Genetics & molecular biology

◉Sheet ○Slide

Number: - 32

Done by: - Farah Almudallal & Bann

Corrected by: -

Doctor: - Diala

Extracellular matrix:

- It is the environment that surrounds the cell.

- It is the space that surrounds the cell which is occupied by different types of molecules such as fibrous proteins, collagen, elastic fibers, adhesion molecules and polysaccharides in a form of proteoglycans (made of core protein and GAGs).

Adhesion molecules: Connect components of ECM together.

Fibronectin

- Consists of 2 polypeptide chains (a dimeric protein) that are crosslinked into fibrils by disulfide bridges (S-S bonds).

→It has many binding sites:

1) one for proteoglycans which is made of GAGs and core proteins (interacts with sugar).

2) one for cell surface (communicates or interacts with cell surface) protein such as integrins linking cells to the ECM.

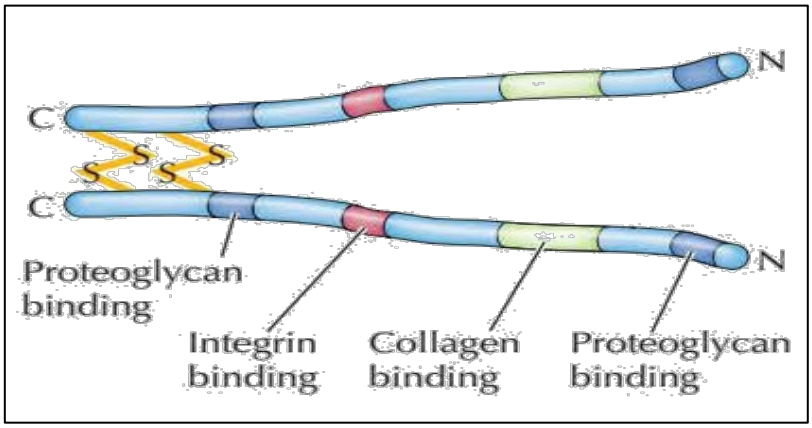
3) one for collagen fibers (interacts with the other component which is fibrous protein).

From slides:

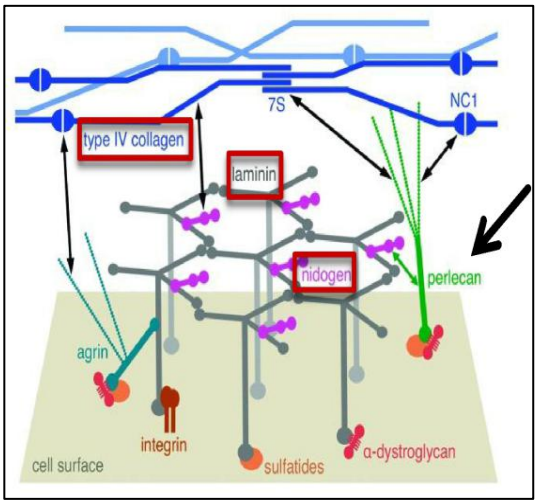
-They link matrix proteins with one another and to the surfaces of cells.

-They **interact with collagen and proteoglycans** and **specify matrix organization** and are **major binding sites for cell surface receptors** such as integrins.

- Fibronectin is considered the principal adhesion protein of connective tissues.



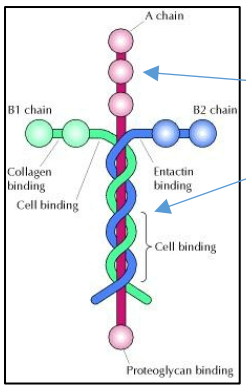
Laminin



- T-shaped or cross-shaped molecules.
- Consists of many chains such as alpha (A), beta 1 (B1) and beta 2 (B2). Every chain has a region presents by itself and another region where they interwind around each other.
- They have many binding sites:

1) For proteoglycans in A chain.
2) Collagen binding site and cell binding site in B1 chain.
3) Cell binding site (present in A,B1, and B2 chains)
4) Entactin binding site.

- This laminin is present in basement membranes (basal lamina), because proteoglycans are abundant in basal lamina underneath the epithelial cells.



