CYTOLOGY

Premed 2018 - JU

◉ Sheet ○ Slides

Number

22+23

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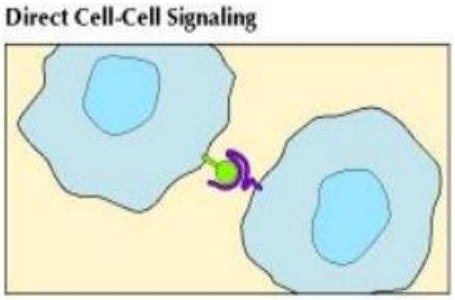
- **This sheet was written based on the last 5 minutes of record 22, in addition to record 23.**

Cell Signaling

❖ Cells interact with each other to transmit messages (either between neighboring or distant cells) or interact with themselves. This interaction is commonly known as **cell signaling**. The **modes of cell signaling** include:

1) Direct cell-cell signaling:

✓ A **signaling molecule** (e.g. surface protein) on the surface of one cell interacts with a **surface receptor** of another cell directly. (direct interaction of a cell with its neighbour).

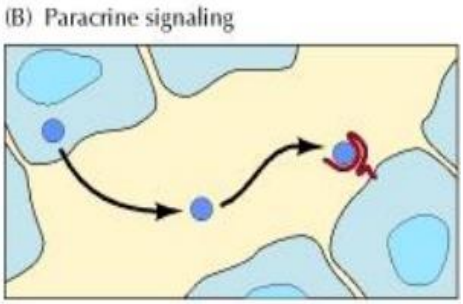


2) Signaling by secreted molecules:

✓ The cell releases a signaling molecule to a target cell. This mode of signaling is divided into three categories **based on the distance of the target cell**:

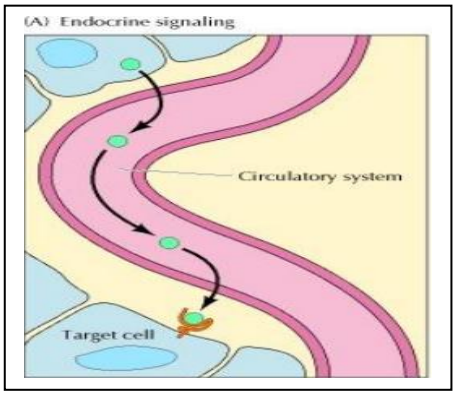
A) Paracrine signaling:

✓ the cell releases a soluble molecule that is transported through the **extracellular matrix** to a (neighboring) target cell that is **relatively close**, and this molecule binds to a receptor on the target cell.



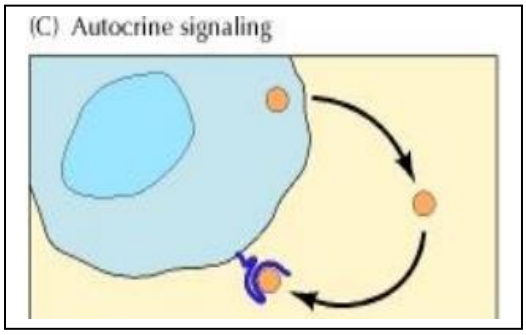
B) Endocrine signaling:

✓ In this case, a molecule is released from one cell into the **blood stream** to a **far or distant** target tissue or cell.



C) Autocrine signaling:

- ✓ The cell releases a compound, this compound binds to a receptor on **its own cell membrane**. (it is basically talking to itself).
- ✓ This mechanism is specifically common in cancer cells.



❖ Classification of signaling molecules:

1) Peptides:

- ✓ A peptide may consist of 2,10, or 40, etc. amino acids.
- ✓ Include **growth factors** (EGF), **peptide hormones** [insulin (which is sometimes considered a protein, sometimes a peptide), glucagon] or **neuropeptides** (oxytocin, enkephalins).

2) Small molecule neurotransmitters:

- ✓ Each of them is **derived** from **one amino acid**.
- ✓ Like **epinephrine** and **thyroid hormone** (both derived from tyrosine), serotonin (derived from tryptophan).

3) Steroids:

- ✓ Derived from **cholesterol**
- ✓ Like estradiol, cortisol, calciferol (vitamin D), testosterone and aldosterone (aldosterone is produced by the adrenal gland).

4) Eicosanoids:

- ✓ Derivatives of **arachidonic acid** (an unsaturated fatty acid containing 20 carbons and 4 double bonds)
- ✓ They are categorized according to chemical structure and function into **prostaglandins**, **leukotrienes**, and **thromboxanes B**.
- ✓ They act as **inflammatory mediators**:

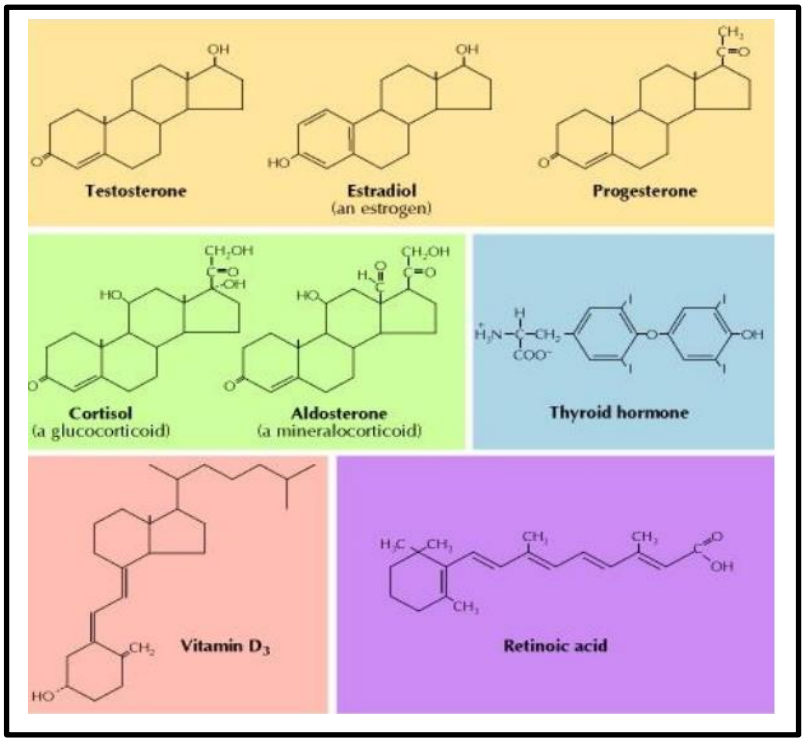
✓ Example: Someone had contaminated and unclean food from a restaurant. The food enters his small intestines. It will not necessarily cause food poisoning, but it

