CYTOLOGY

بسم الله الرحمن الرحيم

MID – Lecture 12

Cell Signaling (Pt.1)

﴿ وَإِن تَتَوَلَّوْا يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوا أَمْثَالَكُم ﴾

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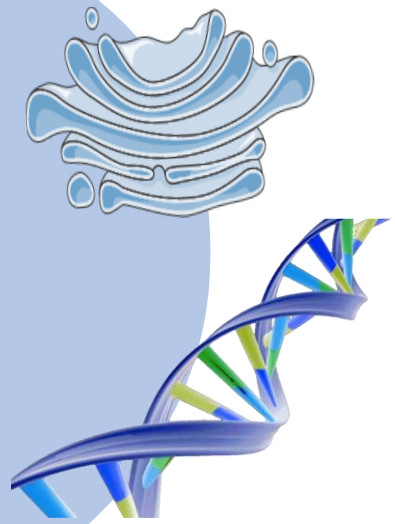


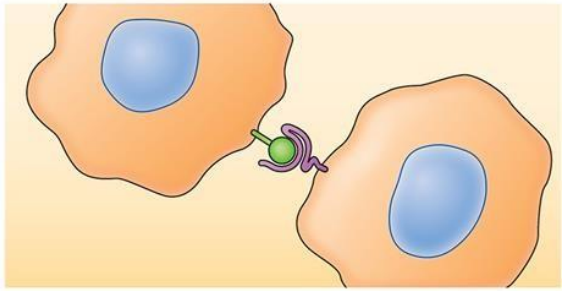
Short Quiz regarding last lecture:

CLICK HERE

Modes of cell signaling

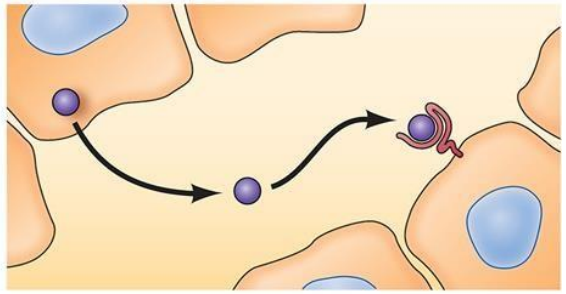
We well study secreted molecule signaling

(A) Direct cell–cell signaling



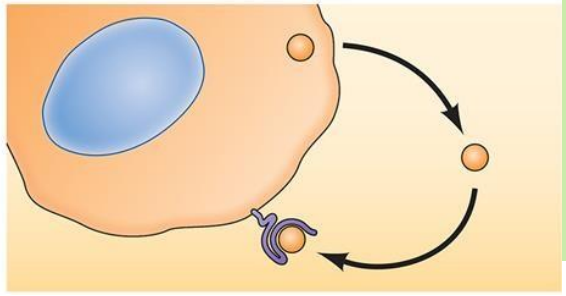
Cell-cell interaction
Direct interaction of a cell with its neighbor

Paracrine signaling



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

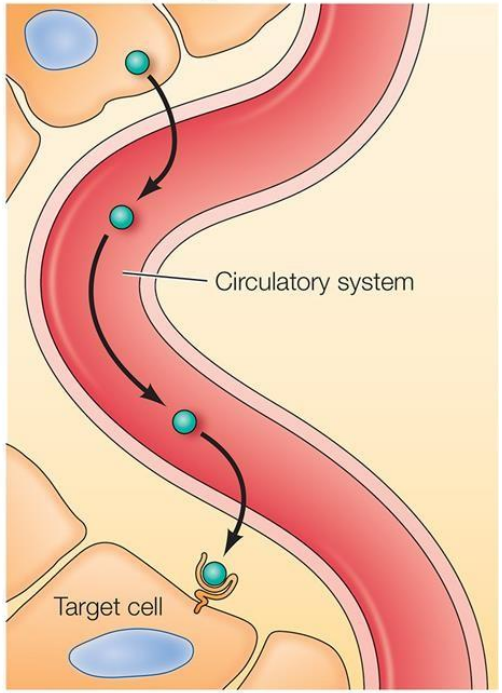
Autocrine signaling



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 circulation to act on target cells at distant body sites.

Blood

Classification of signaling molecules

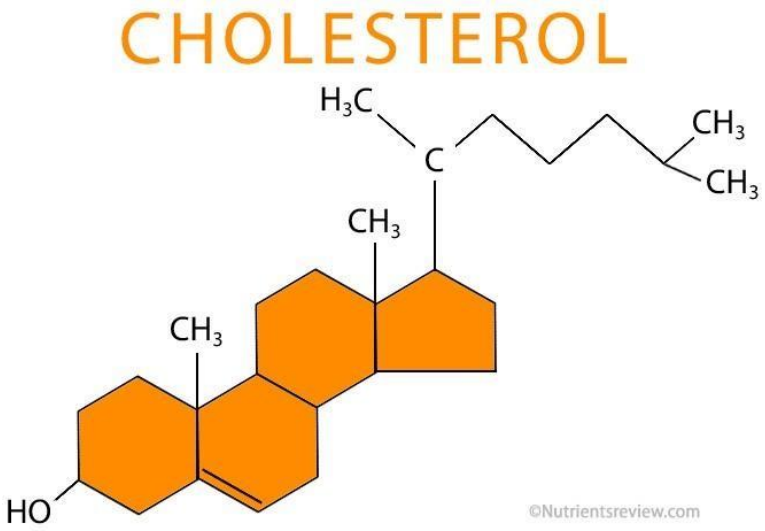
- Peptides: growth factors (EGF), peptide hormones (insulin, glucagon), or neuropeptides (oxytocin, enkephalins) Could be Large (Polypeptide or Protein)
- Small molecule neurotransmitters: derived from amino acids like Epinephrine and thyroid hormone (tyrosine), serotonin (tryptophan).
- Steroids: derived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

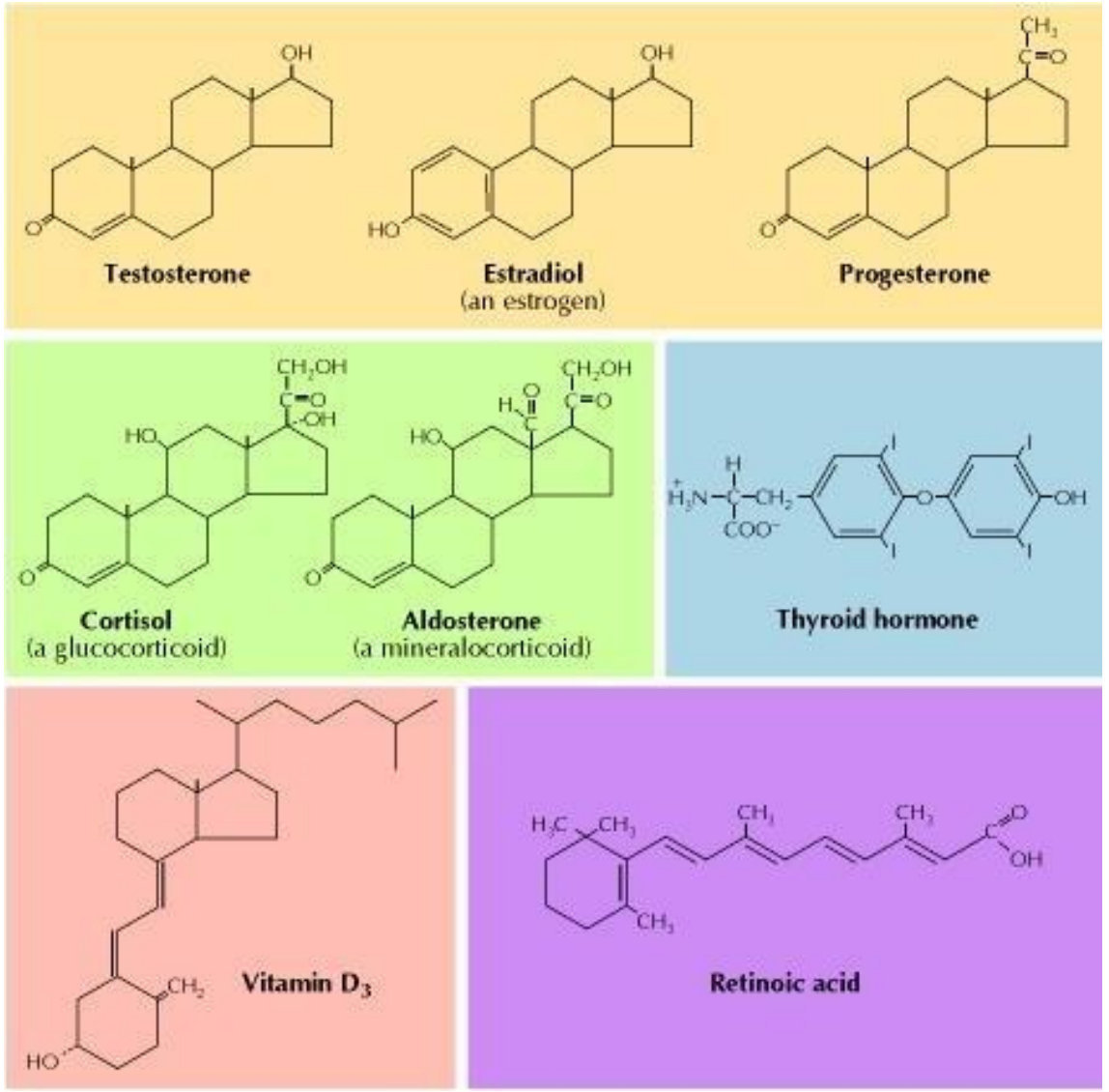
We will take Steroids and small molecule NT signal transduction.

