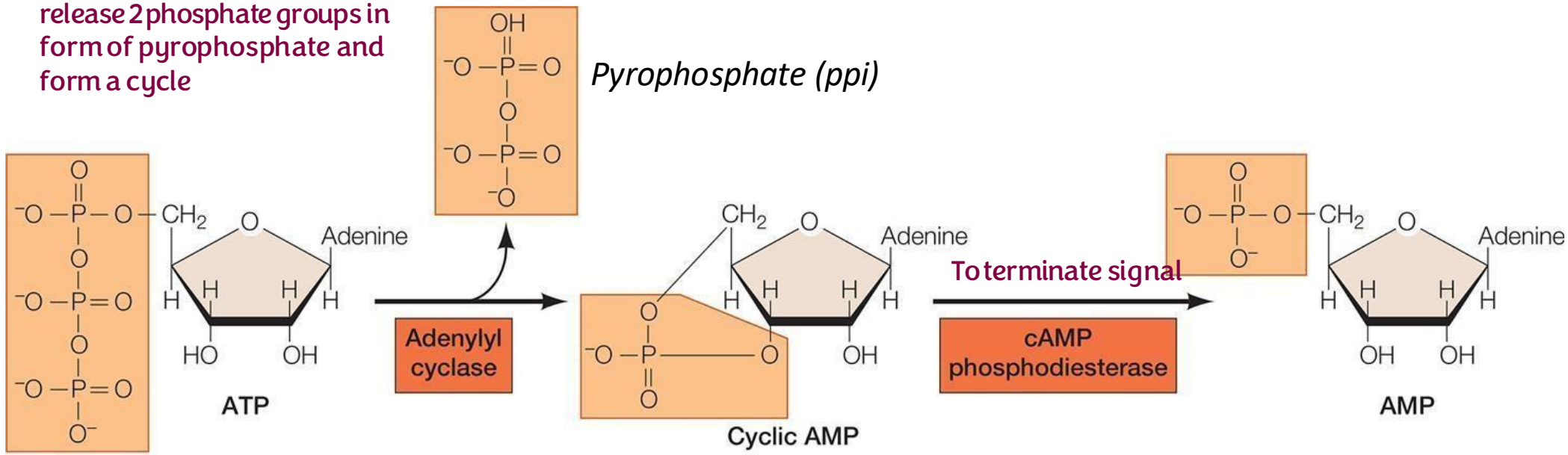
A compound whose metabolism is modified as a result of a ligand-receptor interaction; it functions as a signal transducer by regulating other intracellular processes. Usually small molecules

Note: The first messenger is the hormone itself

The primary messenger

Synthesis and degradation of cAMP

Adenylyl cyclase:
release 2 phosphate groups in form of pyrophosphate and form a cycle



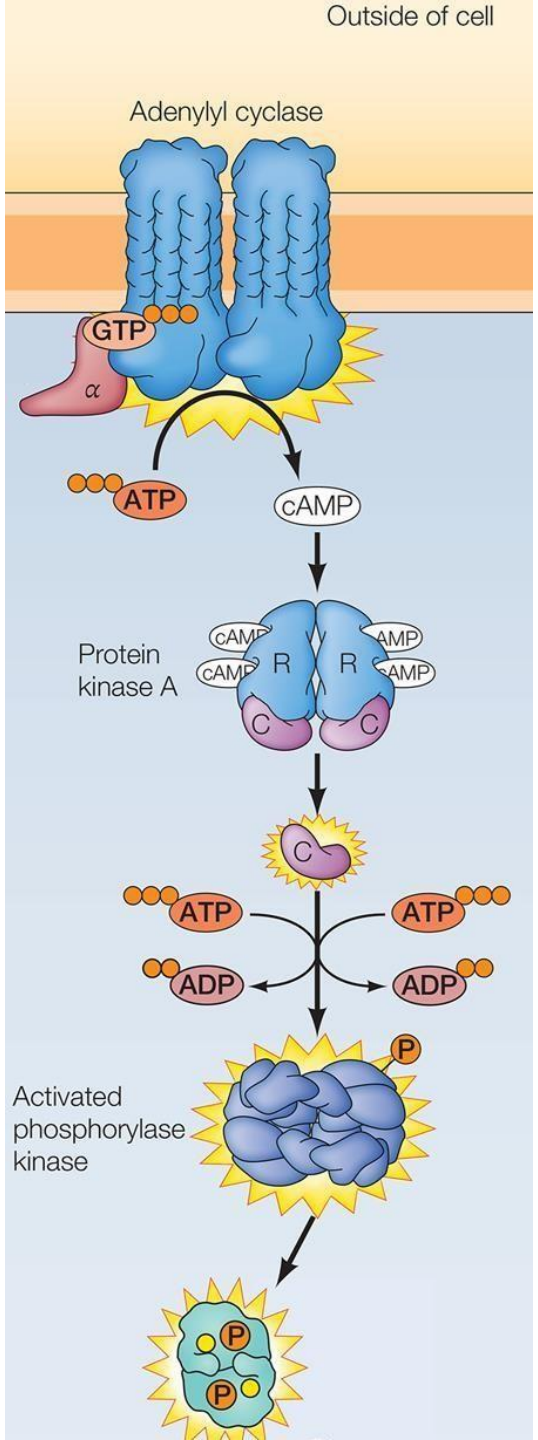
Carbon 3 and 5 linked to phosphate group and make cyclic structure

cAMP, Ca^{+2}, cGMP are examples of secondary messengers.