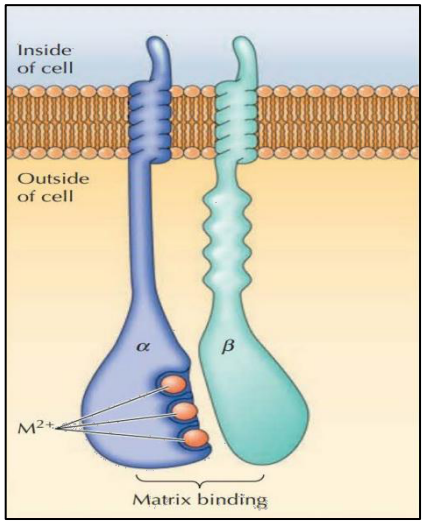
Every chain has a region presents by itself and another region where they interwind around each other.

From slides:

- Laminins are tightly associated with another adhesion protein, called nidogen, which also binds to type IV collagen, all of which form crosslinked networks in the basal lamina.
- Laminins are t-shaped heterotrimers with binding sites for cell surface receptors (e.g. integrins), and ECM components, e.g. type IV collagen, and perlecan.

Cell-ECM interactions

Facilitated through the role of Integrins



From slides:

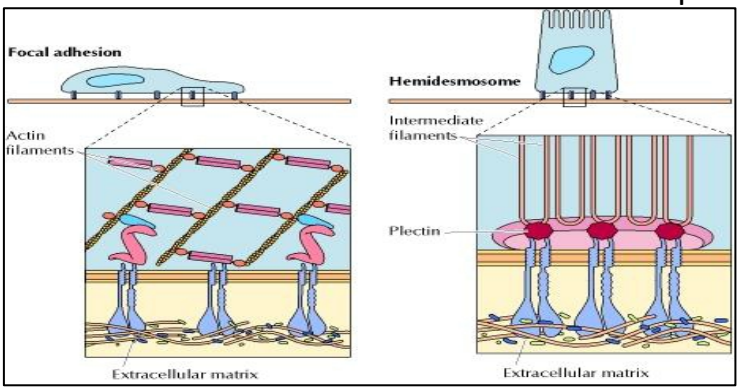
- Integrins are a family of transmembrane heterodimers (α and β).
- They bind to short sequences present in ECM proteins including collagen, fibronectin, laminin and proteoglycans.

- Functions of integrins:

1. The major cell surface receptors that attach cells to ECM
2. They anchor the cytoskeleton at focal adhesions and hemidesmosomes.

1) Hemidesmosomes → **integrin** interacts with ECM from one side and with **plectin** (which interacts with intermediate filaments inside the cell) from the other side.

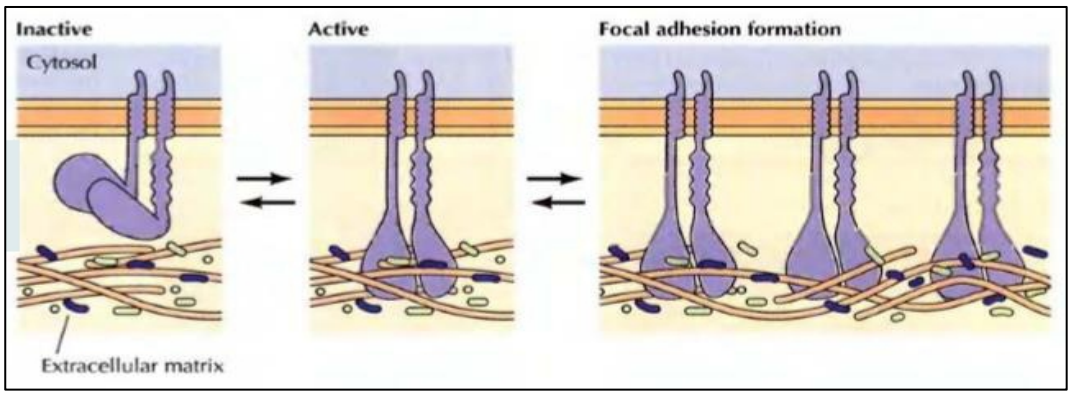
2) Focal adhesion → has membrane proteins "**integrins**" connected to **talin** and to **actin** cytoskeleton inside the cell and interacts with ECM components in the ECM.