Lipophilic hormones

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

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





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

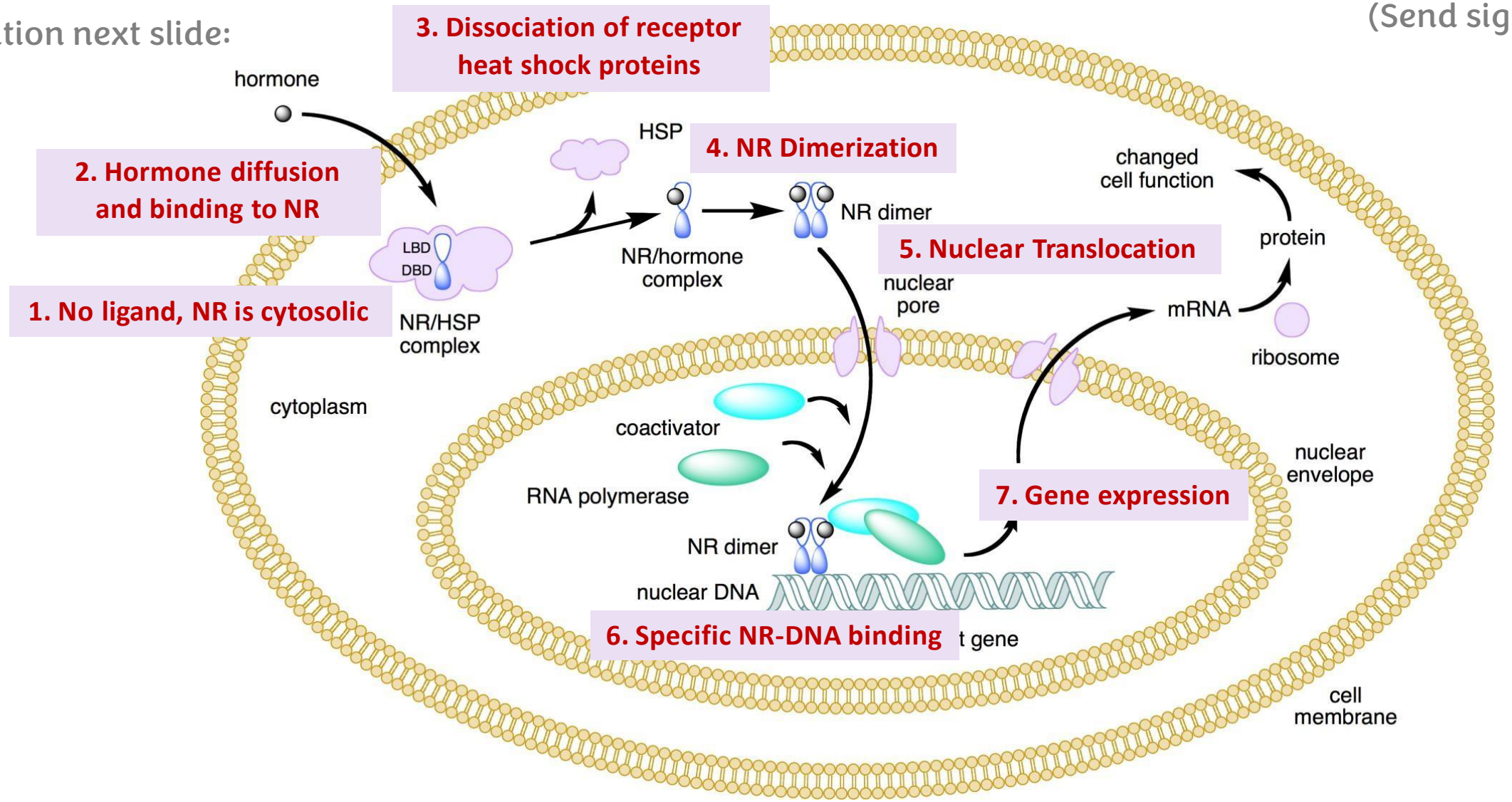
Don't memorize structures, you will come to it in next semester.

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 through the membrane, without needing a channel / carrier.

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

Once hormone binds to receptor:

1. HSP is released.
2. Receptor dimerizes.
3. Receptor gets translocated to Nucleus.
4. Receptor binds to DNA (in a specific place) and controls gene expression.
5. It changes cell behaviours.

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

Summary:

1. Steroid diffuses
2. Binds to (HSP bound) receptor
3. HSP is released
4. Receptor dimerizes
5. Receptor gets translocated to Nucleus
6. it bind to DNA
7. control gene activity and expression
8. Affect is achieved

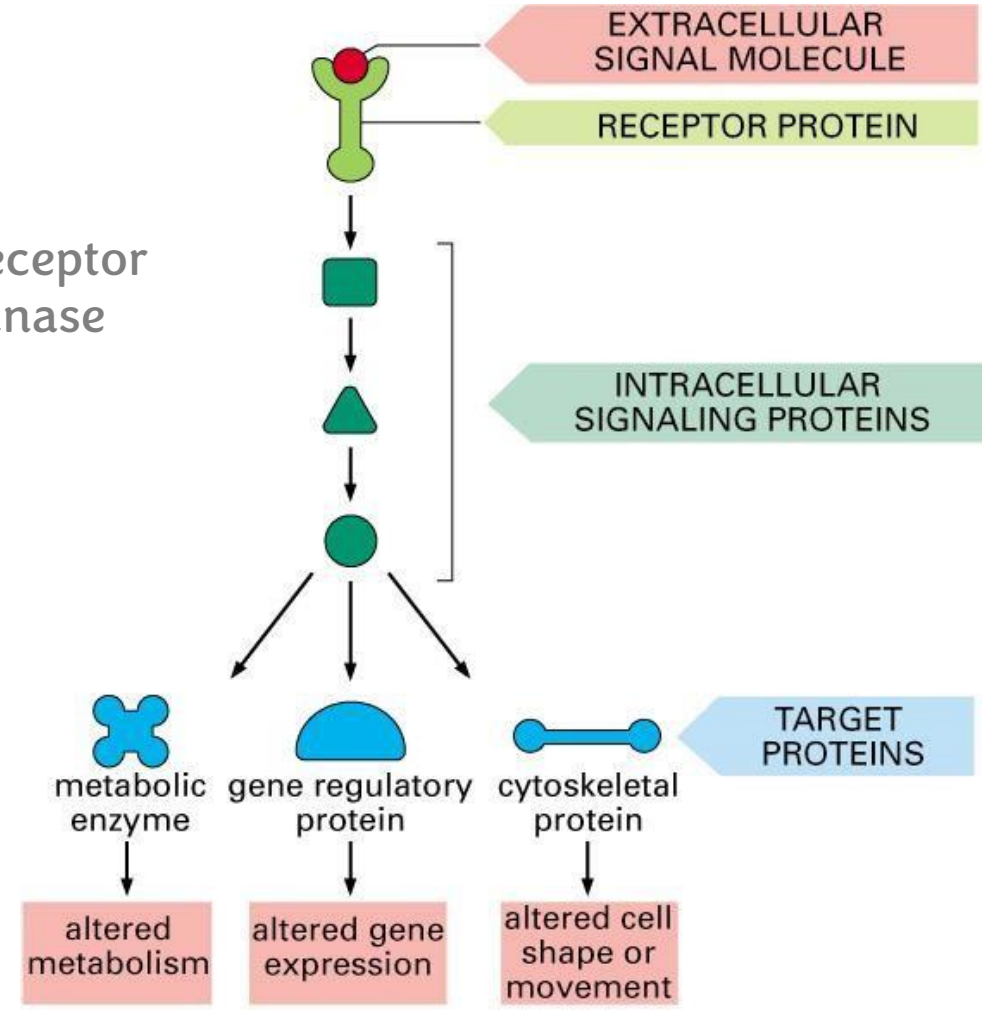
Cell surface receptors

Signal transduction

For ligands that cannot diffuse through membrane

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

- Ligand (hormone, growth factor, Cytokine)
- 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)

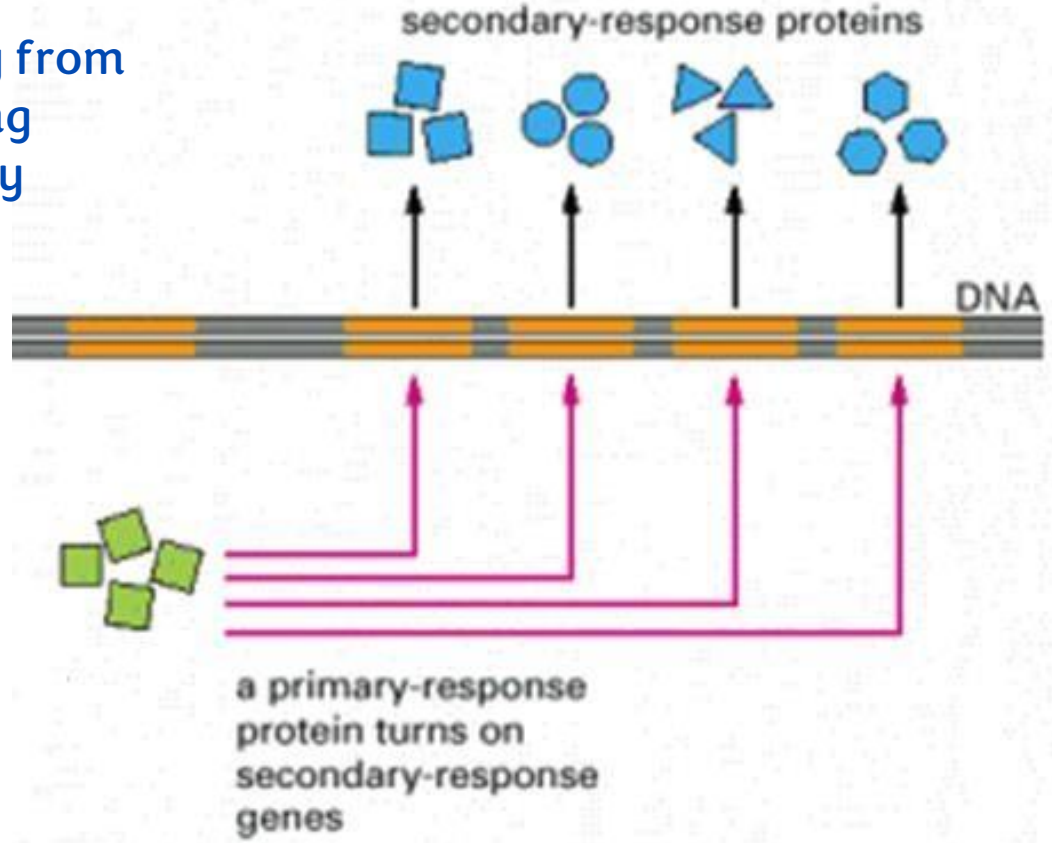


- Transducers: just sends a signal
- Effectors molecules: are enzymes (mainly) which can act on other pathways
- Secondary messengers are present in some pathways

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 may not be what the cell wants, but 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

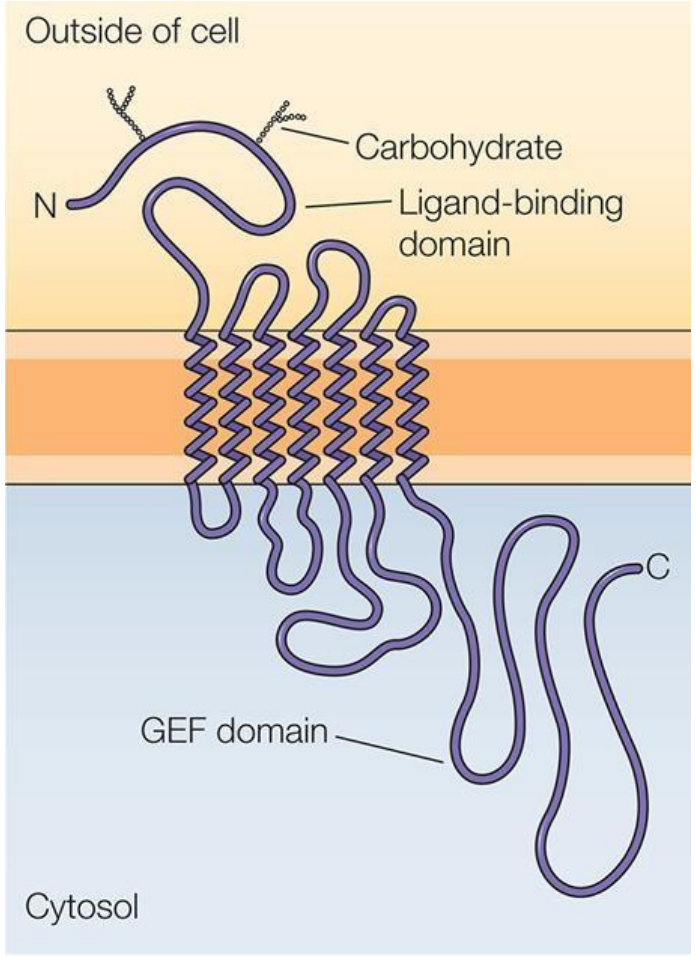
Outlines:

- 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)

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).