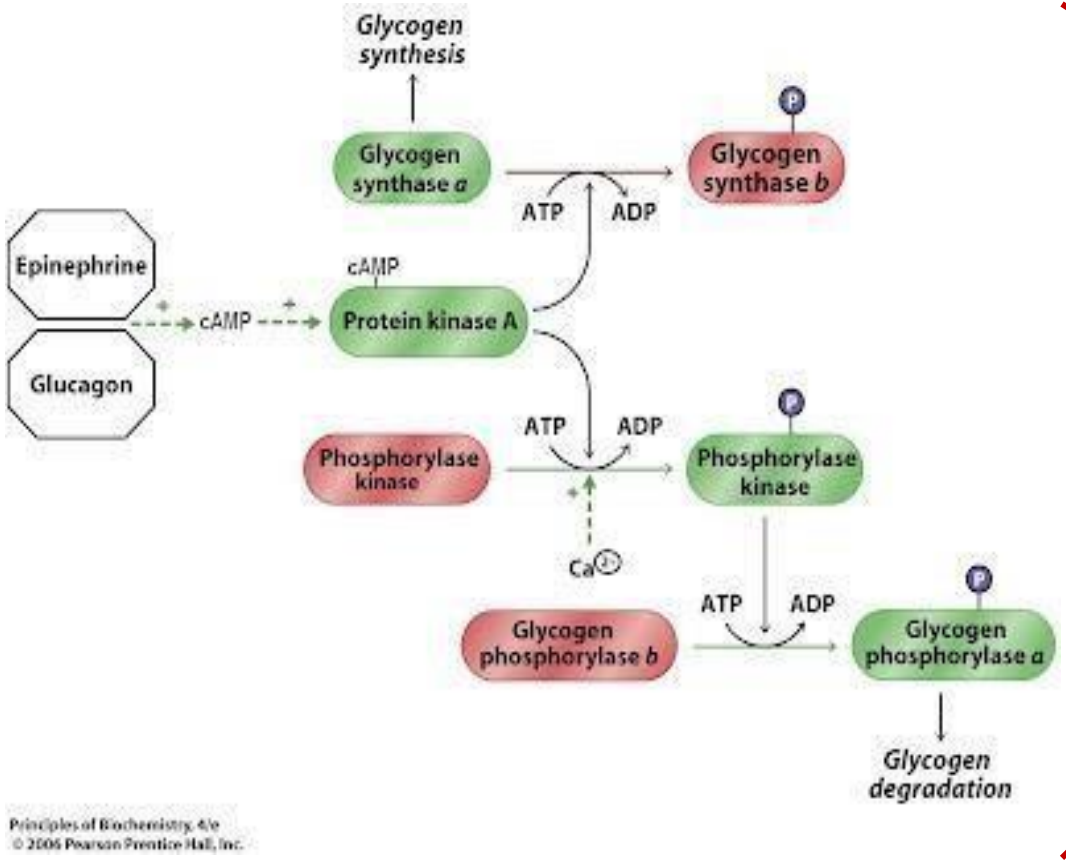
Regulation of protein kinase A by cAMP

- cAMP activates effector proteins (primarily enzymes) such as: 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 to phosphorylate other molecules, which may be effector molecules (other enzymes) themselves.
- Protein kinase A is a serine/threonine kinase that has many targets in numerous cells and tissues.