will induce an inflammatory response, which includes a sequence of events such as: **swelling** (edema), **redness** and **hotness**. Note that inflammation is different from infection, because no microorganisms are involved in inflammation. The changes that accompany inflammation need chemical substances that mediate them. These substances are prostaglandins, etc. Also called "cytokines".

5) Gases:

- ✓ Nitric oxide (NO) and carbon monoxide (CO).

- The signaling molecules mentioned previously include a group of molecules that are **lipophilic** (opposite to hydrophilic). They prefer interacting with lipids. These lipophilic hormones include:



- ✓ **<u>sex hormones</u>**: testosterone, estrogen,progesterone.
- ✓ **<u>Adrenal gland hormones</u>**: like cortisol and aldosterone**.**
- ✓ **<u>Other examples</u>** include vitamin D, retinoic acid (known as vitamin A, notice the structure: it is hydrophobic except for the COOH part) and the thyroid hormone.
- All these lipophilic molecules will have **their receptors inside the cell** rather than the cell surface because their hydrophobic structure allows them to penetrate the lipid bilayer of the membrane (their receptors are soluble proteins in the cytosol).

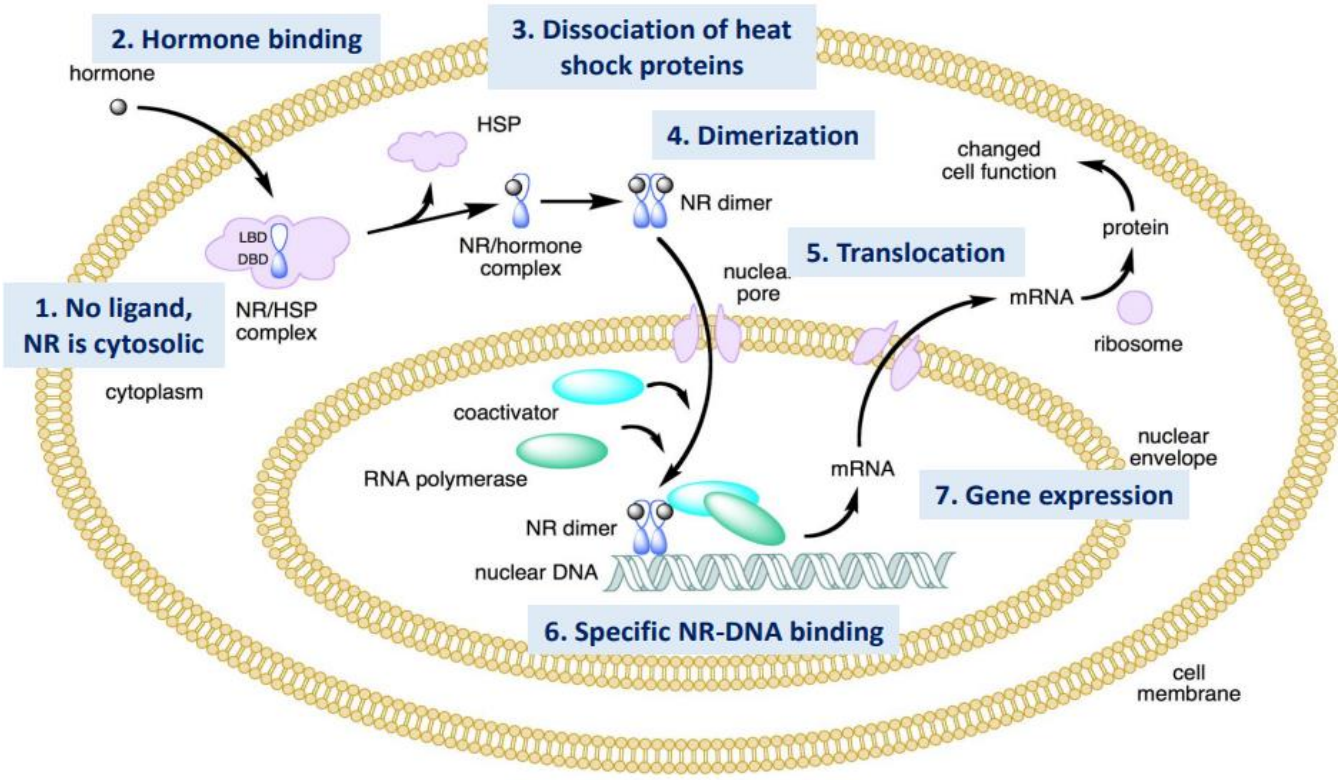
❖ Receptors:

➢ Mechanism of action of steroid receptors:

- ✓ We are going to start with the **receptors of lipophilic molecules**, which are called **nuclear receptors (NR)**, because the receptor-ligand complex is going to enter the nucleus.

❖ **Mechanism:**

- The nuclear receptor is present in the **inactive state** in the **cytosol** bound to a **protein complex called (HSP).**
- Once the hormone enters inside the cell, it is going to **bind** to the receptor and **dissociate the protein complex**.
- We now have a **receptor-hormone complex** that can **dimerize** with another receptor-hormone complex.
- This dimer can enter the nucleus through nuclear pores, and it has the ability to bind certain **regions of DNA** and activate the **synthesis** of certain **proteins** of target **genes**.
- With the action of polymerase, **mRNA is transcribed** and exits through the nuclear pore, then it is **translated** by the ribosome.