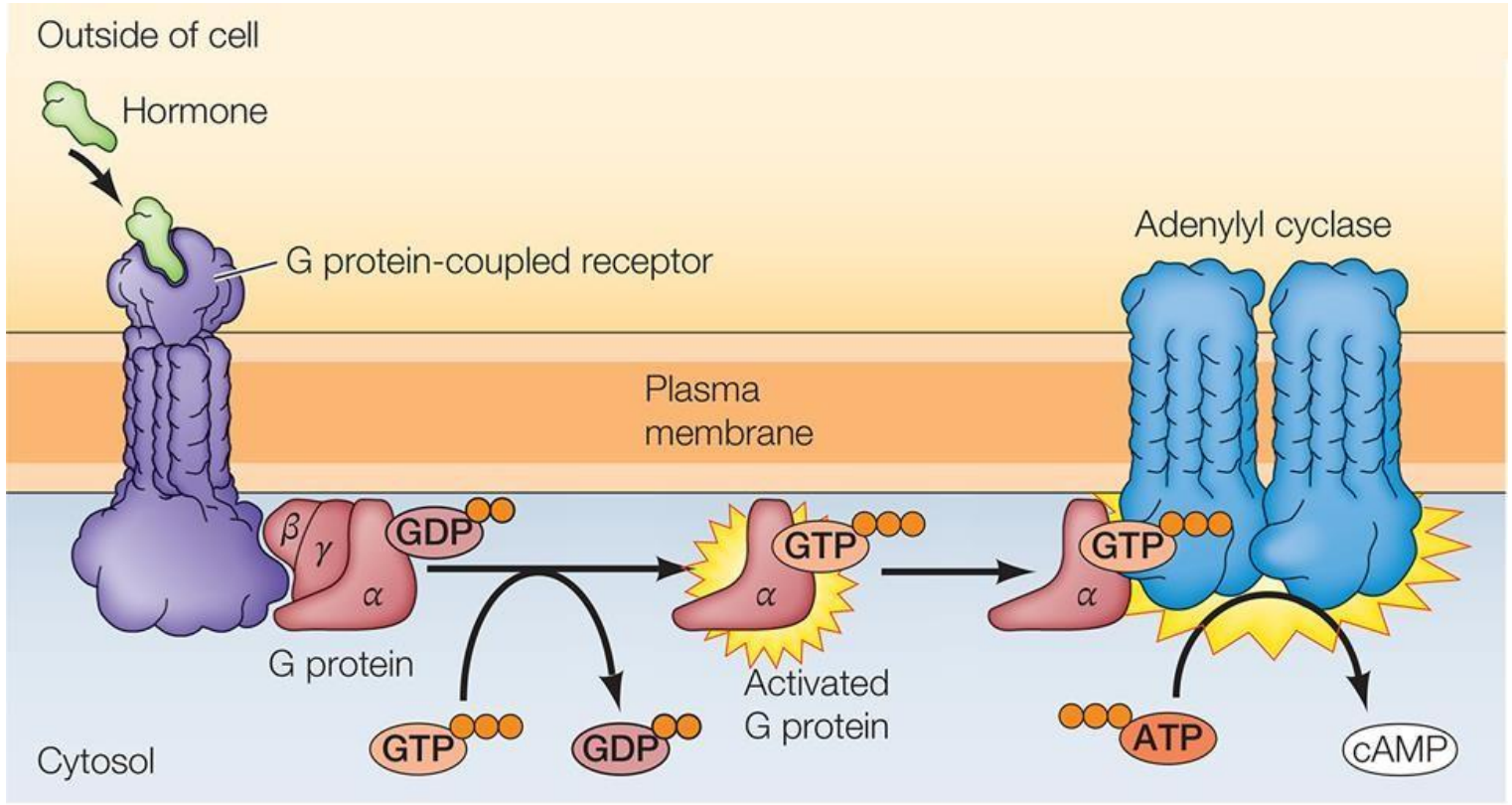
- G proteins are composed of three protein subunits—α, β, and γ.
- In the unstimulated state, the α subunit has GDP bound and the G protein is inactive.
- When stimulated, the α subunit releases its bound GDP, allowing GTP to bind in its place.
- This causes the trimer to dissociate into an α subunit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

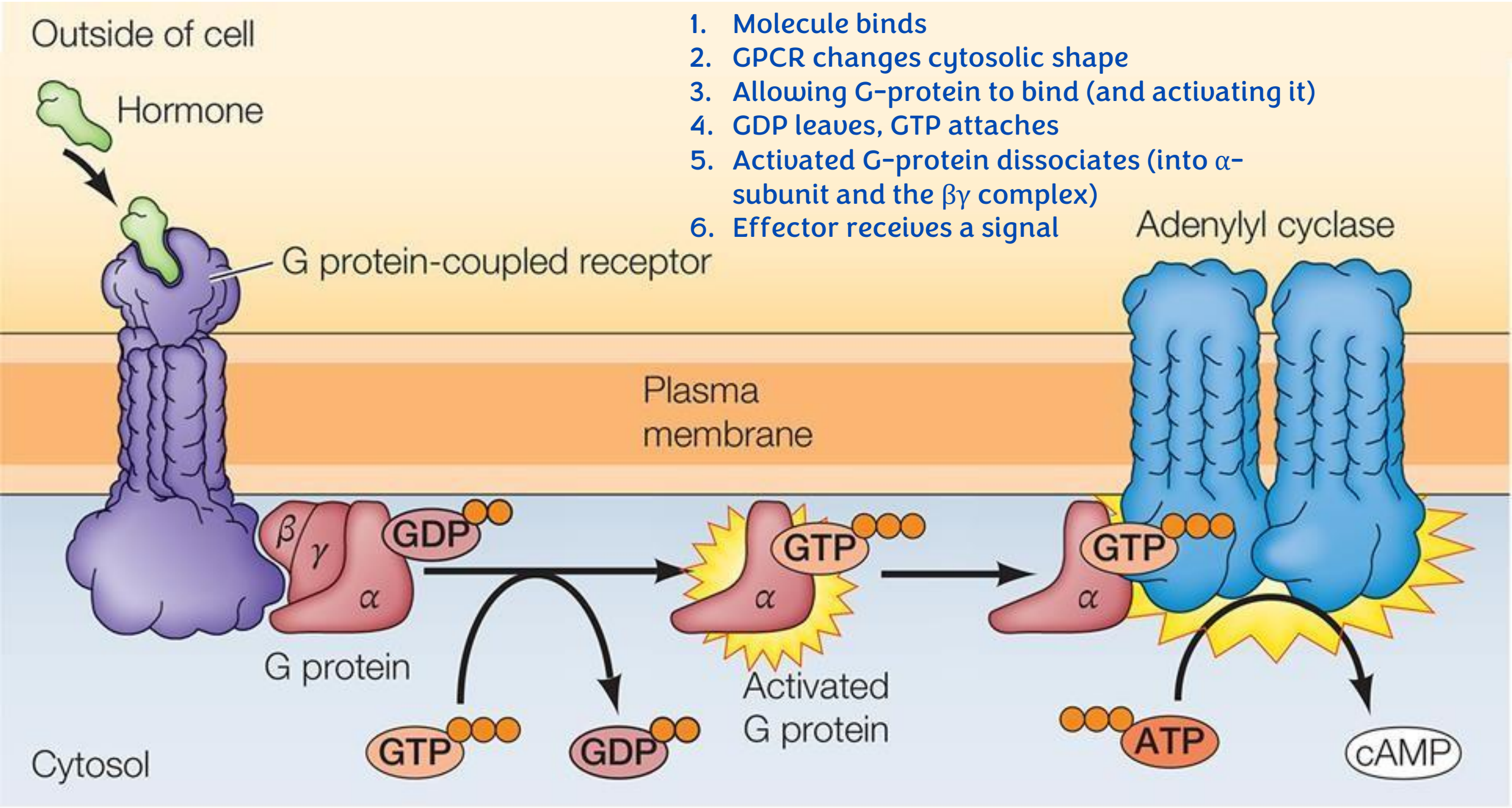
Before binding of ligand

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

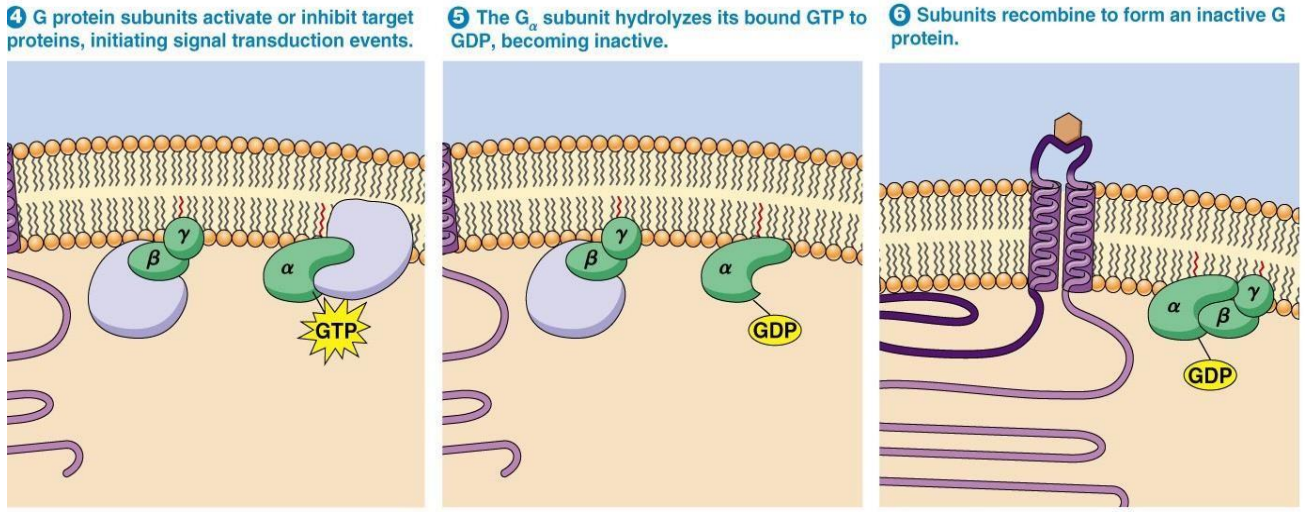
G protein inactivation

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.