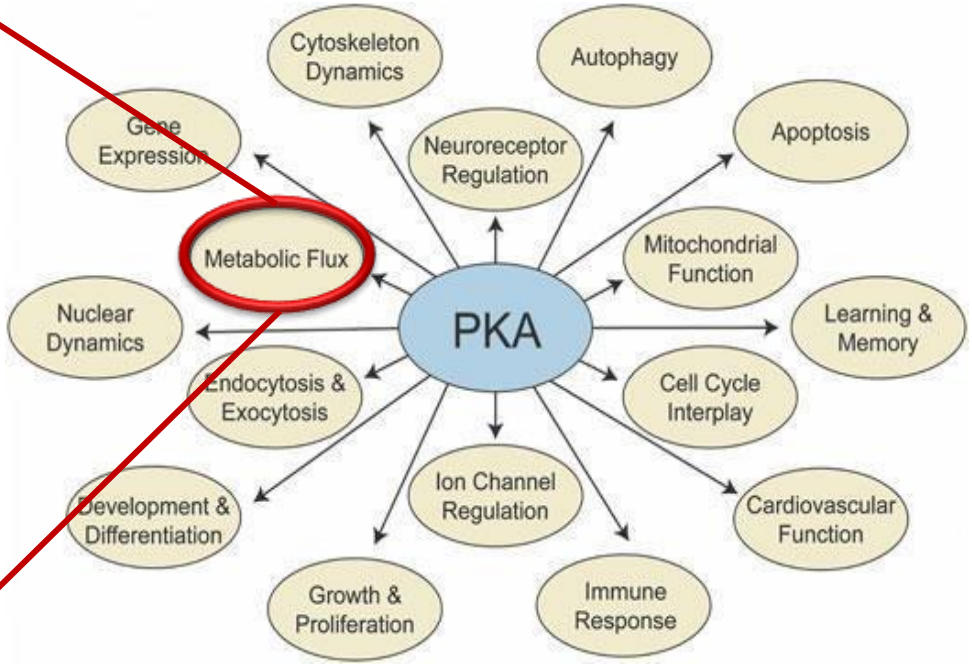
This whole process is called Signal transduction; as signal is sent from a molecule to another



The many targets of PKA



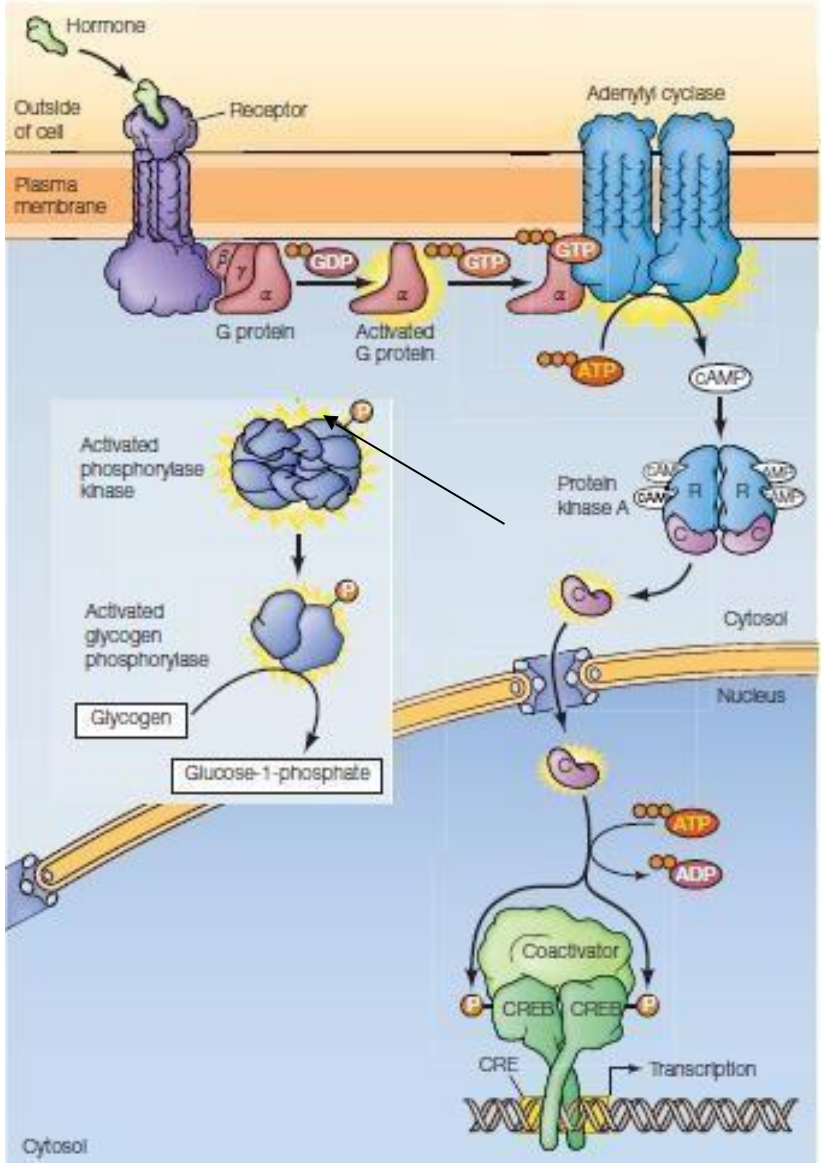
This is an example of PKA's effect on metabolic flux.



PKA has so many effects depend on the type of cell, making its effect on an organ different than its effect on another organ or pathway.
There's harmony between all these pathways.

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.