✓ **Note:** Target genes are different when different molecules bind to a receptor. For instance, target genes of estrogen are different from that of testosterone, and thus different molecules will induce different responses.

❖ Cell Surface Receptors:

- Now we are going to deal with receptors that are on the cell surface (the receptor is a membrane protein)

- **General Scheme:**

✓ A **ligand** binds to its cell surface **receptor,** but the message cannot be transmitted directly to the nucleus as in nuclear receptors, because the receptor is part of the membrane and cannot leave it.

✓ Instead, it activates **downstream** effectors, transducers, second messengers until it reaches a transcription factor or a protein with an NLS (Nuclear Localization Signal) causing it to enter the nucleus.

✓ This protein or transcription factor then binds to a certain region of DNA to activate gene expression. (Genes could be related to metabolism, cytoskeletal proteins, etc).

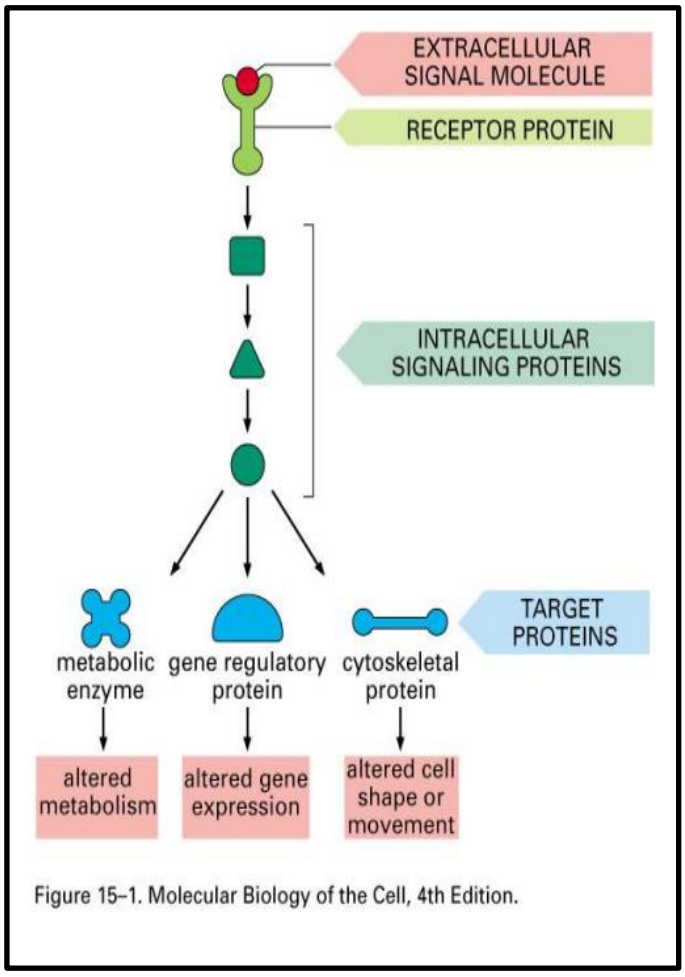


Figure 15–1. Molecular Biology of the Cell, 4th Edition.

- Many molecules participate in signaling by cell surface receptors, these molecules are also called **Players of Signaling by Cell Surface Receptors** and include:

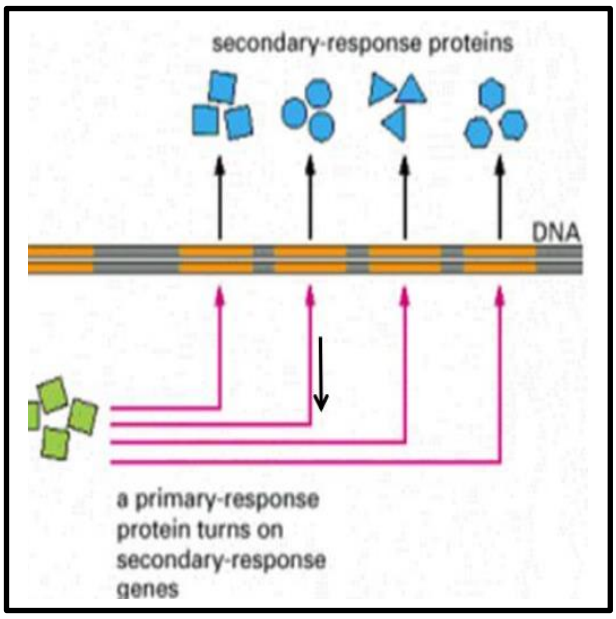
✓ **Ligands** (e.g. hormone, growth factor)
✓ **Receptor** (e.g. GPCR, RTK)
✓ **Transducers** (e.g. G protein, Ras)
✓ **Effector molecules** (e.g. adenylate cyclase, MAPK)
✓ **Second messengers** (e.g. cAMP, cGMP, Ca^{+2})
✓ **Final target molecules** (e.g. DNA, channel → Response)

❖ Types of Response:

✓ A **response** is the final event that takes place in a cell signaling pathway, and it is divided into **two types**:

1) Primary response:

✓ The activation of a receptor, downstream effectors and transcription factors is followed by direct activation of a small number of specific genes and synthesis of a protein. This is a primary response which resulted from the first gene expression directly. The proteins that result are called **primary response products**. It is quick (30 minutes).



2) Secondary response:

✓ If the primary response product binds to **ANOTHER** region of DNA, it activates other genes and causes synthesis of newer proteins (**secondary response products**). This is a secondary response because it resulted from the newer (second) gene expression which occurred indirectly.