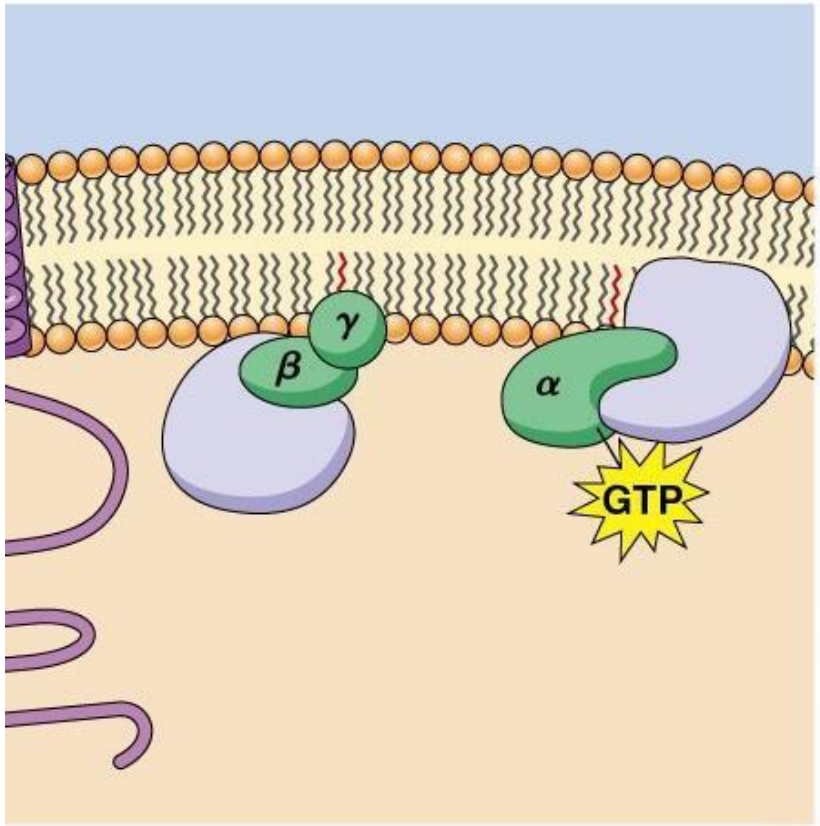
Decreasing length of activity

Or Activators:

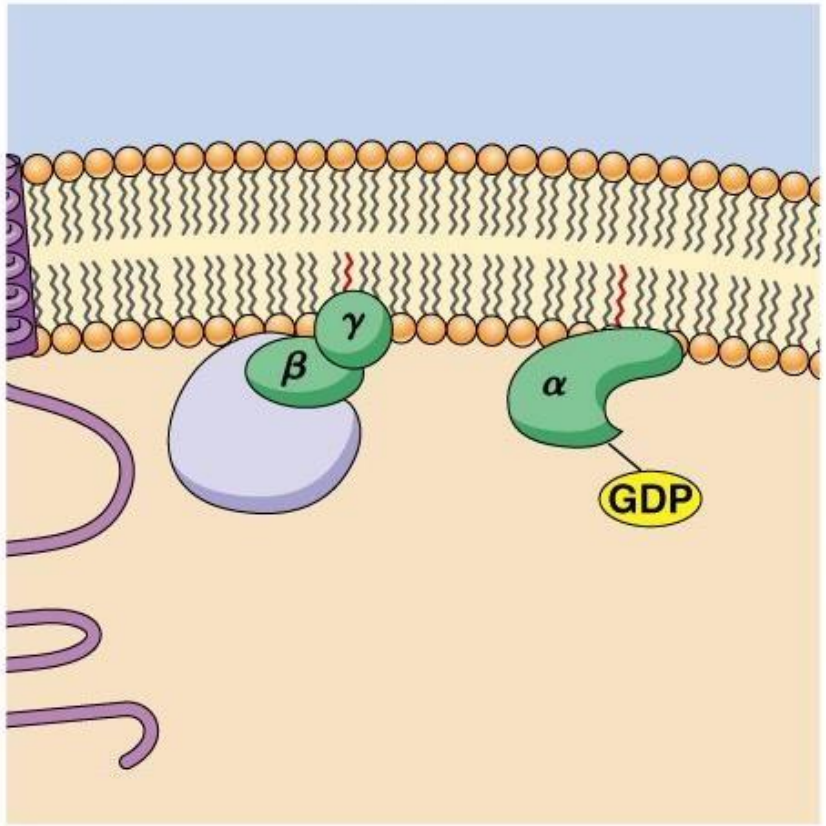
There are also regulators that regulate dissociation of GDP and binding of GTP on G proteins

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

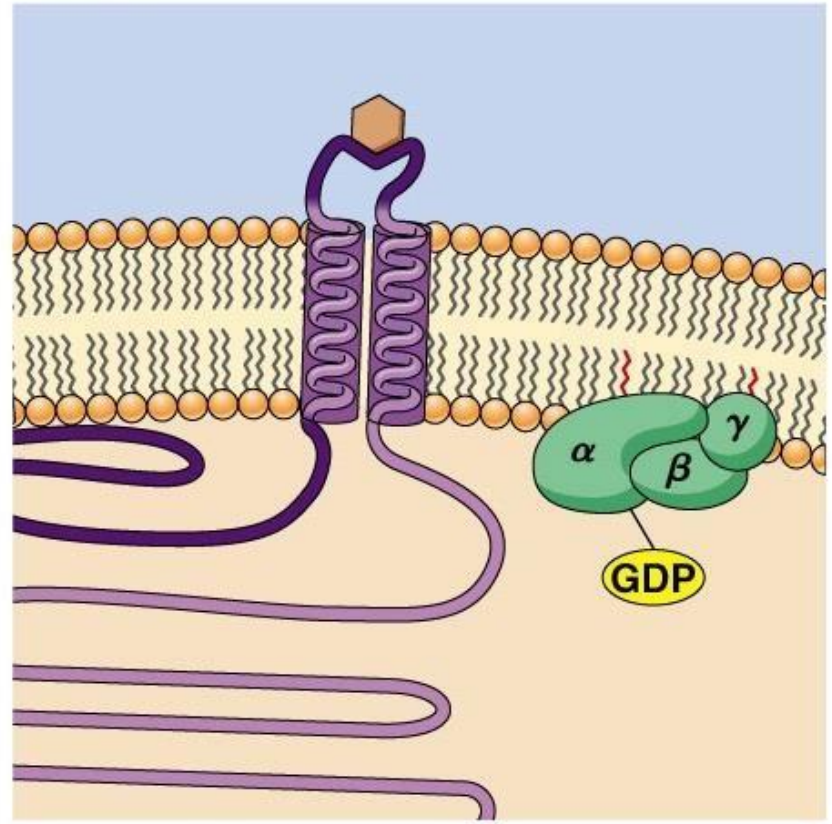
4 G protein subunits activate or inhibit target proteins, initiating signal transduction events.



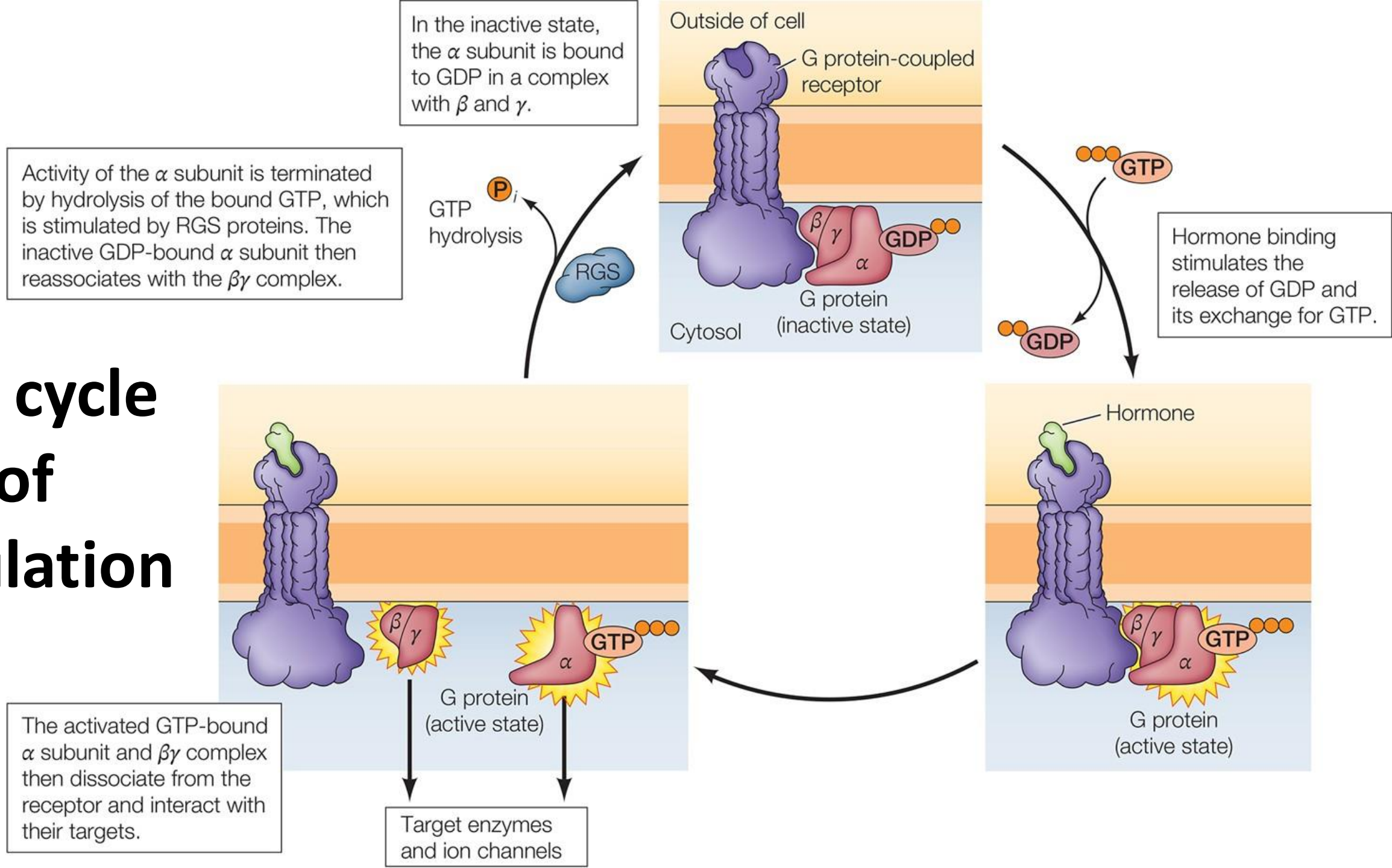
5 The G_α subunit hydrolyzes its bound GTP to GDP, becoming inactive.