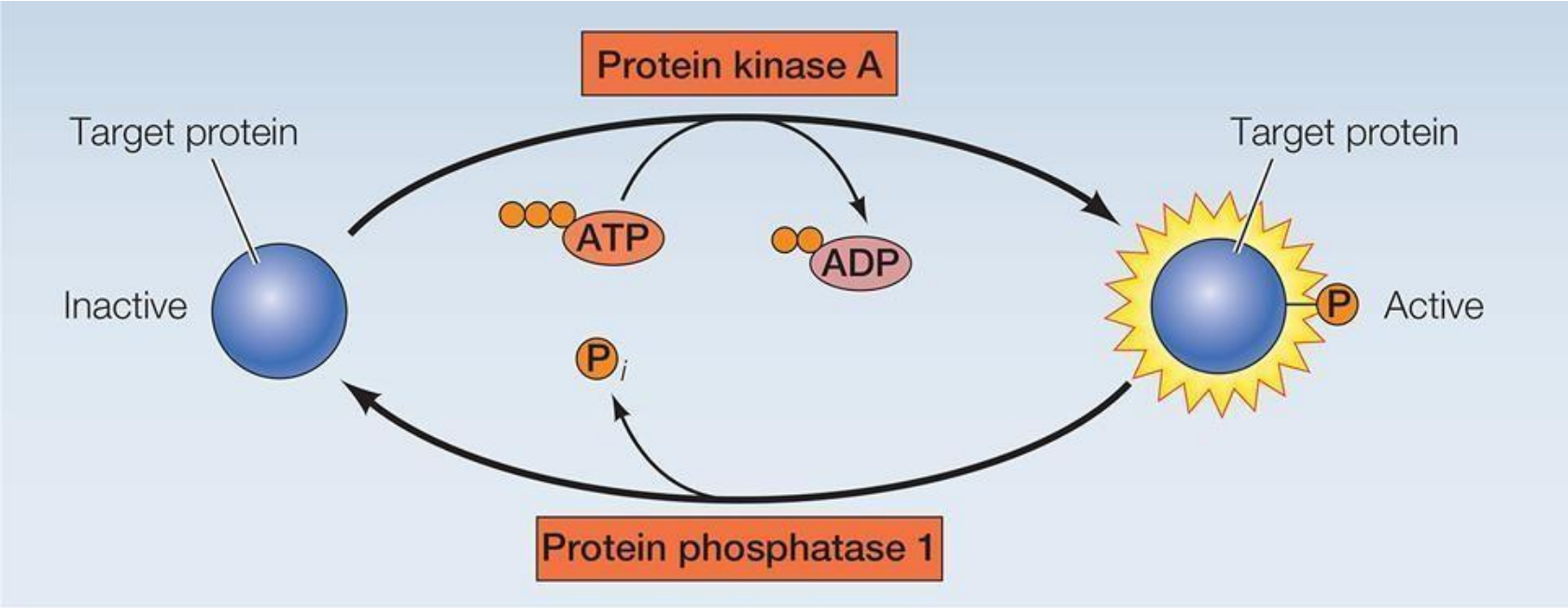


When catalytic subunit is released from regulatory subunit enter the nucleus and phosphorylates DNA binding proteins (transcription factors) transcription : production of mRNA
Affects gene activity and work on long term
So this enzymes in signal transduction could be quick response , quick effect or slow effect.

Regulation by dephosphorylation

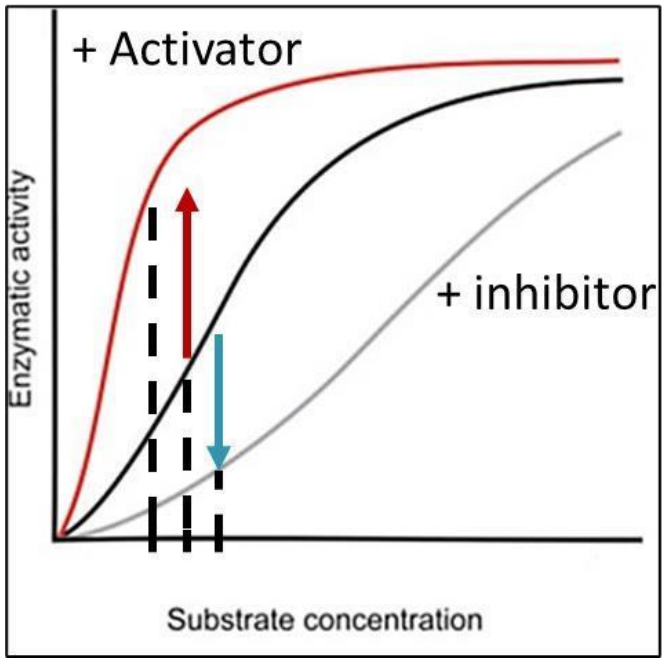
cell must terminate the signal as it doesn't want the response to stay forever

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

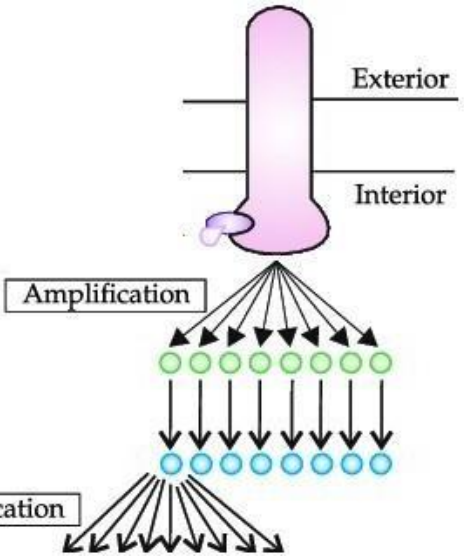


Why are effectors enzymes?