✓ Example:

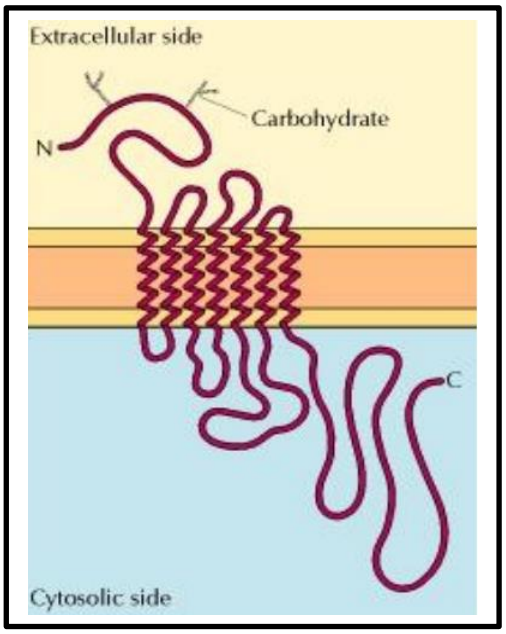
✓ After the activation of a series of receptors, effectors, etc. we reach **transcription factor (1)** which activates **gene (1)**, and results in synthesis of **protein (X)**. This is a **primary response**. However, protein X may not be the desired protein.

✓ The desired protein may require the expression of another gene (gene (2) for instance), but transcription factor (1) cannot bind to this gene. That is why we need the primary response product (protein X) as an intermediate which will bind to **gene (2)** and activate it causing synthesis of **protein (Z)** which is the desired product. This is a **secondary response**.

- **The <u>secondary response</u> needs more time than the <u>primary response.</u>**

❖ **There are many types of cell surface receptors, including:**

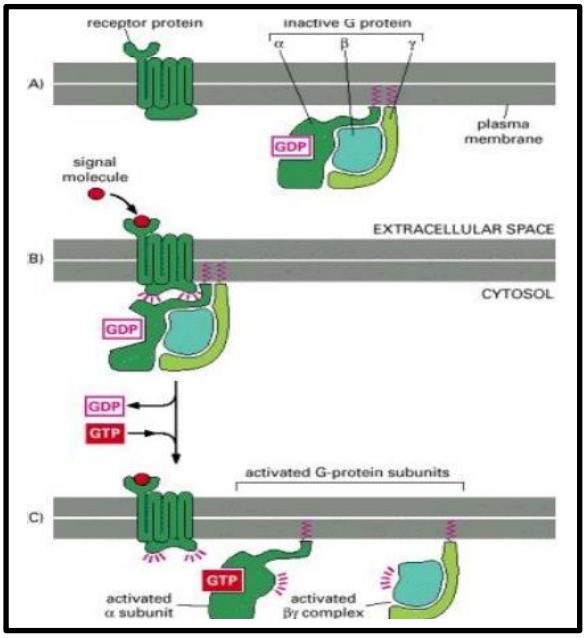
A) G protein-coupled receptors (GPCR):



- ✓ A family of proteins composed of **seven membrane-spanning α helices**.
- ✓ Most abundant type of cell surface receptors
- ✓ **N-terminus**, **sugar components and ligand-binding site** are directed outside (**extracellularly**).
- ✓ **C-terminus** is **intracellular** and has the ability to bind to G-proteins.
- ✓ When the signaling pathway associated with GPCR is **inactive**, the **G-protein is not bound** to the receptor (GPCR) and is **away from it**.
- ✓ **G-protein** is an **anchored membrane protein** composed of **three subunits (heterotrimeric): α, β, γ units.**

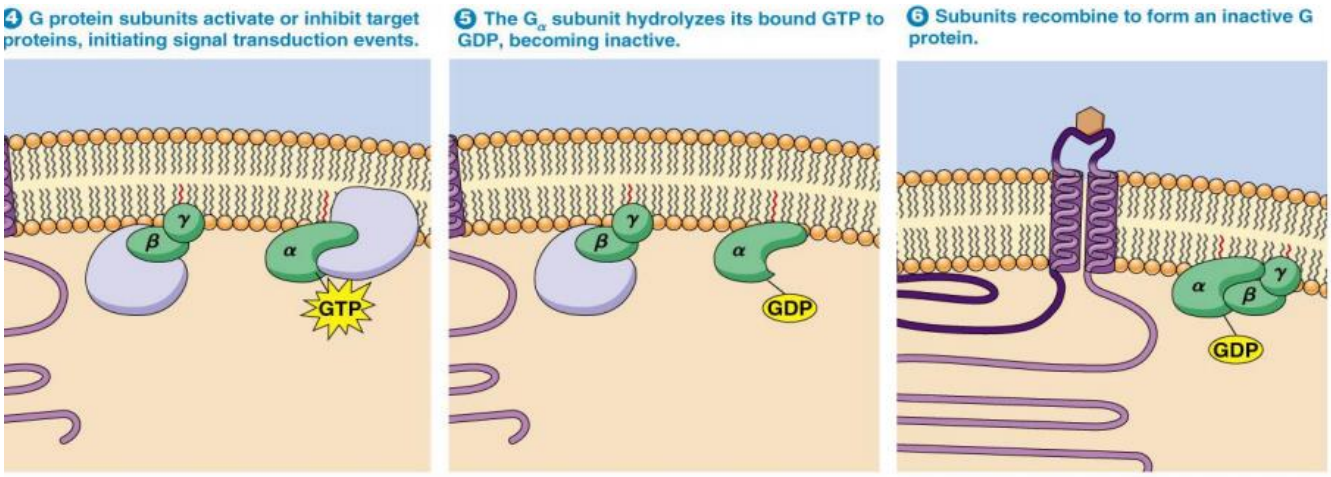
- ✓ **G-proteins have two states**:
- In the unstimulated state, the **α subunit is bound to GDP**, and **the G-protein is inactive.**
- When stimulated, the α subunit **releases its bound GDP**, allowing **GTP** to **bind** in its place**. G-protein is in the active state**.