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

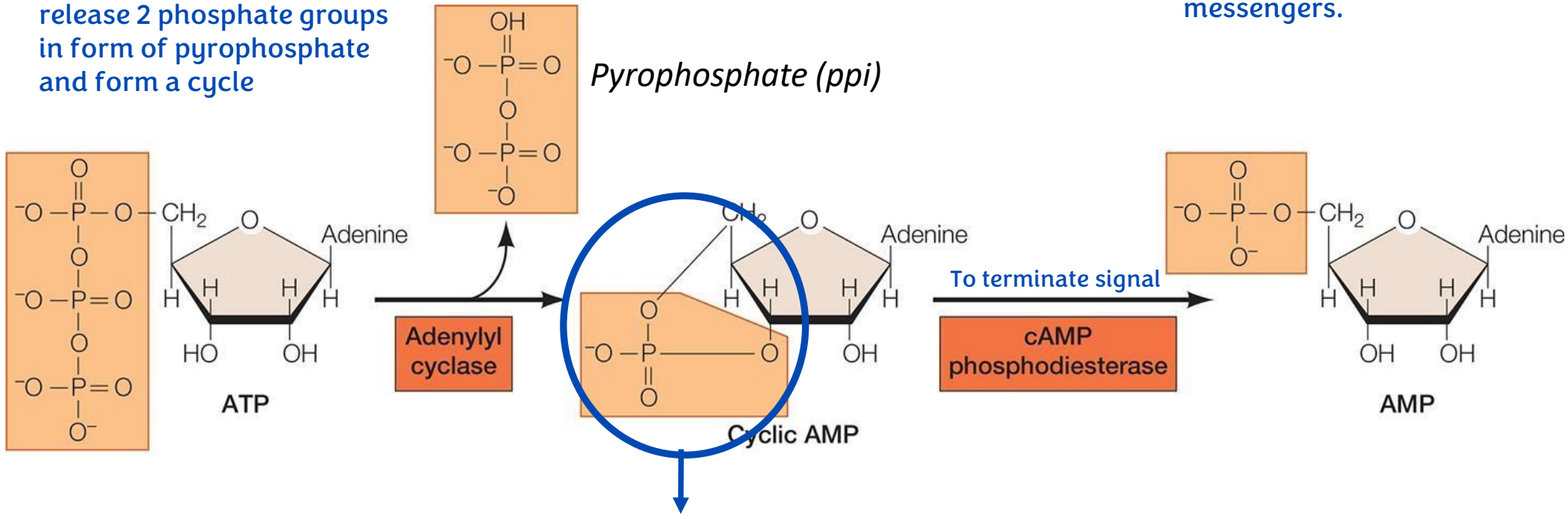
Note: The first messenger is the hormone itself

The primary messenger

Synthesis and degradation of cAMP

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

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

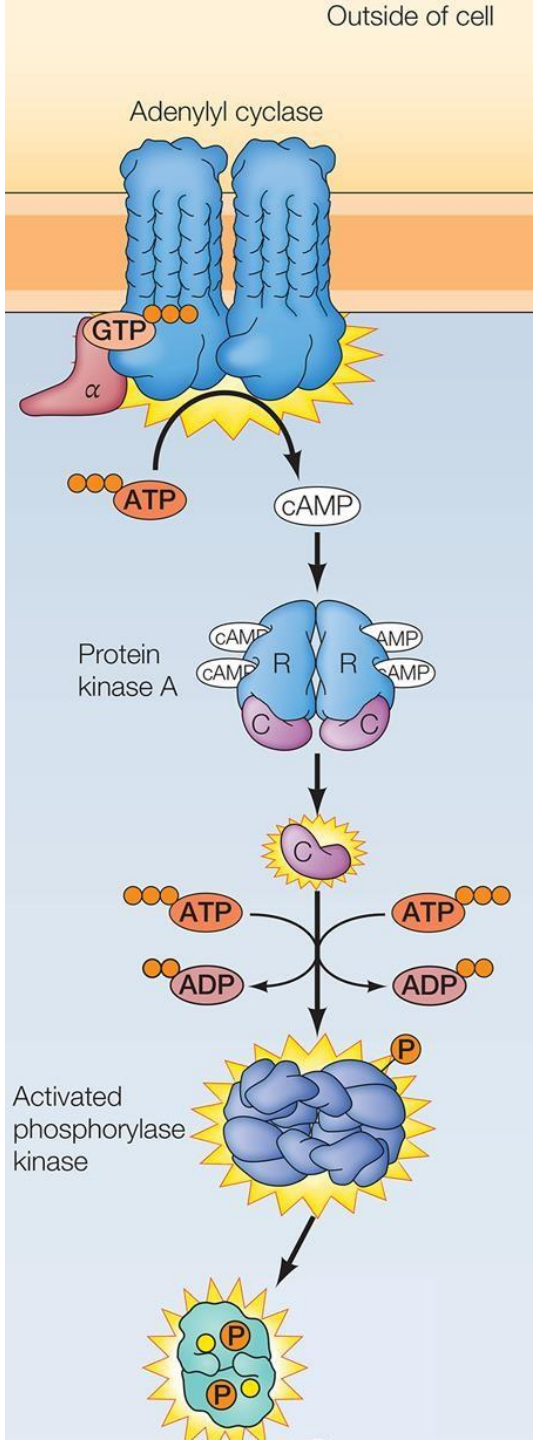


The cycle; the reason of "cyclic" in naming

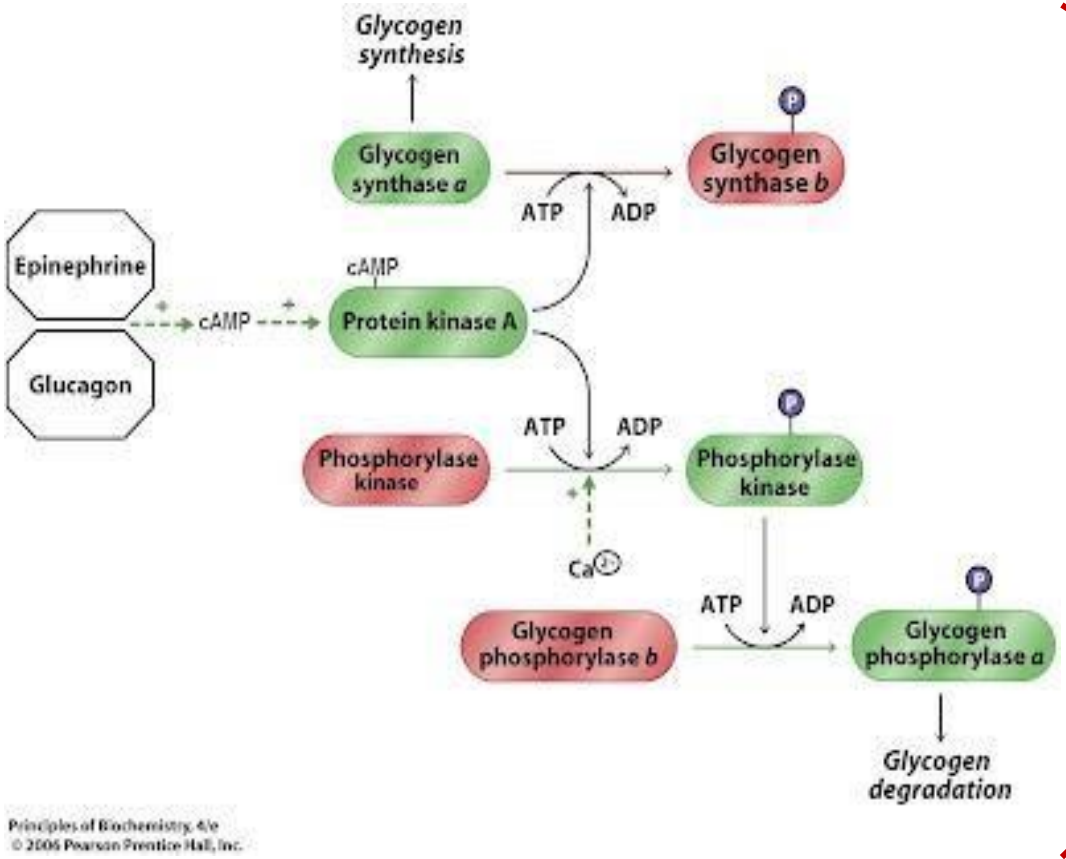
Regulation of protein kinase A by cAMP

- 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.

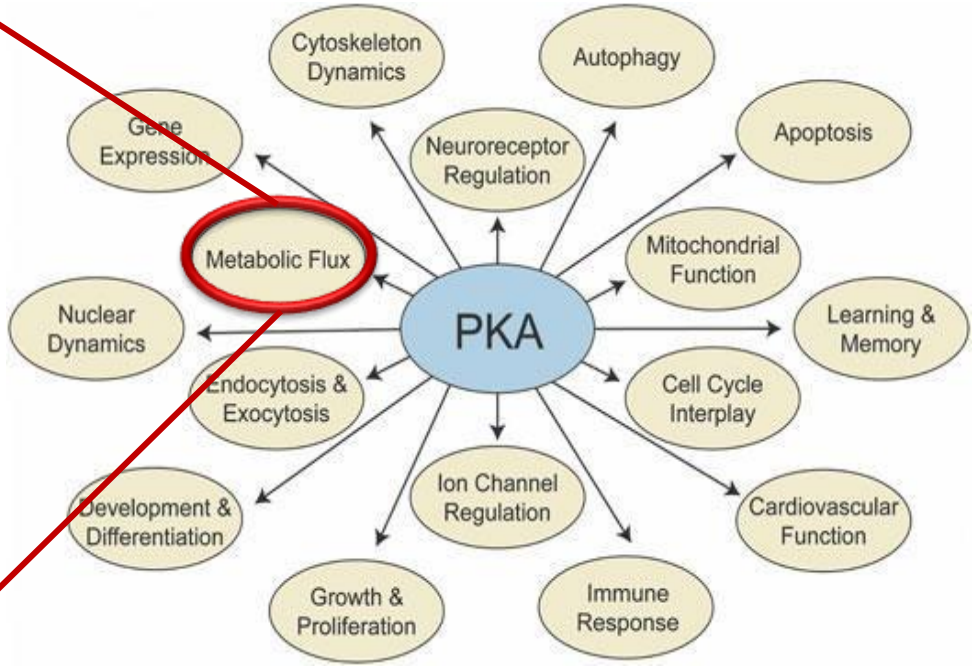
This whole process is called Signal transduction; as signal is sent from a molecule to another



The many targets of PKA (Protein Kinase A)



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



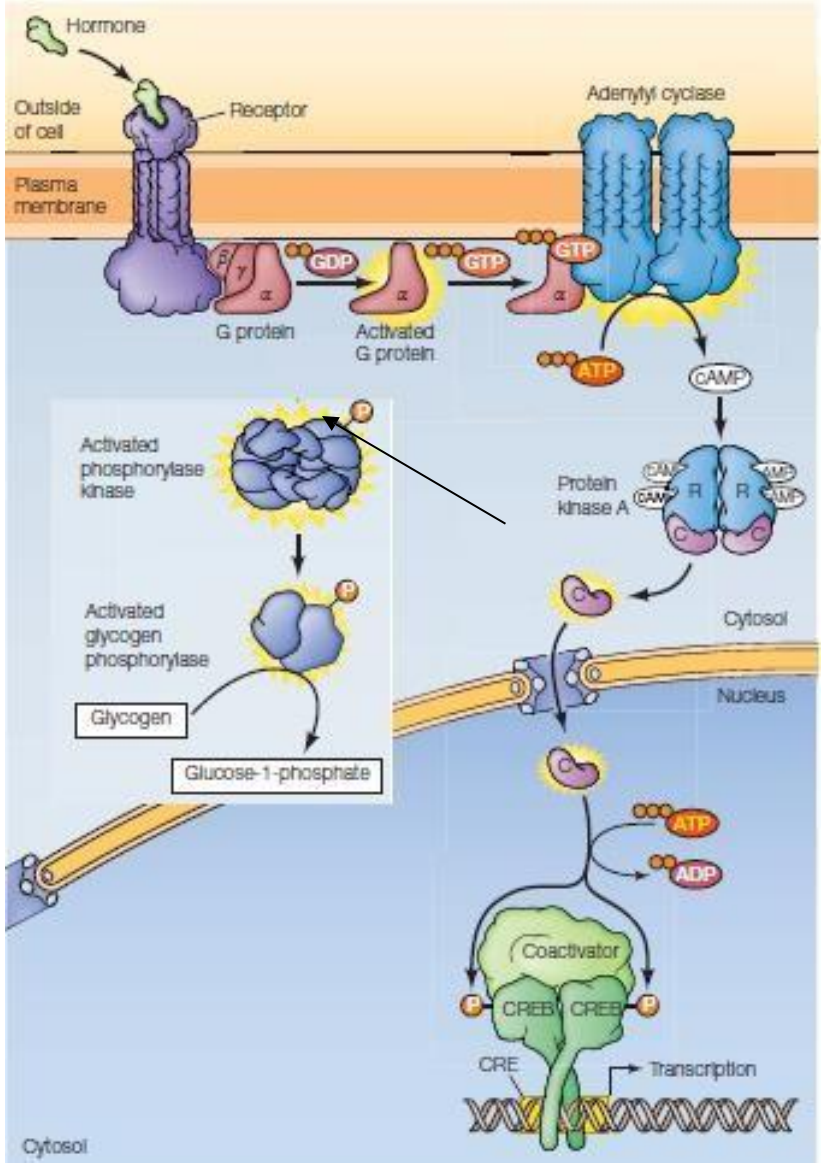
PKA has so many effects that are cell specific, making its effect on an organ different than its effect on another organ or pathway.