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

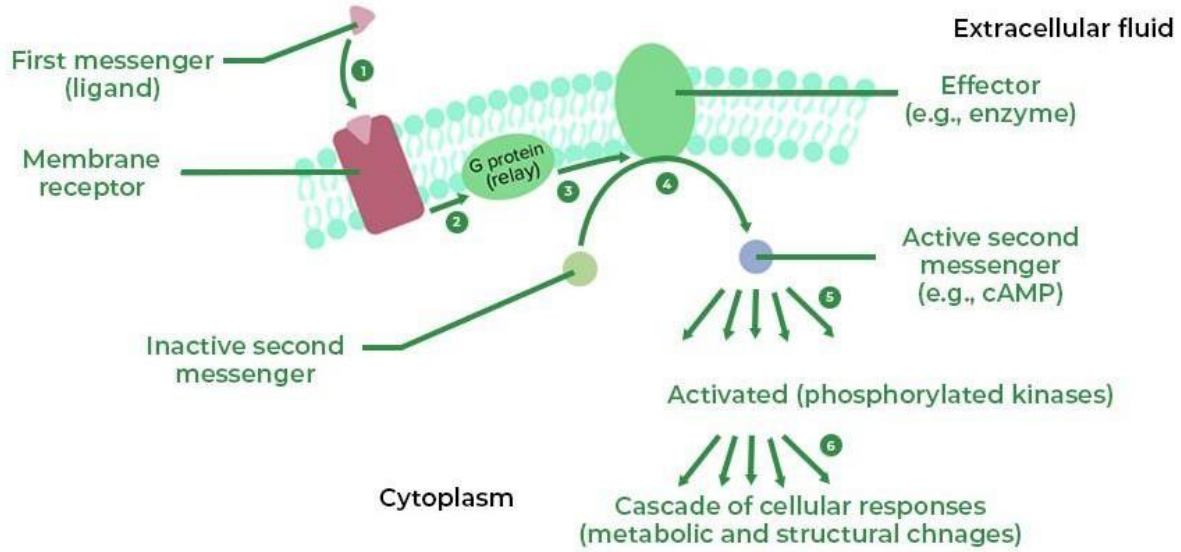


Allosteric enzyme, sigmoidal curve . If we put an activator we'll get a large increase in enzyme activity because it's allosteric .
Also it's the same thing when we put inhibitor the enzyme activity change in large amount



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)

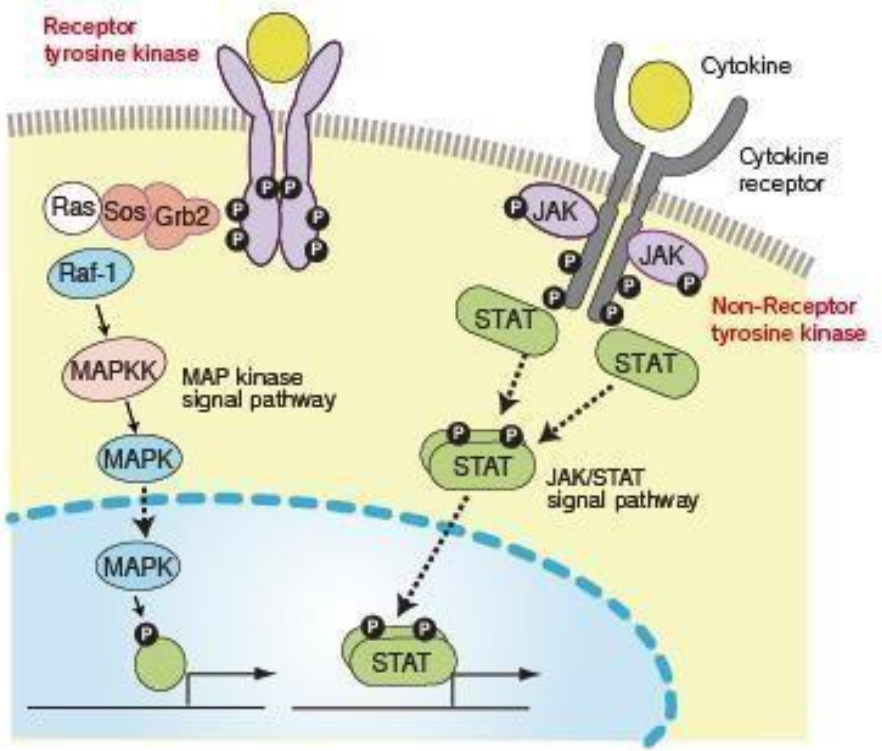
Some receptors either

- Receptor tyrosine kinases (have an intrinsic tyrosine kinase activity)
- A receptor tyrosine kinase is a type of receptor that also has intrinsic enzymatic activity ,it functions both as a receptor (binding a ligand) and as a kinase enzyme