Movement of the cell;

Assisted via Focal Adhesions

- When the cell moves, formation and distortion of focal adhesion occurs. This happens through the regulatory sites and the binding sites that exist on these integrins allowing integrins exist in two states; an active state and inactive one.
- To break the focal adhesions, the inactive conformation is required. (the focal adhesion dissociates from the ECM)
- Once the regulatory sites are bounded with a ligand, they are activated and moved towards the active conformation where they form focal adhesion for e.g.



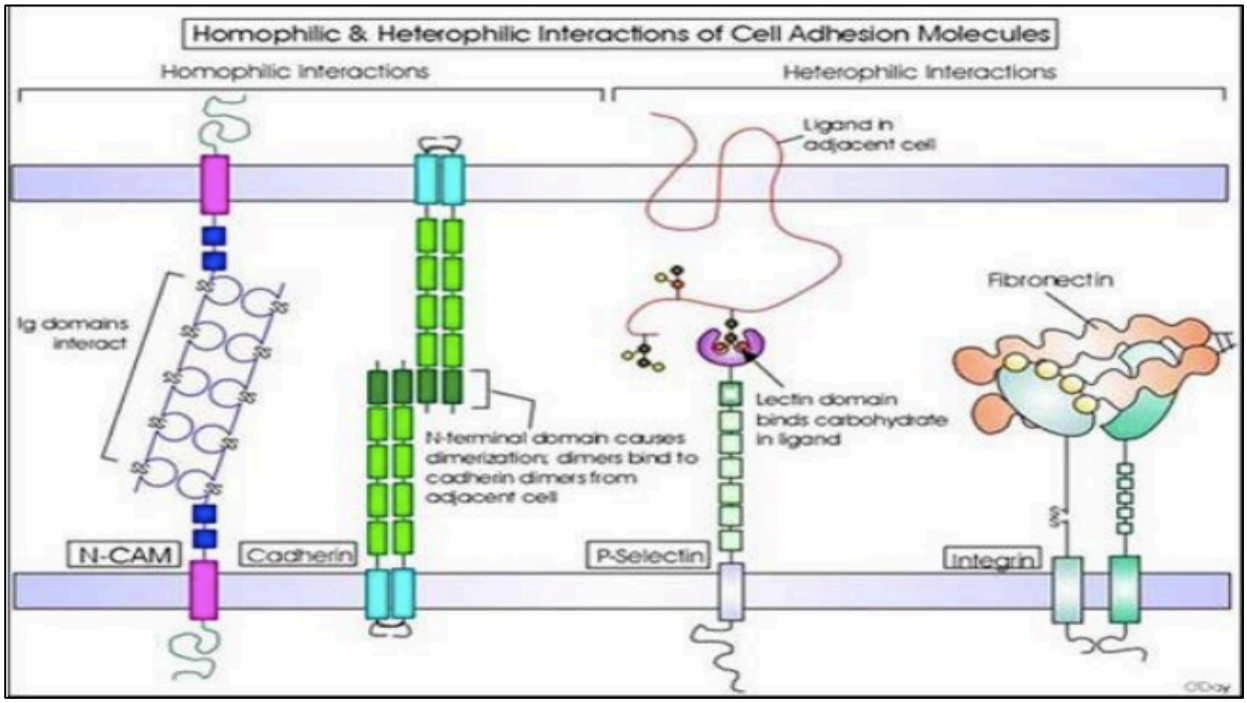
1. **Activation of integrin and binding to ECM.**
2. **Recruitment of additional integrins forming focal complex.**
3. **Development of small integrin clusters called focal complexes.**
4. **Development of focal adhesions by the recruitment of formin, talin, vinculin and a-actinin.**

Cell-cell adhesion is a selective process Cell Adhesion Molecules

- Interaction between the same type of proteins between adjacent cells is called **"homophilic interaction"** → e.g. cadherin dimers from adjacent cells.