Example: cAMP-inducible gene expression

The free catalytic subunit of protein kinase A (released when cAMP binds PKA) can:

1. Phosphorylate other enzymes or molecules, such as: glycogen synthase or glycogen phosphorylated kinase.
2. Translocate into the nucleus and <u>phosphorylates</u> *transcription factors* (proteins that regulate gene expression) like CREB (CRE-binding protein), leading to the expression of cAMP-inducible genes which may produce more of the same enzyme that was phosphorylated outside the nucleus.

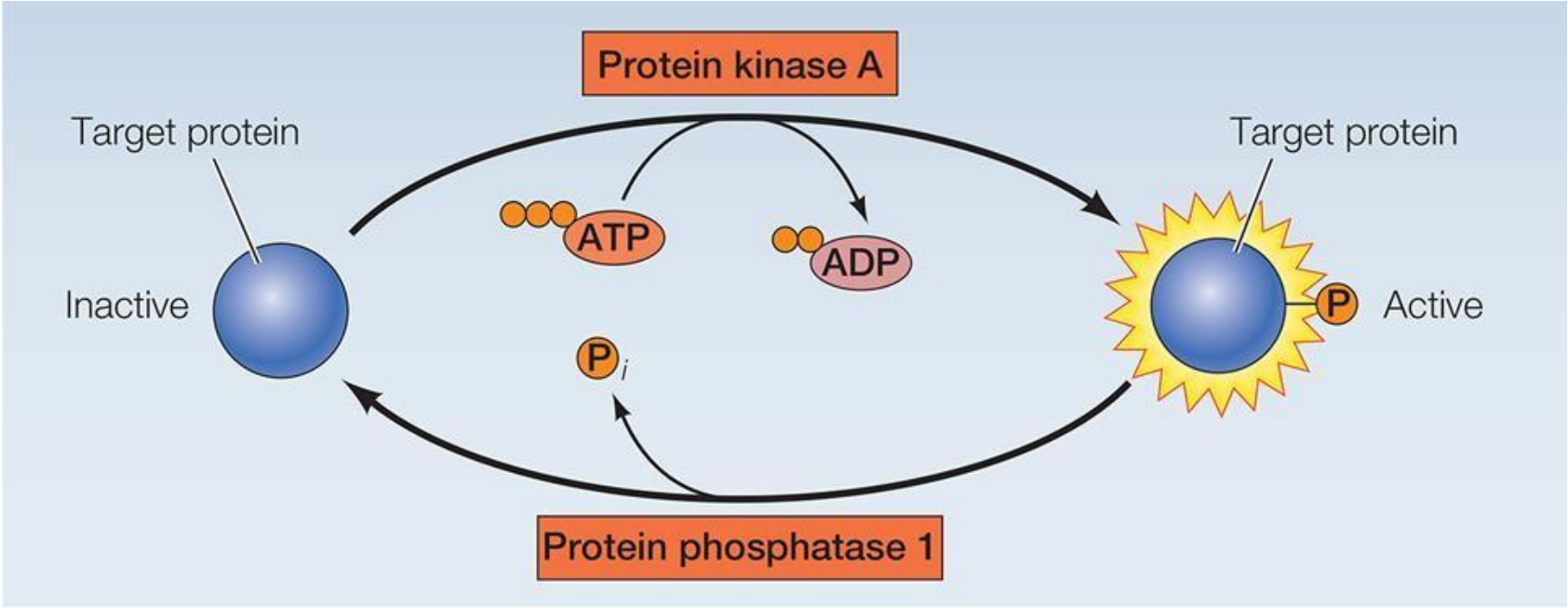
We have a rapid effect (the phosphorylation of the enzyme) and a slower effect (gene expression & synthesizing new enzymes).



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?

- **How well created our bodies are: over 10 billion reaction per second undergo in the body, and they are all well regulated.**
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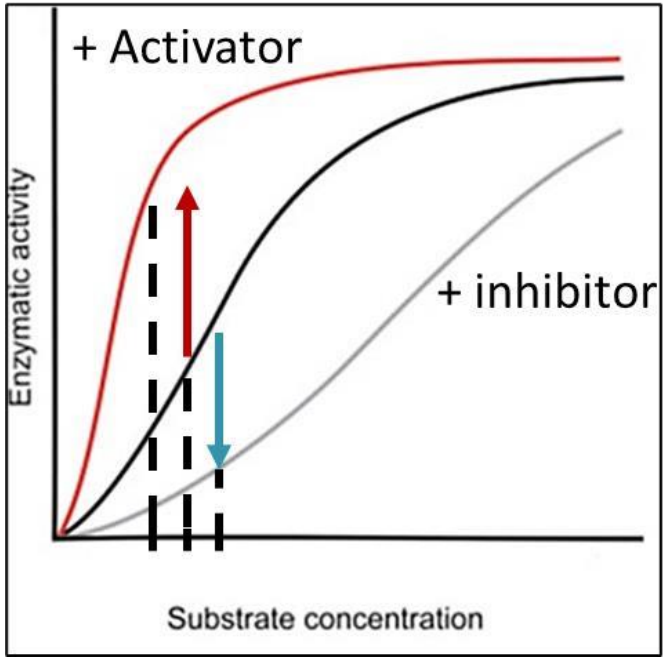
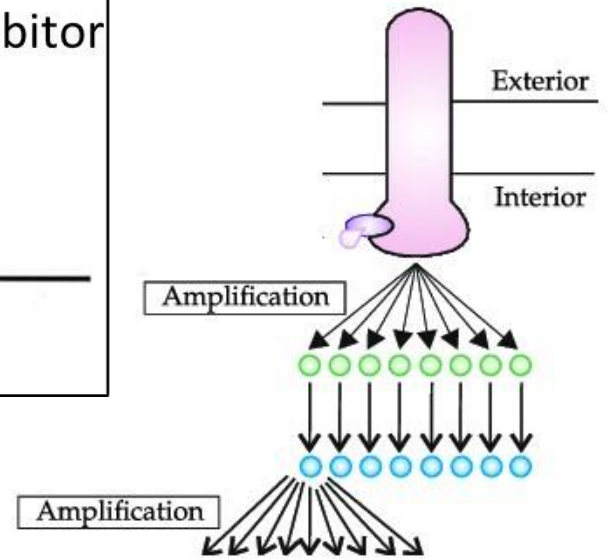
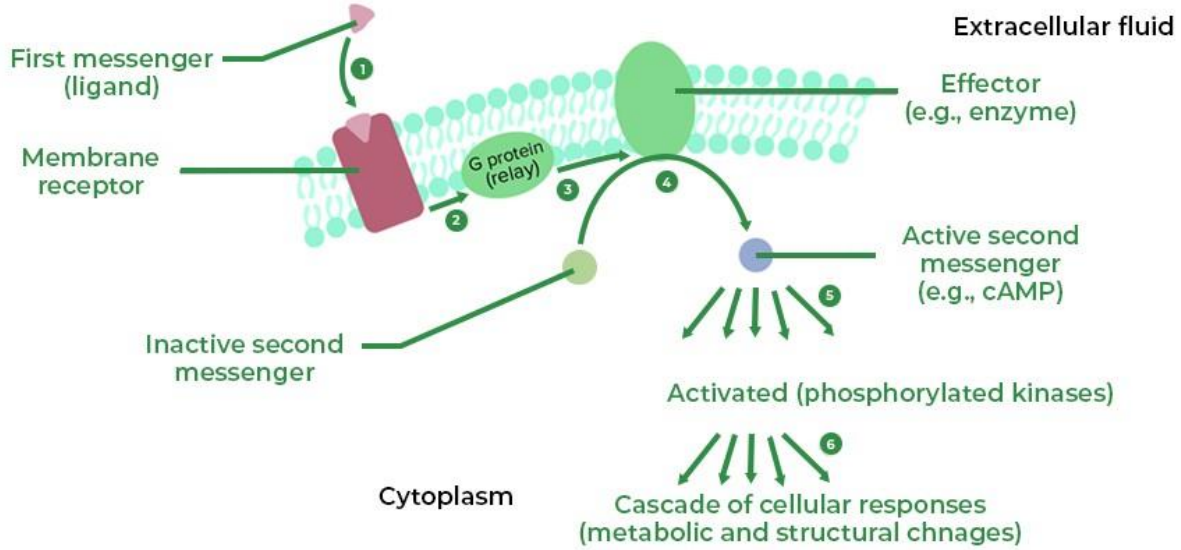
- Easy and quick regulation
  - Reversible covalent modification (e.g., phosphorylation)
  - Binding to small molecules (e.g., cAMP)
- Sensitive
  - Allostery
- Amplification





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)