OR

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

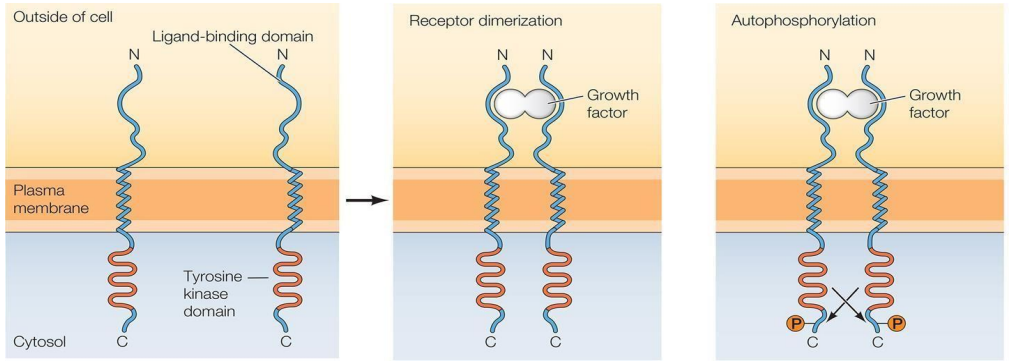
Binding of ligands extracellularly activates the kinase activity resulting in a phosphorylation cascade.



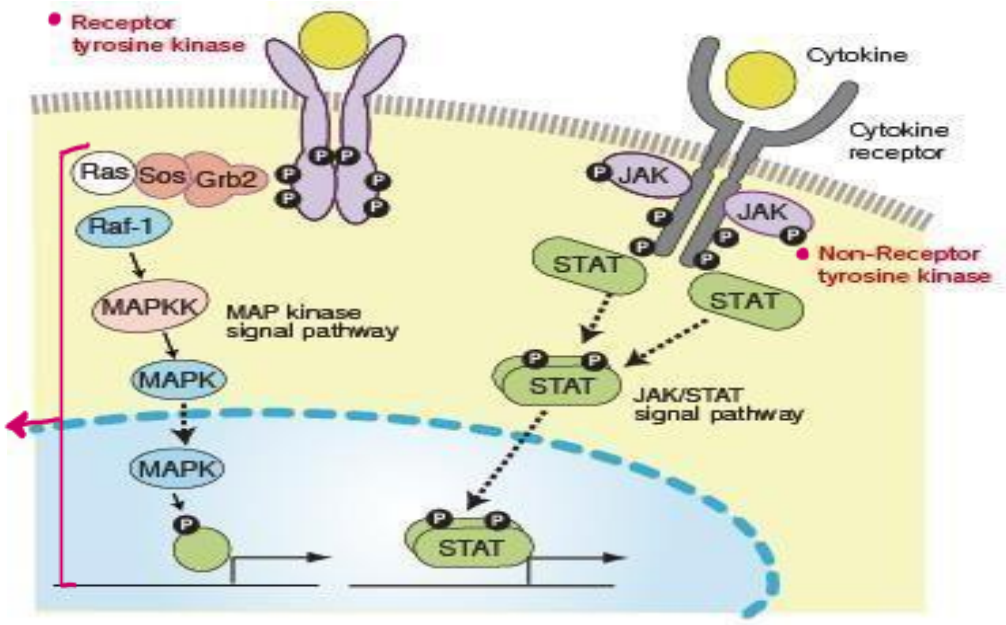
Kinase = Phospholorates

Receptor tyrosine kinases

When a ligand binds to the receptor, it causes two receptor monomers to come together (dimerize). This dimerization activates their tyrosine kinase domains, allowing each receptor to phosphorylate tyrosine residues on the other (a process called autophosphorylation).