- ✓ Once the **ligand** (signaling molecule) **binds** to the receptor GPCR, **conformational changes** occur that **recruit the G-protein** allowing it to **bind to the receptor**. Now, the G-protein is **active** and **exchanges GDP with GTP**. This exchange causes the G-protein trimer to **dissociate** into active components: **α subunit alone** and **βγ complex**. These activated components will cause downstream events and bind to other effectors.



❖ **G-protein inactivation:**

✓ At some point, we need to **inactivate** the GPCR pathway. This is accomplished by **hydrolyzing GTP to GDP**. This allows the subunits (α, β, γ) to assemble again and get away from the GPCR.



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 reassociates with the βγ complex.

- As long as the ligand is bound to the GPCR, the G proteins remain active and the signal continues to induce changes in the cell.

B) Receptor protein tyrosine kinase (RTK):

✓ The receptor itself has the ability to **phosphorylate tyrosine** amino acids present within the receptor.

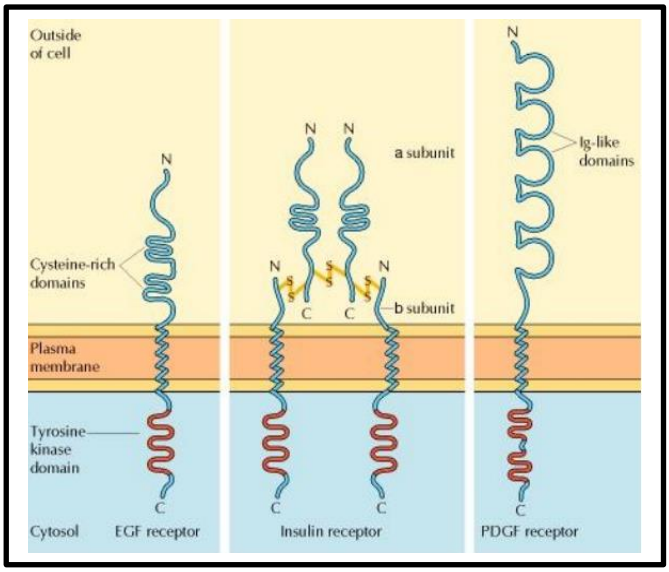
✓ Some receptors are directly linked to intracellular enzymes.

✓ RTKs are also enzymes (they have **enzymatic activity** as part of the protein itself).

✓ **There are several subtypes of RTKs:**

1) **Epidermal Growth Factor (EGF) receptor**
2) **Insulin receptor.**
3) **Platelet-derived Growth Factor (PDGF) receptor.**

✓ Notice that they all **share** the **intracellular part** (similar), which represents the **kinase domains.**

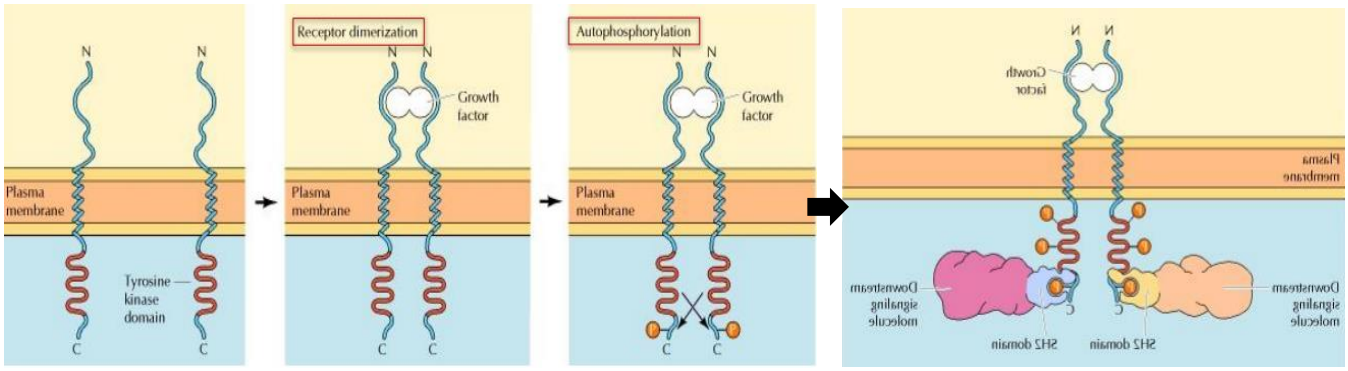


✓ On the other hand, **the extracellular part** (that binds the ligand) **varies** among the different receptors, because they have different ligands.

❖ Mechanism of activation of RTKs:

- ✓ The RTKs are present as single molecules in the plasma membrane in the **inactive state**.
- ✓ Once the growth factor binds to one receptor, it induces the **dimerization** with another receptor. (Now we have a dimer with a growth factor (signaling molecule) bound to it).
- ✓ The receptors are activated, and each receptor phosphorylates tyrosines of the other receptor in the dimer **(autophosphorylation) which includes:**

1) **Phosphorylation** of **tyrosines outside the kinase domain** which creates high affinity binding sites for the binding of other signaling molecules, effectors and downstream molecules.
2) **Phosphorylation** of **tyrosines within the kinase domain** which increases the kinase activity.