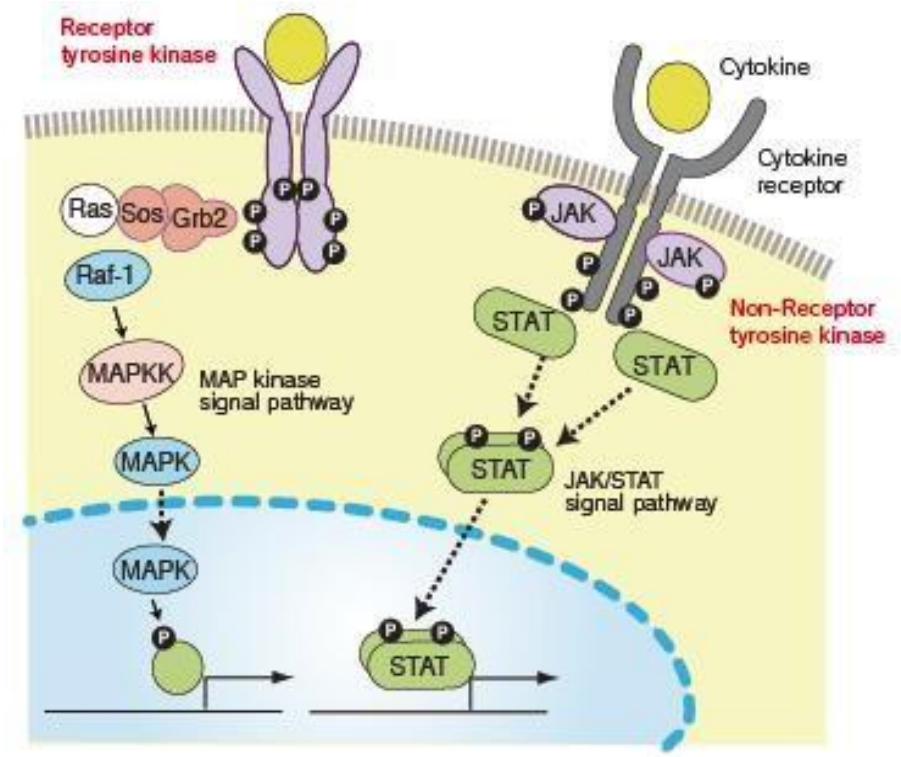
Some receptors either

- Receptor tyrosine kinases (have an intrinsic tyrosine kinase activity) **the receptor itself is also a kinase.**

OR

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

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



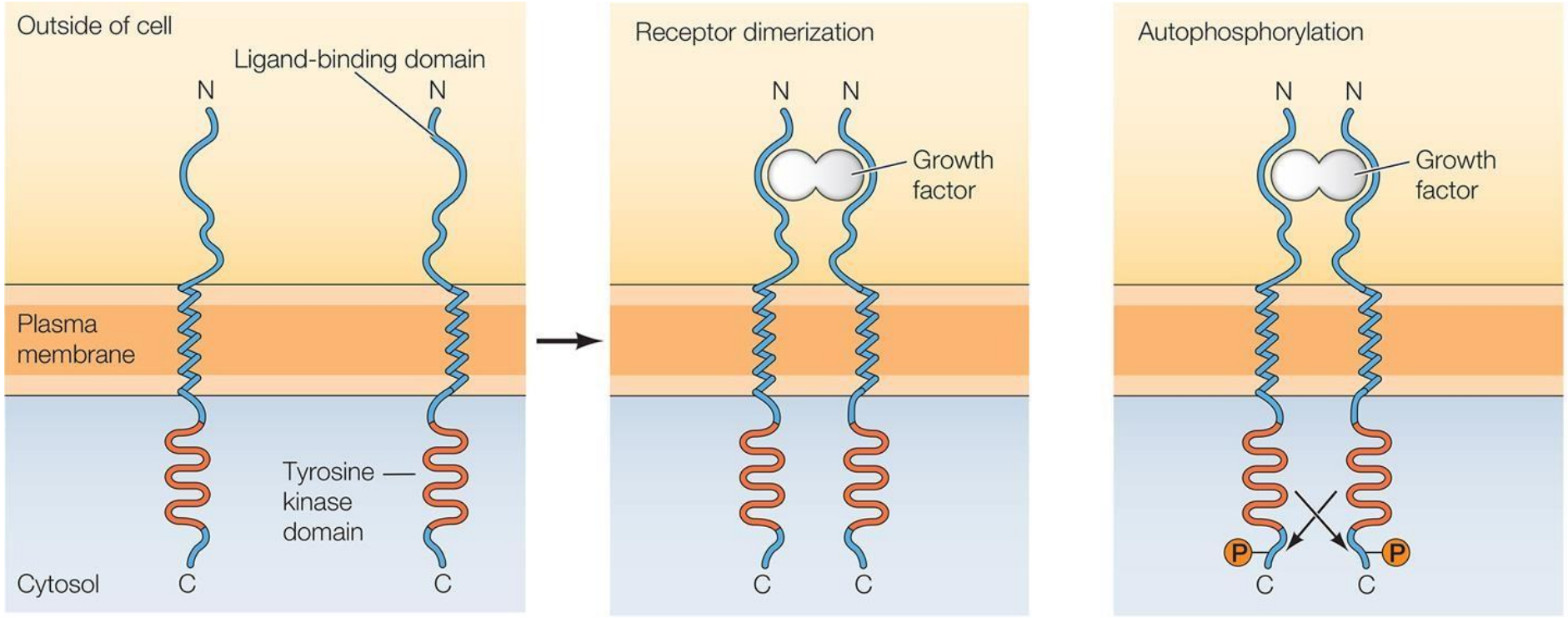
Kinase = Phospholorates

There are 3 amino acids that can be phosphorylated (they all have a hydroxyl group):

1. **Tyrosine**
2. **Serine**
3. **Threonine**

And it is mentioned that PKA is a serine/threonine kinase

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. 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

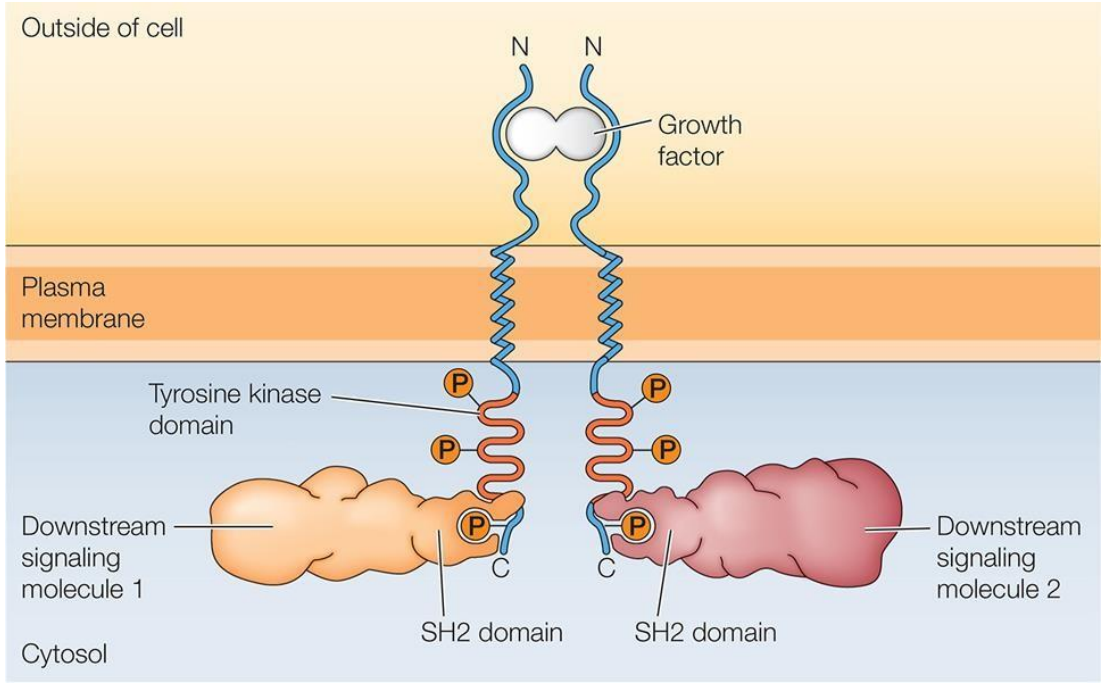
What is the effect of autophosphorylation?

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.

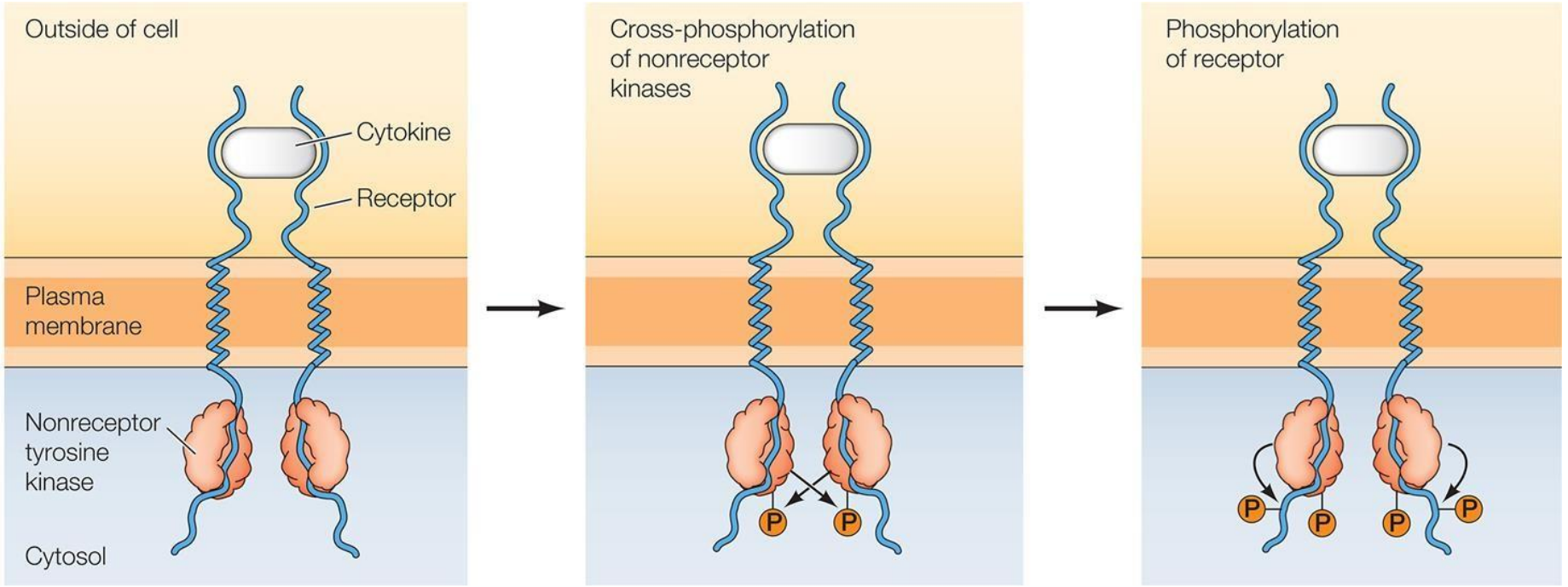
  Phosphate group has a negative charge, which facilitate electrostatic interactions with other proteins.

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.