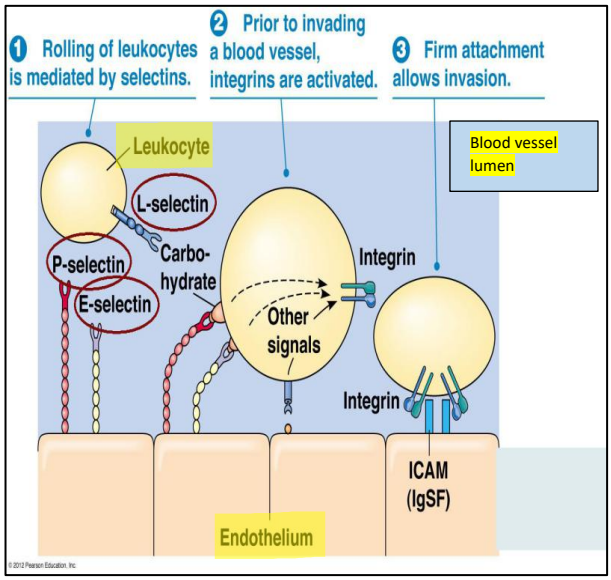
-Interaction between different types of protein or between proteins and sugars is called **"heterophilic interaction"** → e.g. interaction between integrins and Ig superfamily; interaction between integrins and ECM components (focal adhesion and hemidesmosomes)

Family	Ligands recognized	Stable cell junctions
Selectins	Carbohydrates	No
Integrins	Extracellular matrix	Focal adhesions and hemidesmosomes
	Members of Ig superfamily	No
Ig superfamily	Integrins	No
	Homophilic interactions	No
Cadherins	Homophilic interactions	Adherens junctions and desmosomes



*** Just remember the difference between two types of interactions not the examples.**

Selectin-mediated interaction between leukocytes and endothelial cells



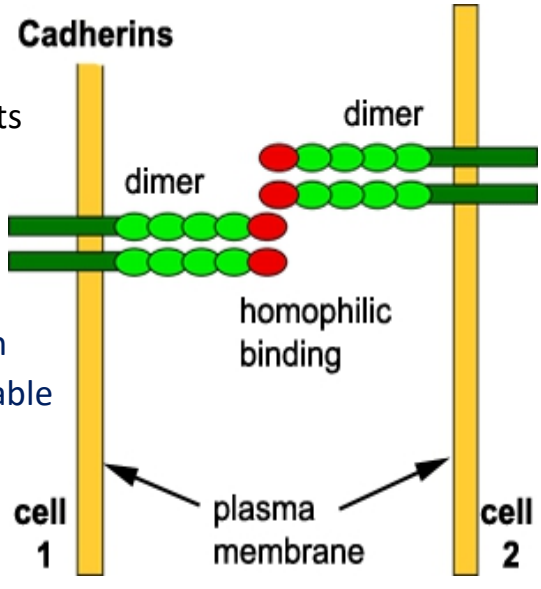
- **Selectins** are proteins that interact with carbohydrates.
- They mediate extravasation (leakage) of leukocytes from blood vessels to the injury site.
-This process starts by the interaction between the selectins present on the leukocyte surface (L-selectins) with the sugar molecules present on the surface of endothelial cells, and the selectins (E and P-selectins) on the endothelial cell surface of blood vessels (simple squamous) interact with sugar molecules on the leukocyte's surfaces.

⟶ this interaction is going to anchor the leukocyte next to the endothelial cell ⟶ this binding will trigger signals inside the leukocyte that bring the integrin close to the surface membrane of endothelial cells ⟶ these integrins start to interact with Ig superfamily on the endothelial cells (heterophilic interaction), specifically "ICAM" which is an intercellular adhesion molecule ⟶ firm attachment of the leukocyte to the endothelial cell occurs ⟶ this will facilitate the movement of leukocyte toward the target site of injury.

Cadherins adhesion molecules

- Mostly participate in **homophilic interactions.**

- A specific domain from a cadherin molecule interacts with the same domain of another cadherin molecule on another cell.



From slides:

*Cadherins are responsible for the selective adhesion between embryonic cells and for the formation of stable junctions between cells in tissues.