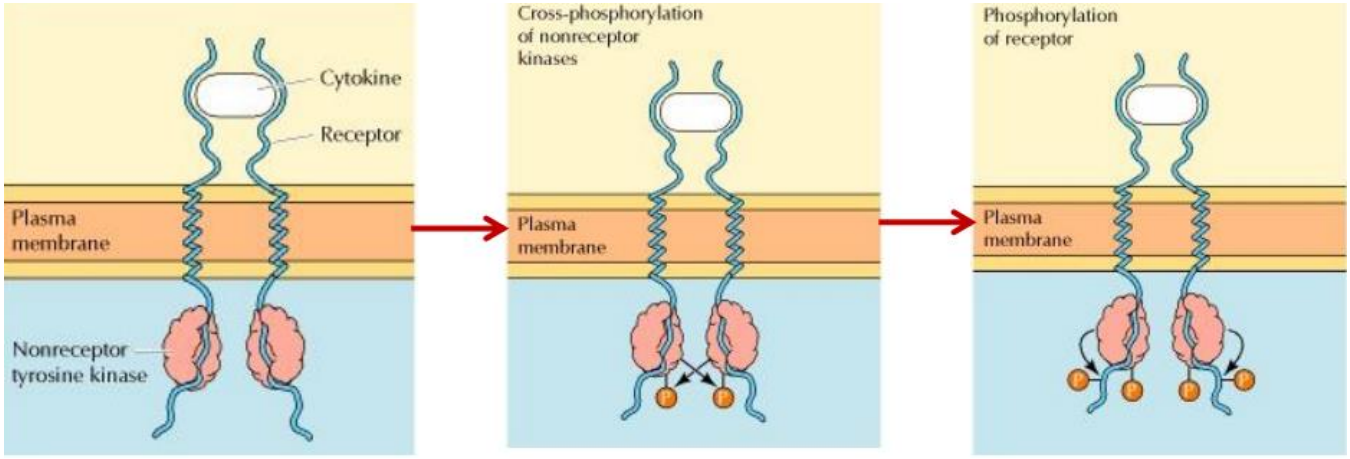
C) Cytokine receptor superfamily (associated with nonreceptor protein tyrosine kinases):

- ➢ Tyrosine kinase is not part of some receptors like cytokine receptors. So, the enzymatic activity is not part of the receptor itself (unlike RTKs)
- ➢ Cytokine receptors respond to Eicosanoids (prostaglandins, etc.)
- ➢ They are similar to RTKs, as they have cytosolic, transmembrane and extracellular domains.
- ➢ They are present as single molecules in the membrane in the inactive state.

❖ **Mechanism:**

- ✓ Once the ligand binds, **dimerization** occurs (dimers of receptors are formed).
- ✓ This brings in intracellular molecules called **nonreceptor protein tyrosine kinases**. These molecules will carry out phosphorylation.
- ✓ The **nonreceptor protein tyrosine kinase** bound to one receptor **phosphorylates the kinase** bound to the other receptor (this type of phosphorylation is called **cross-phosphorylation**)
- ✓ Once the **kinases** are **phosphorylated**, they become **activated**, and each kinase **phosphorylates tyrosines on the receptor to which it is bound.**
- ✓ The **phosphorylated tyrosines** on the receptor now act as **attractive sites** for the **binding** of effectors and downstream molecules.

- **Examples of nonreceptor tyrosine kinases: JAK, Src.**



❖ **Other examples/types of receptors and regulators:**

- ✓ **Protein-tyrosine phosphatases:**
  - ➢ Activation and inhibition roles (such as inactivating receptor tyrosine kinases by removing phosphate group from tyrosine causing the receptor to become inactive)
- ✓ **Protein Serine/Threonine kinase:**
  - ➢ It carries out phosphorylation on serine and threonine amino acids.
  - ➢ An example of a signaling molecule associated with this kinase **is TGF-β (transforming growth factor β)**
- ✓ **Receptor guanylyl cyclases**
- ✓ **Protease-associated receptor:**
  - ➢ Tumor necrosis factor (TNF)

❖ Second Messengers:

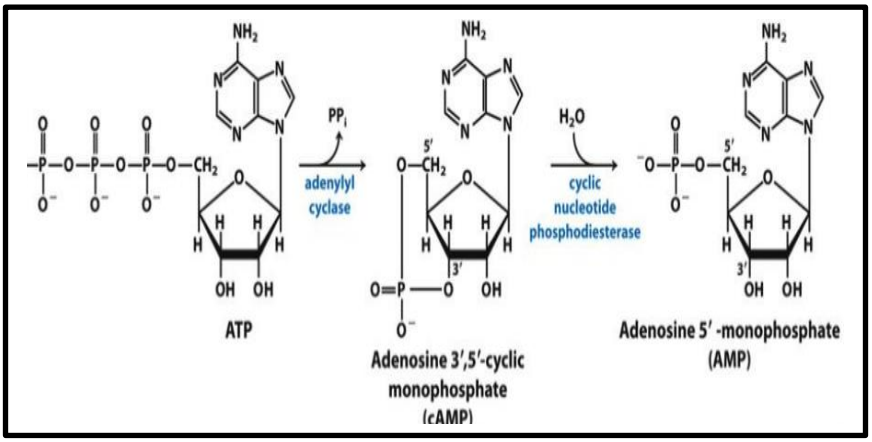
Why are second messengers important? Why do we need them?

- ✓ **The receptor is fixed** in the membrane, so we need **second messengers** which are often **free to diffuse** to other compartments of the cell until we reach the intended target (transcription factor).
- ✓ **Signal amplification** (each activated receptor may activate multiple second messengers, and each secondary messenger may interact with multiple downstream molecules amplifying the response).
- ✓ **Common second messengers** in multiple signaling pathways often result in **cross-talk** between different signaling pathways. (A second messenger of one pathway may interact with a second messenger of another pathway and activate it).

❖ Synthesis and degradation of cAMP:

- ➢ An example of second messengers is **cAMP**, which is a **nucleotide** molecule. (second messengers are not necessarily proteins).

- **Steps:**



- ✓ cAMP is produced from ATP molecules.
- ✓ ATP (which has 3 phosphate groups) loses **pyrophosphate (2 phosphates)** by the action of the enzyme **adenylyl cyclase**. It will induce **cyclization** of the structure and form cAMP. (Normally, one of the phosphates in **ATP** makes **one bond** with the sugar, but in **cAMP**, the phosphate makes **two bonds** with the sugar which causes a cyclic structure).