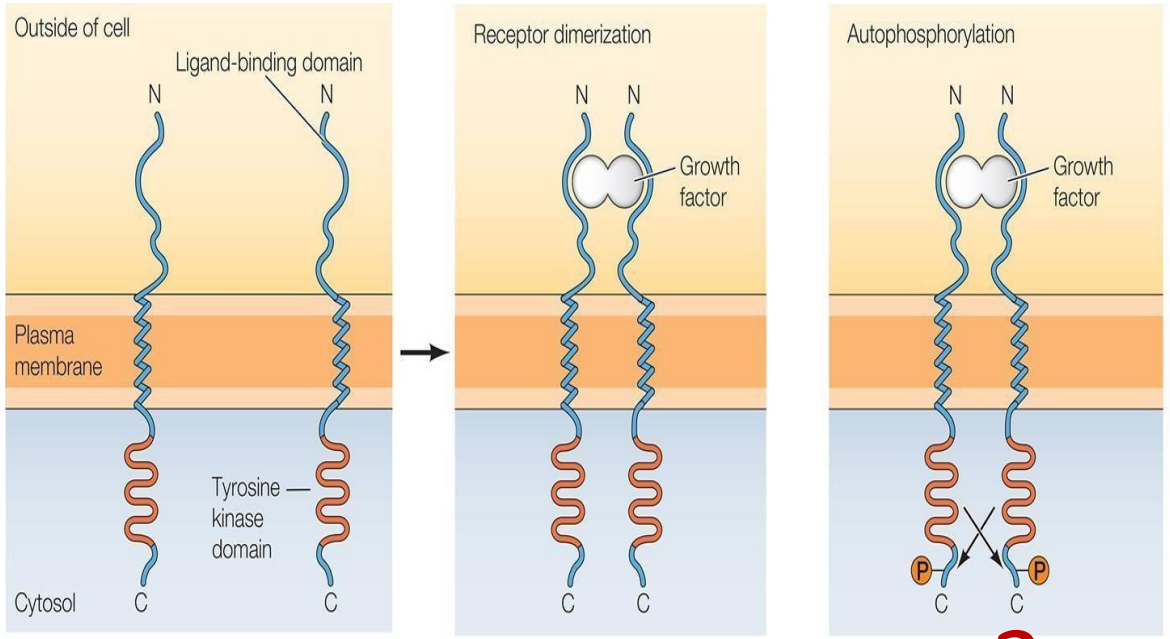


The phosphorylated tyrosines then serve as docking sites (binding sites)for intracellular signaling proteins, often called signal transducers.
These transducers include guanine nucleotide exchange factors (GEFs), such as SOS, which activate RAS.
Activated RAS then stimulates RAF-1 and other downstream kinases in a cascade that eventually reaches the final targets often transcription factors like STAT which regulate gene expression.



Mechanism of action of receptor tyrosine kinases

In non-receptor tyrosine kinase signaling, the receptor itself has no kinase activity.
The kinases are independent proteins already bound to the receptor.
When a ligand binds, it changes the receptor's structure, which activates the associated kinases.
These kinases phosphorylate each other, increasing their enzymatic activity, and the phosphorylated sites on the receptor become docking sites for other signaling proteins.



1

Each receptor consists of an extracellular ligand-binding domain, a single transmembrane α helix, and a cytosolic domain with tyrosine kinase activity. We have 2 of them

Growth factor binding induces receptor dimerization.

2

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

3

What is the effect of autophosphorylation?

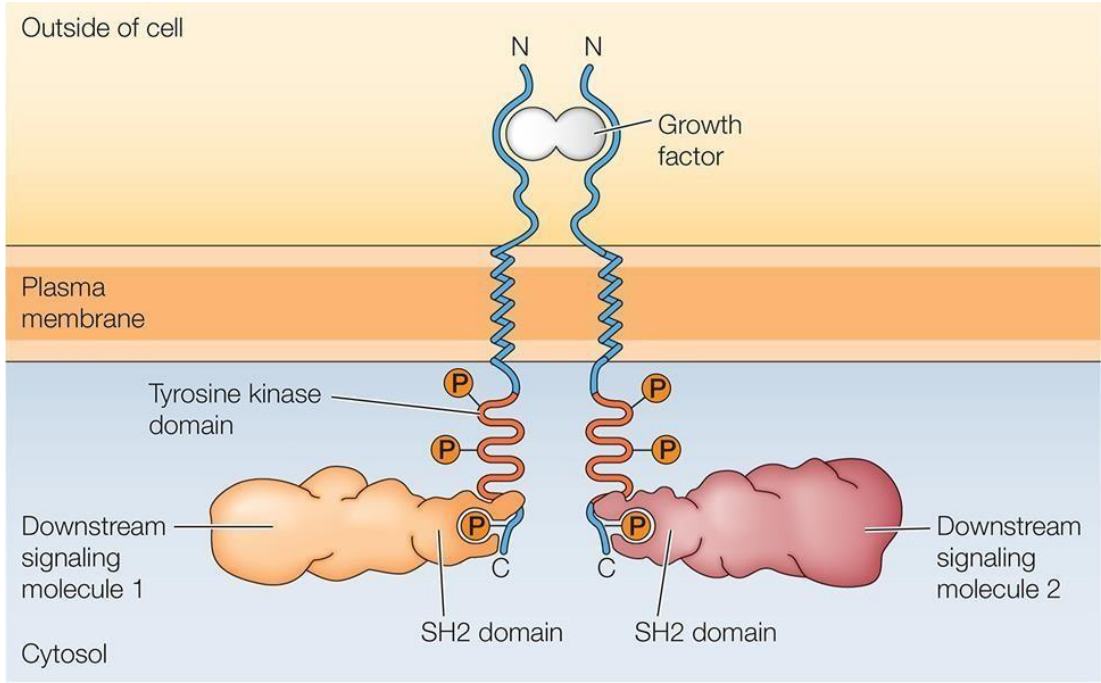
Autophosphorylation of the tyrosine residues has two effects.