- Several subfamilies

1)Classic Cadherins

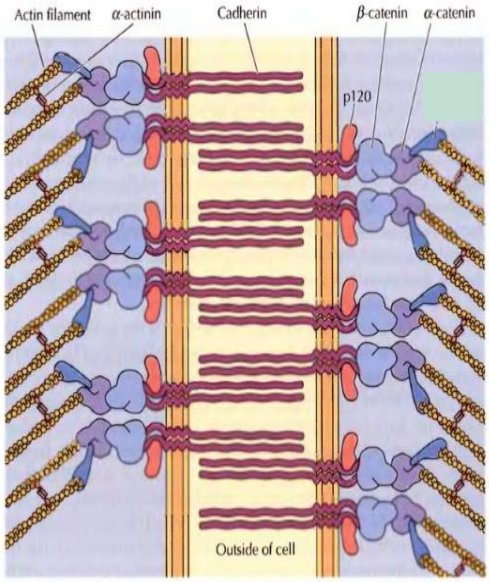
→**E-cadherin**: epithelial cells

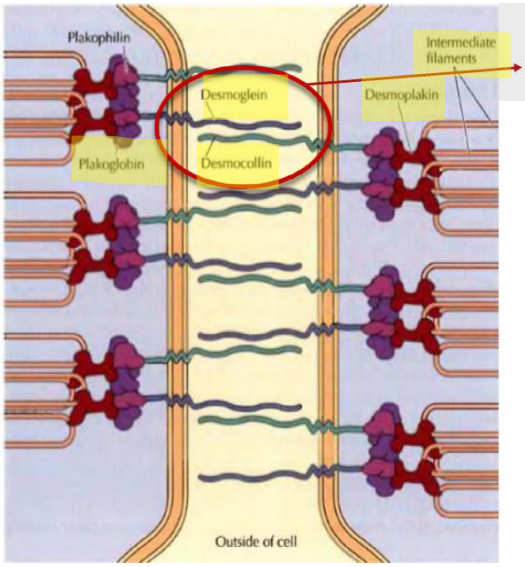
→**N-cadherin**: neural cells

→**P-cadherin**: placental cells

2) Nonclassical types: Desmosomes, fat-like and 7-Transmembrane cadherins

Classic cadherin





Desomosomal cadherin (heterophilic interaction)

*Both desmocollin and desmoglein interact with plakoglobin and desmoplakin (which are linked to intermediate filaments)

Tight Junctions

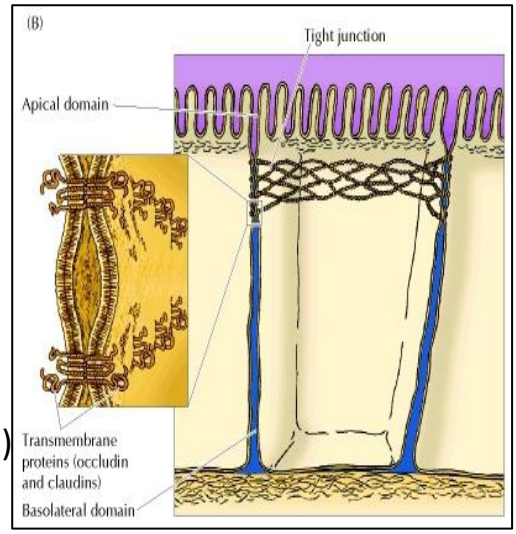
-Tight, impermeable

- Connecting cells together, separate the apical part from the basolateral part of membranes

-They don't allow the movement of molecules in between epithelial cells

- They have many types of membrane proteins (what differentiates them from other types of junctions) such as **claudins, occludin, and JAMs** "Junctional Adhesion Molecules".

- These proteins (claudins, occludin and JAMs) interact with the actin cytoskeleton via **zonula occludins** proteins "ZO".

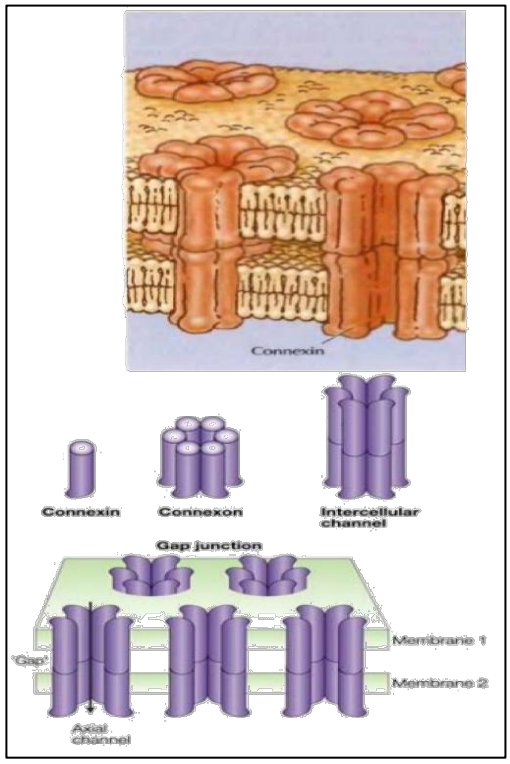


From slides:

- A network of protein strands that continues around the entire circumference of the cell

- Each strand in these networks is composed of transmembrane proteins (claudins, occludin, and JAMs) that bind to similar proteins on adjacent cells, thereby sealing the space between their plasma membranes.