The JAK/STAT pathway

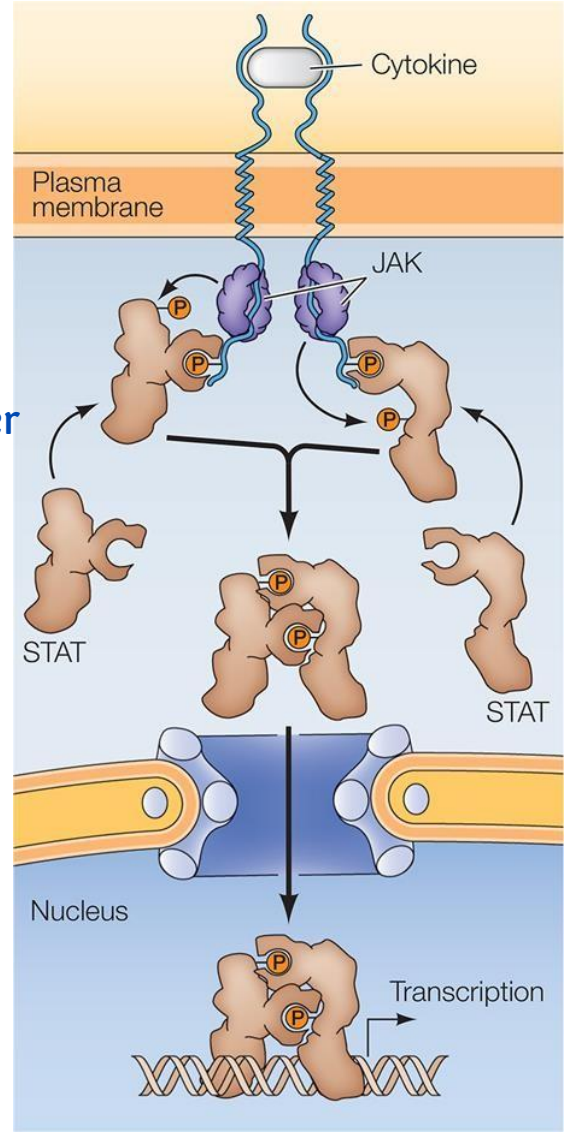
JAK is a kinase, its pathway is activated by cytokines

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.

STAT: signal transducer and activator of transcription proteins

JAK: Just another kinase/Janus kinase

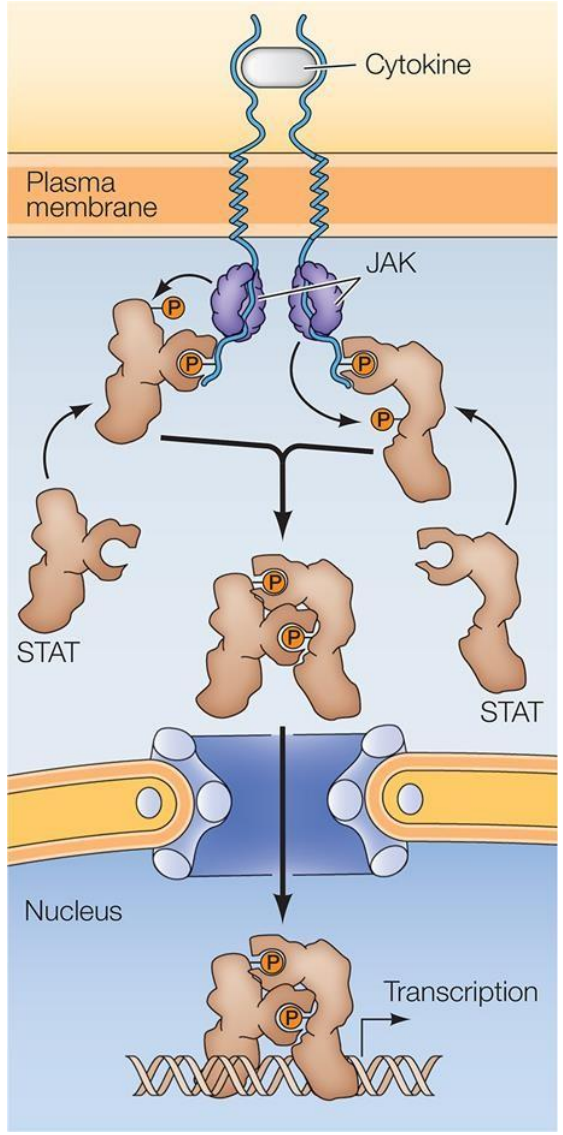


The JAK/STAT pathway

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

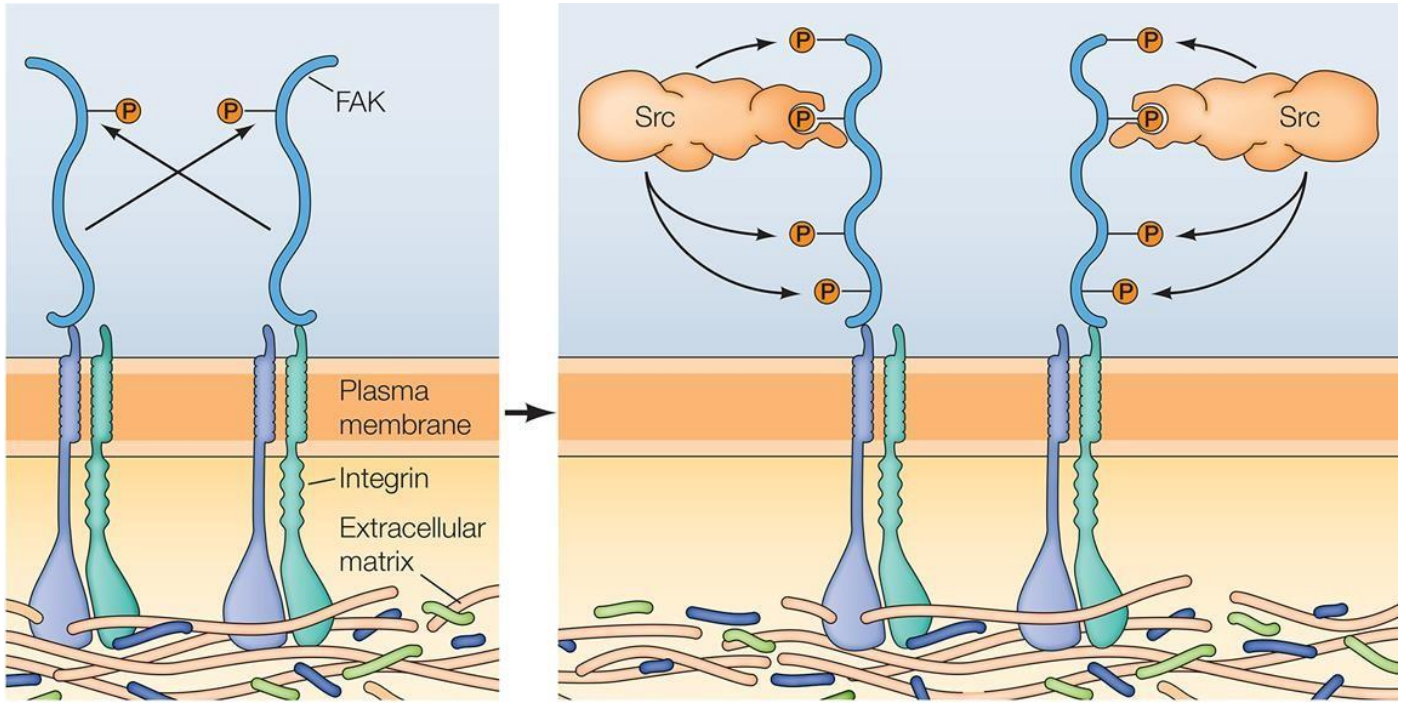
Explanation:

1. Cytokine binds to receptor
2. JAK cross-phopholorate and then phosopholorate the receptor
3. Transcription factor (STAT) binds to phosphotyrosine-binding sites on the receptor
4. STAT proteins are phopholorated by JAK
5. STAT dimerizes and goes into the nucleus
6. Affecting gene expression



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*

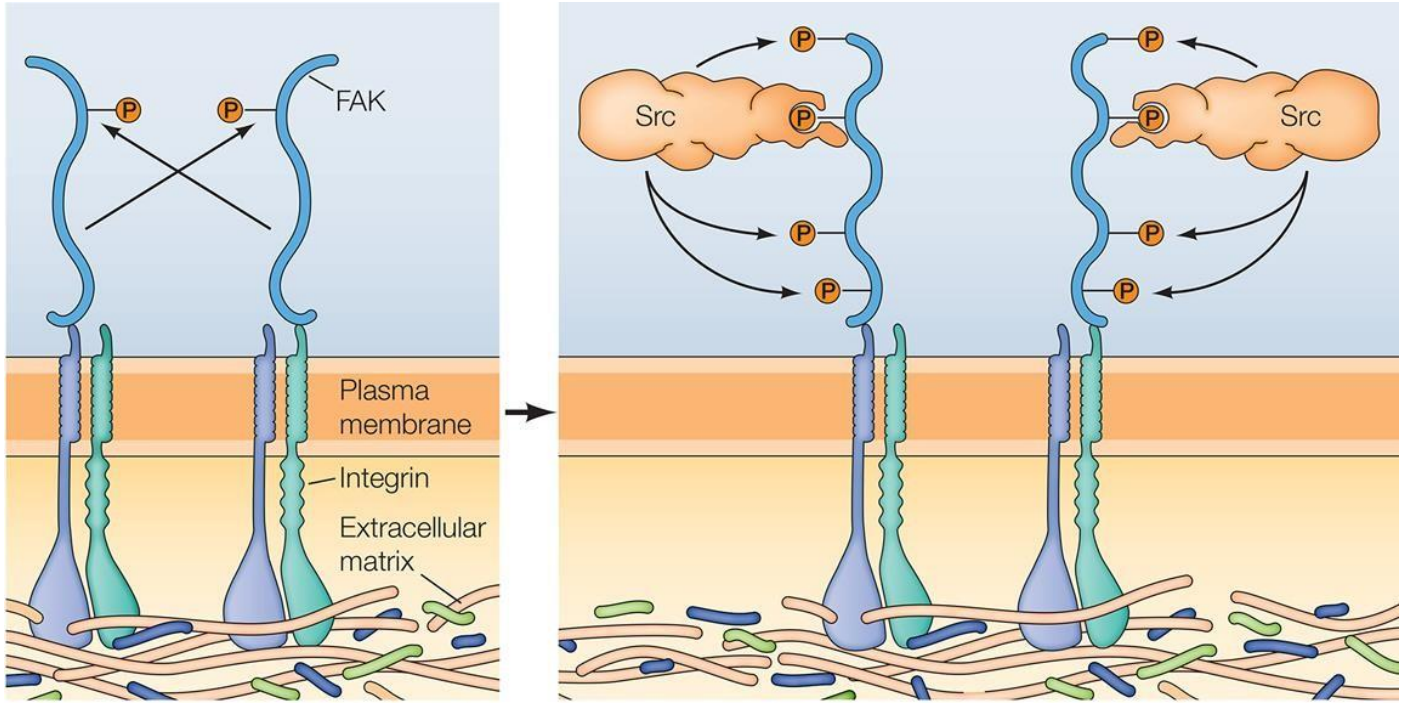


These receptors transmit signals in a similar way

Integrin signaling:

Simple Explanation:

1. Integrin binds to ECM, causing integrins to cluster and FAK (NRTK) to be activated by autophosphorylation
2. Src Binds to FAK and phospholorates FAK more
3. Now there is binding site for signalling mollecules
4. Signal molecule binds and gets activated
5. Signal is transmitted



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			

Additional Resources:

رسالة من الفريق العلمي:

و لو وافيت ربك دون ذنبٍ
و ناقشك الحساب إذًا هلكتا
و لم يظلمك في عمل و لكن
عسيرٌ أن تقوم بما حملتا
و لو قد جئت يوم الفصل فردًا
و أبصرت المنازل فيه شتى
لأعظمت الندامة فيه لهفًا
على ما في حياتك قد أضعتا