- It increases the protein kinase activity.
- It creates specific binding sites "docking site" for additional proteins that transmit intracellular signals downstream of the activated receptors.

  Phosphate group has a negative charge, which facilitate electrostatic interactions with other proteins.

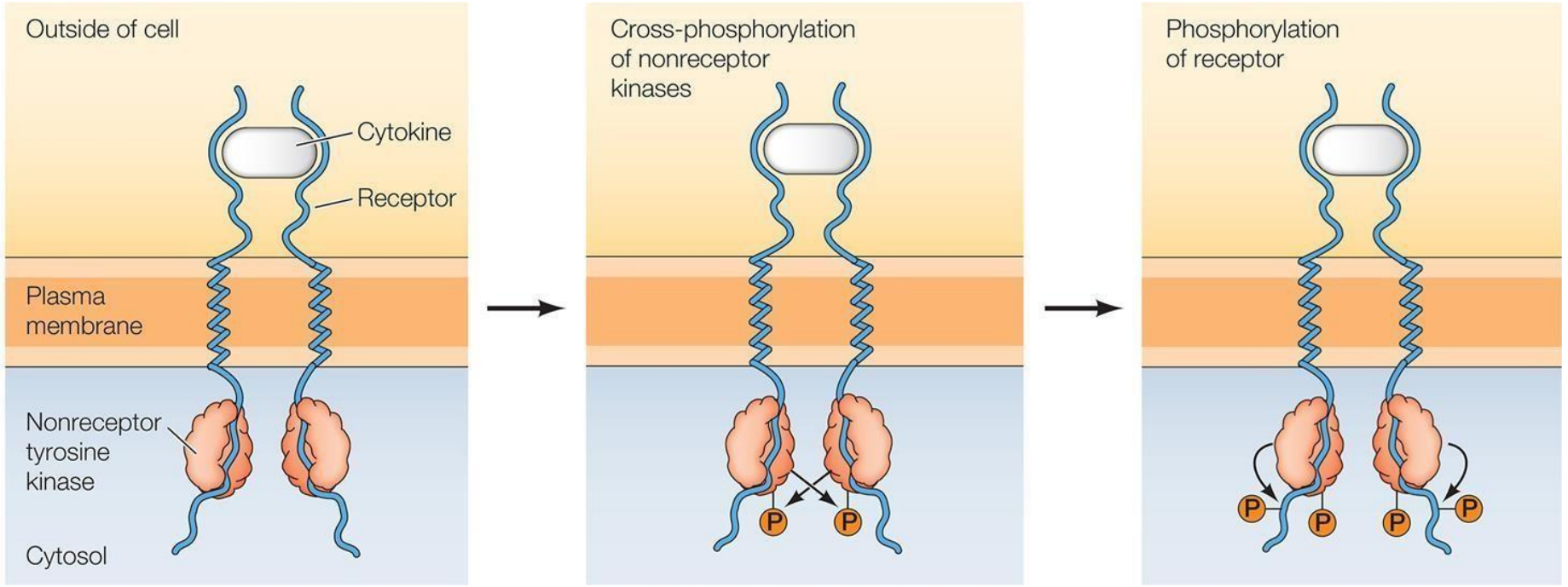
The consequence of protein association with activated receptor tyrosine kinases:

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.

Here, Kinases phosphorylate each other (cross-phosphorylation) first, then they phosphorylate the receptors.

Additional Resources:

رسالة من الفريق العلمي:

قال الرسول صلى الله عليه وسلم :
يا شدَّادُ بنُ أوسٍ ، إذا رأيتَ النَّاسَ قد اكتنزوا الذَّهبَ والفضَّةَ؛ فاكنِز هؤلاء الكلماتِ : اللَّهمَّ إنِّي أسألُك الثَّباتَ في الأمرِ ، والعزيمةَ على الرُّشدِ ، وأسألُك موجِباتِ رحمتِك ، وعزائمَ مغفرتِك ، وأسألُك شُكرَ نعمتِك، وحُسنَ عبادتِك، وأسألُك قلبًا سليمًا، ولسانًا صادقًا، وأسألُك من خيرِ ما تعلَمُ، وأعوذُ بك من شرِّ ما تعلَمُ، وأستغفرُك لما تعلَمُ؛ إنَّك أنت علَّامُ الغيوبِ

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			