Gap Junctions



-These junctions **communicate** cells together by opening the cytosol of one cell to the cytosol of another cell (what differentiates them from other types of junctions).

- They are channel-shaped, a **channel** (protein complex) from one cell opposing a channel from another cell making the whole structure of a gap junction.

- Extracellular sides of the channel protein complexes of the two cells communicate with each other, so this doesn't permit the formation of adhesion between phospholipids present on the membranes (just the proteins are connected together and there is a space where the phospholipids of the two membranes face one another)

-Molecules of sizes up to 1 thousand Da (small number) can move through gap junction up such as ions and water molecules.

- Most proteins and mRNAs can't move through gap junction because the average size of proteins is between 50 to 60 kDa.

-Gap junctions are important in the transport of action potential and the changes in ion concentration across different cells such as cardiac cells (in the heart so they function together simultaneously as one unit) as well as smooth muscle cells.

Gap Junctions in terms of structure:

- They are made of transmembrane proteins called **connexins**.

- **Six connexins** assemble to form a **connexon**

(channel like structure, a cylinder with an open aqueous pore in its center)

- **Two connexons** on adjacent cells make a **gap junction (**one from each cell)

→ Histological Technique mentioned by the doctor on Gap Junctions:

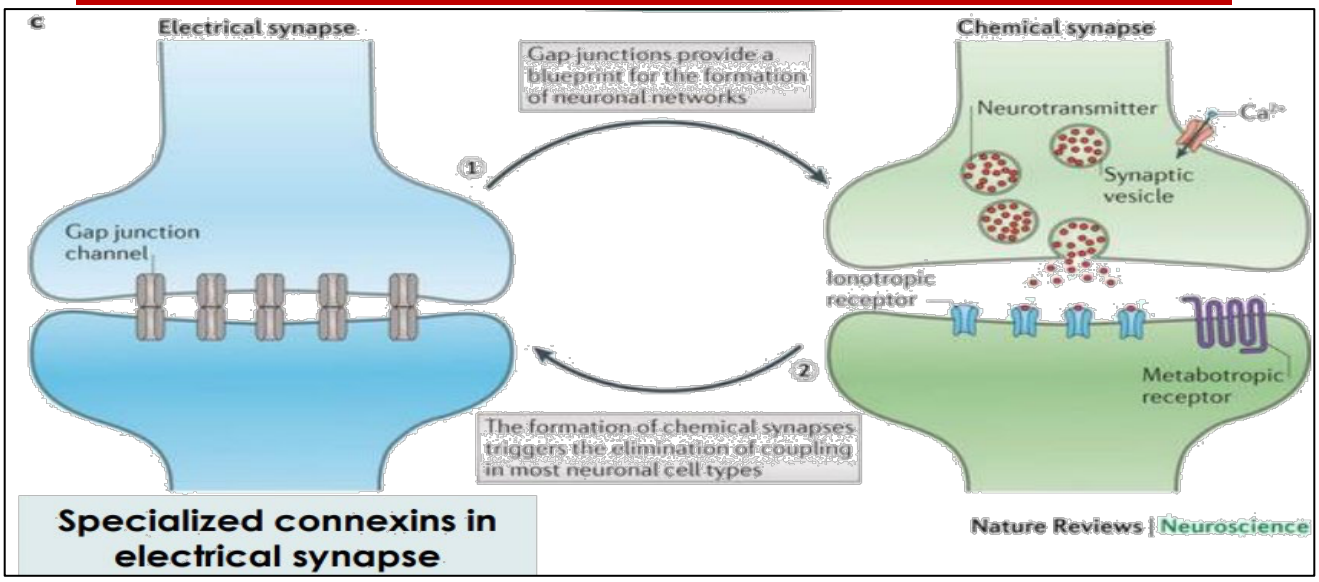
A microinjection of a specific dye will be inserted into a cell (<1000 Da) to determine the presence of gap Junctions in that cell. If gap junctions are present and function well → every cell will take the color. If gap junctions are not present or their function is distorted → only the injected cell will take the color. If the dye is more than 1000 Da and gap junctions are present → the cells will not take the color.