- **How can we destroy cAMP and degrade it? (because at some point we have to stop the signal)**

  - ✓ By the enzyme **cyclic nucleotide phosphodiesterase,** which converts **cAMP** back to **AMP**, and the response is stopped.

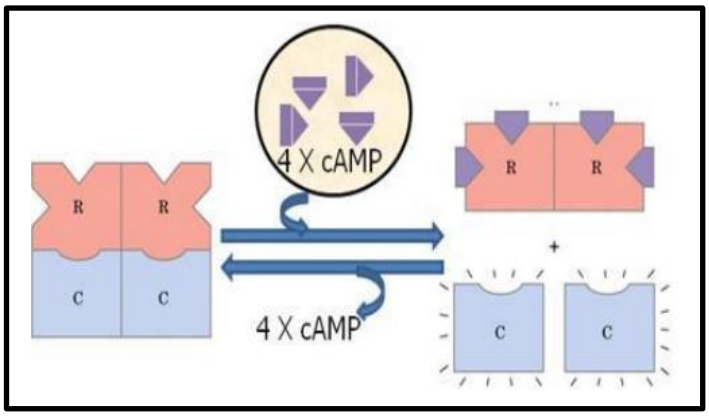
❖ cAMP-inducible gene expression:

➢ cAMP binds to downstream molecules, such as Protein kinase A.

Protein kinase A consists of 4 subunits:

✓ **2 regulatory subunits (R)**, each one has 2 cAMP binding sites (total of 4)

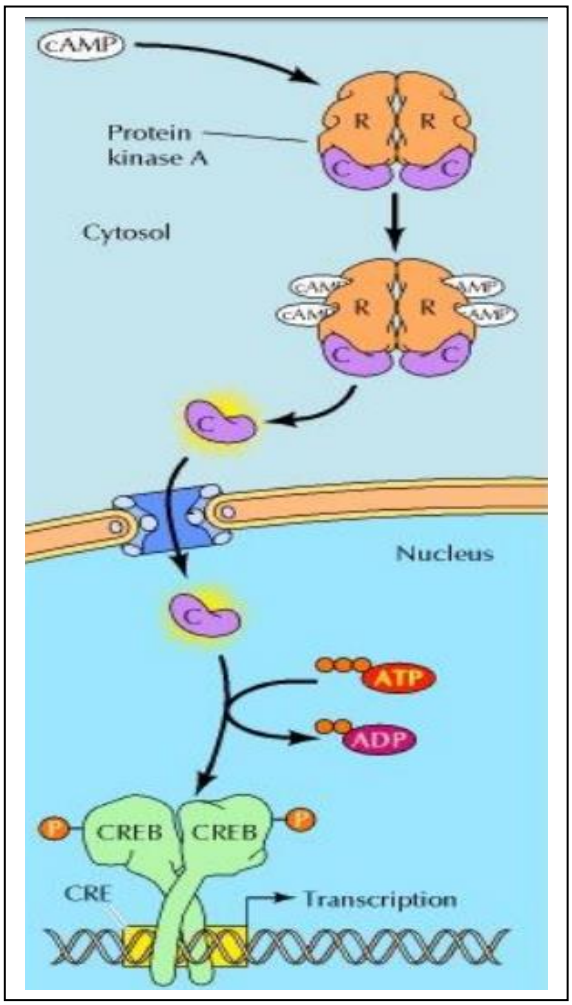
✓ **2 catalytic subunits (C)** that have the ability to phosphorylate)



✓ The binding of **4 cAMP** molecules to the **regulatory subunits** is going to **detach** the **catalytic subunits**.

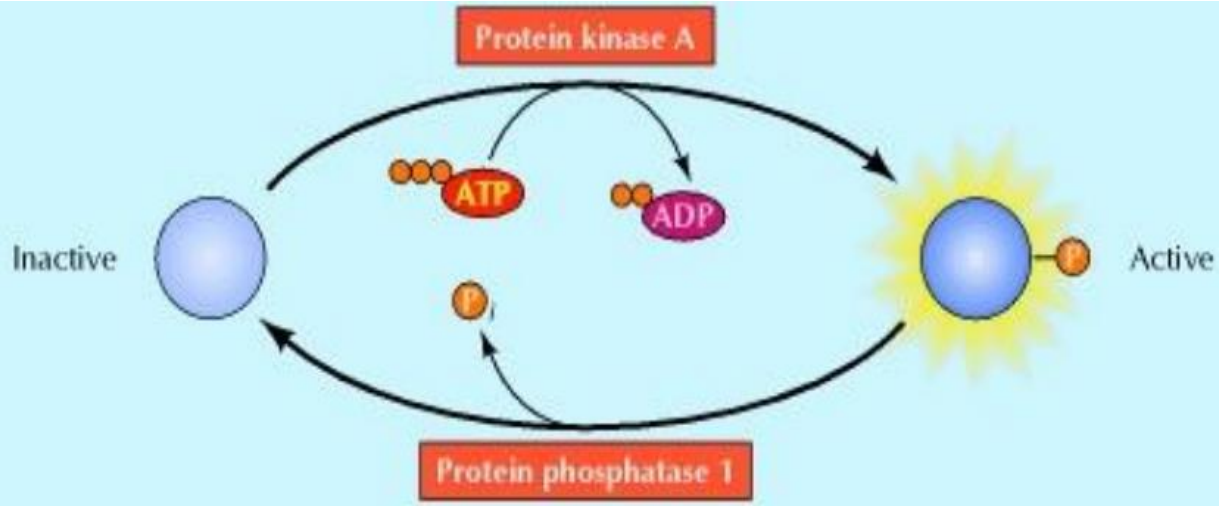
✓ The **detached (free) catalytic subunits** of protein kinase A are now **active** and **translocated** into the nucleus through nuclear pores, then they **phosphorylate the transcription factor CREB** (CRE-binding protein).