From slides:

-They provide direct connections between the cytoplasm of adjacent cells as open channels allowing ions and small molecules (<1000 Da) including signaling molecules to diffuse freely between neighboring cells, but preventing the passage of proteins and nucleic acids.

-Present in cells like epithelial cells, endothelial cells, cardiac cells and smooth muscle cells.

Gap junctions and electrical synapses



- Initially, for blueprinting and fast connection of neurons together, gap junctions are used between neural cells before the formation of a chemical synapse occurs.

- Gap junctions are put on both presynaptic and postsynaptic neurons.

***Gap junction → electrical synapse**

***Other components → chemical synapse**

- When all components of the synapse are synthesized, inhibition of gap junction occurs to allow the formation of a chemical synapse (gap junctions act as a transient step before a chemical synapse occurs)

Gap Junction Diseases

Mutations in different types of connexins result in many diseases such as:

Charcot-Marie-Tooth disease:

- degeneration of peripheral myelinated nerves

Deafness:

- inability to rapidly exchange K+ which is important in the hearing process due to a problem in gap junctions.

Cataracts*:*

- inability to obtain nutrients from epithelial cells through gap junctions to the lens (avascular tissue needed for the transmission of light) due to an abnormal structure of gap junctions so cells of the lens will die and it becomes translucent which affects the transmission of light.

- one of the most important causes of blindness worldwide.

Skin disease:

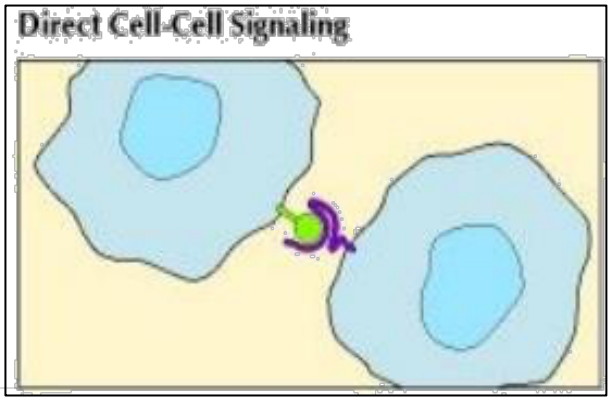
- transport of nutrients to the skin happens through **gap junctions**.

- a problem in gap junctions will lead to a problem in nutrient availability in skin cells.

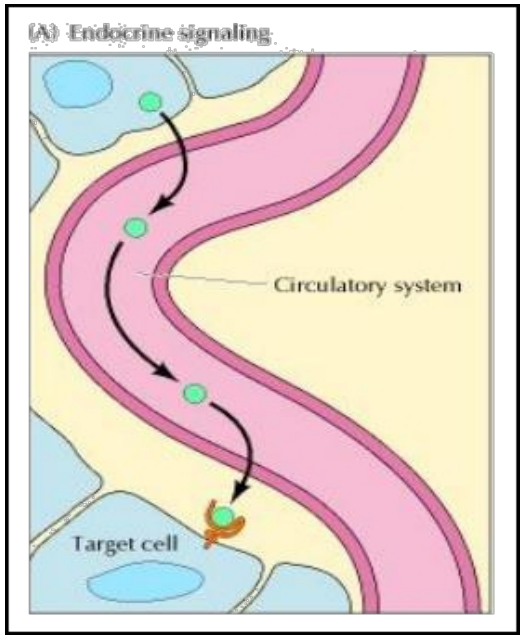
(new slide) Cell signaling

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 neighbor).