✓ **The transcription factor CREB** binds to a region of DNA called **CRE**, leading to **transcription** of mRNA and **expression of cAMP-inducible genes**.



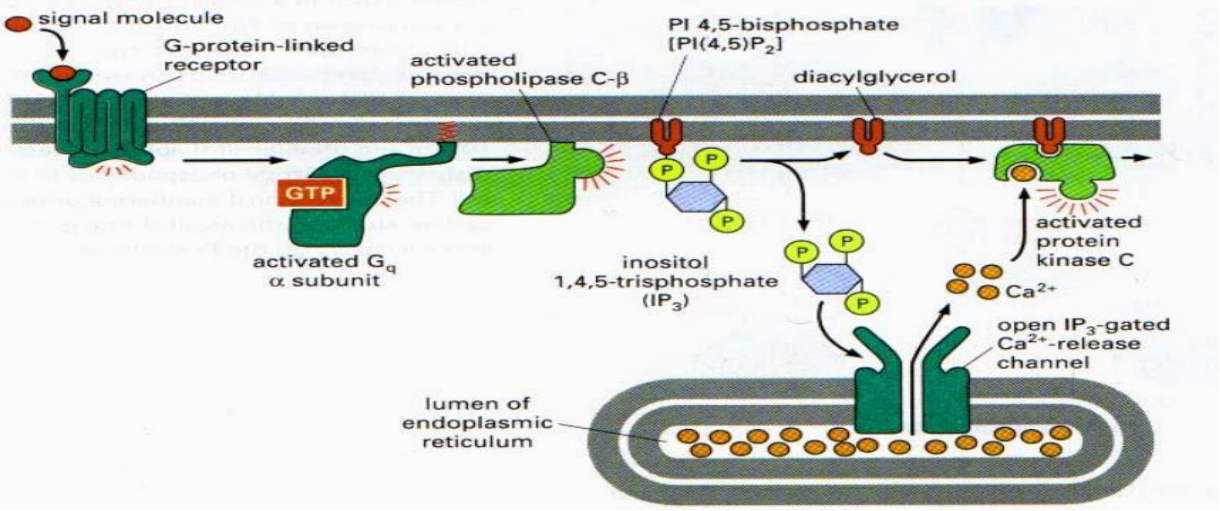
✓ **PKA regulation by dephosphorylation:**

- Protein kinase A phosphorylates CREB and activates it through phosphorylation. In order to deactivate CREB, it is dephosphorylated by **Protein phosphatase 1** which removes the phosphate group and CREB is inactivated. See the following image:



❖ Phospholipids and Ca^{+2}:

- ✓ **The ligand** (signaling molecule) **binds** to the **GPCR**, and then the **G-protein** is recruited and **activated** causing the **exchange** of GDP with GTP.
- ✓ The G-protein trimer then **dissociates** into an **α subunit alone**, and a **βγ complex**.
- ✓ The activated α subunit activates the enzyme **phospholipase C-β**, which splits the membrane phospholipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) or more shortly 'PIP2' into:
  - **Diacylglycerol** (composed of glycerol and two fatty acids): it remains in the membrane and binds to **protein kinase C**.
  - **Inositol 1,4,5-triphosphate (IP3):** it is detached from the membrane and is no longer part of the phospholipid, and it can now bind to **IP3-gated calcium ion channels** present on the **ER membrane**, opening them releasing calcium into the cytosol. Ca^{+2} then binds to **protein kinase C** and activating it along with diacylglycerol. Protein kinase C is going to phosphorylate downstream molecules.



❖ Ca+2/Calmodulin:

- Another example where calcium plays a role in signaling is **calcium/calmodulin signaling.**

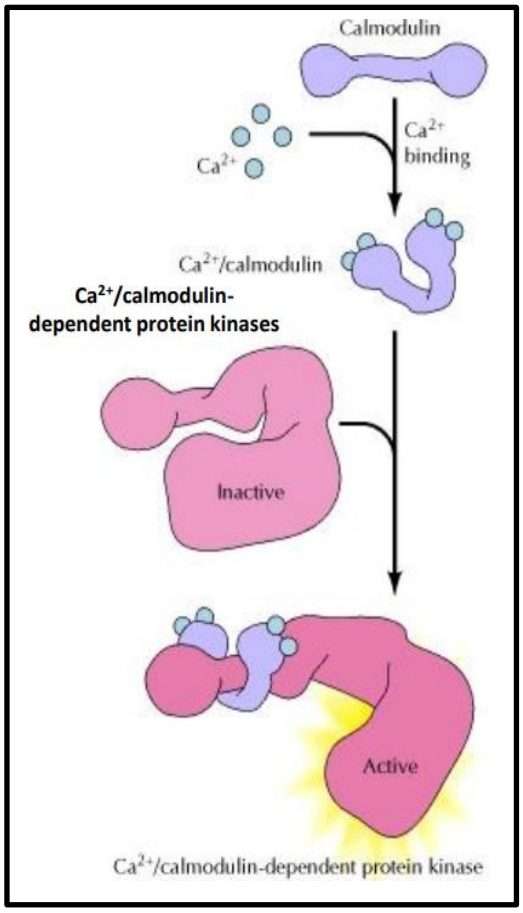
✓ Once calcium is released from the ER due to a certain stimulus (as we mentioned previously), it can bind to different molecules.

✓ In the previous example, it bound to protein kinase C.

✓ Calcium can also bind to other proteins like **calmodulin**.

✓ The **binding** of calcium to calmodulin induces **conformational changes** in **calmodulin** (notice how calmodulin becomes bent) and becomes active.

✓ **Calcium/Calmodulin** in the active state can bind to **Ca+2/calmodulin-dependent kinase**, **activating** it, and causing it to **phosphorylate** downstream molecules.



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