2) Signaling by secreted molecules:

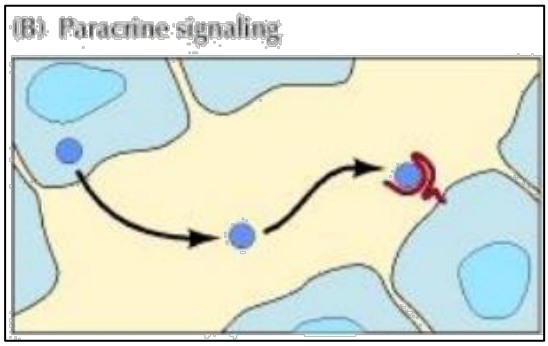


✓ The cell releases a signaling molecule to a target cell. This mode of signaling is divided into three categories based on the distance of the target cell:

A) Endocrine signaling:

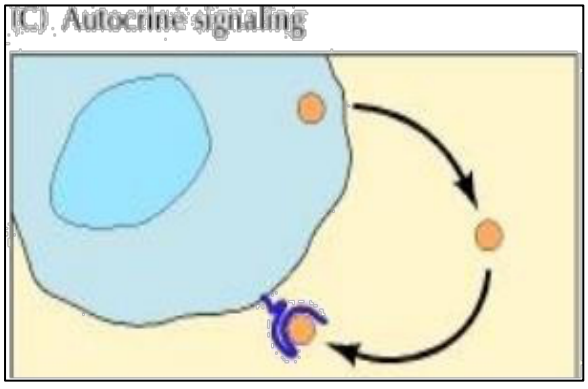
✓ In this case, a molecule is released from one cell into the blood stream to a far or distant target tissue or cell.

B) 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.

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 parathyroid hormone** which are considered peptides (20-50 amino acids)] 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) such as cytokines.

✓ They are categorized according to chemical structure and function into prostaglandins, leukotrienes, and thromboxane B.

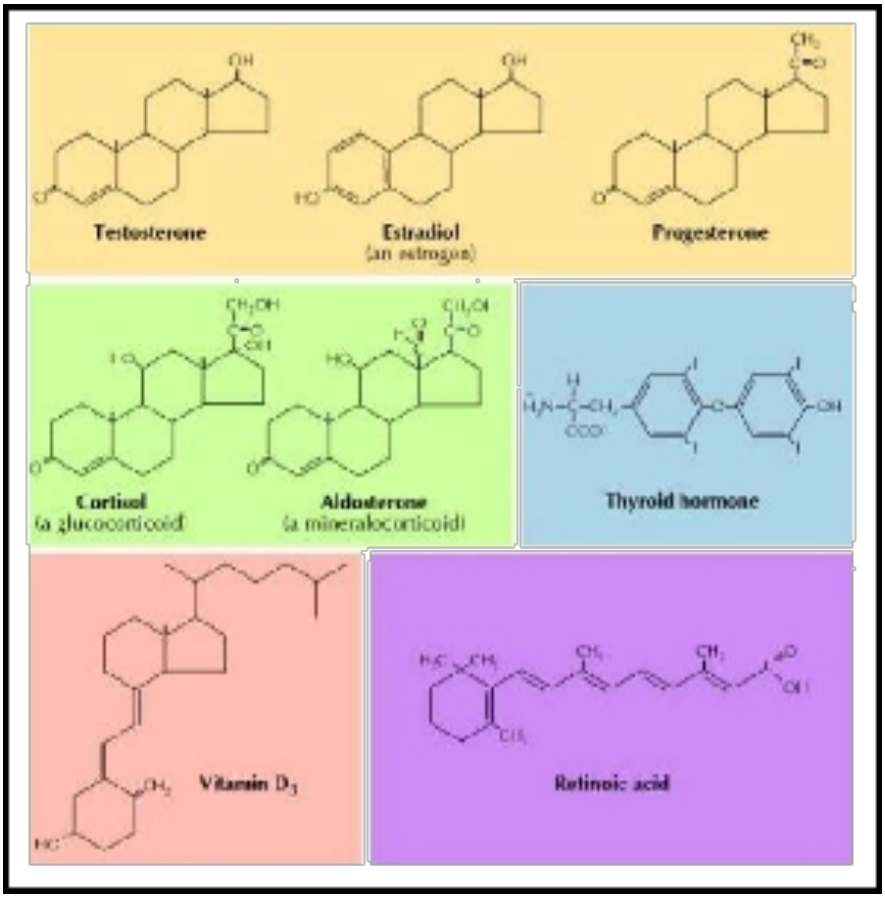
✓ They act as inflammatory mediators

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:

✓ **sex hormones**: testosterone, estrogen, progesterone.



✓ **Adrenal gland hormones**: like cortisol and aldosterone.

✓ Other examples 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

❖ 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:

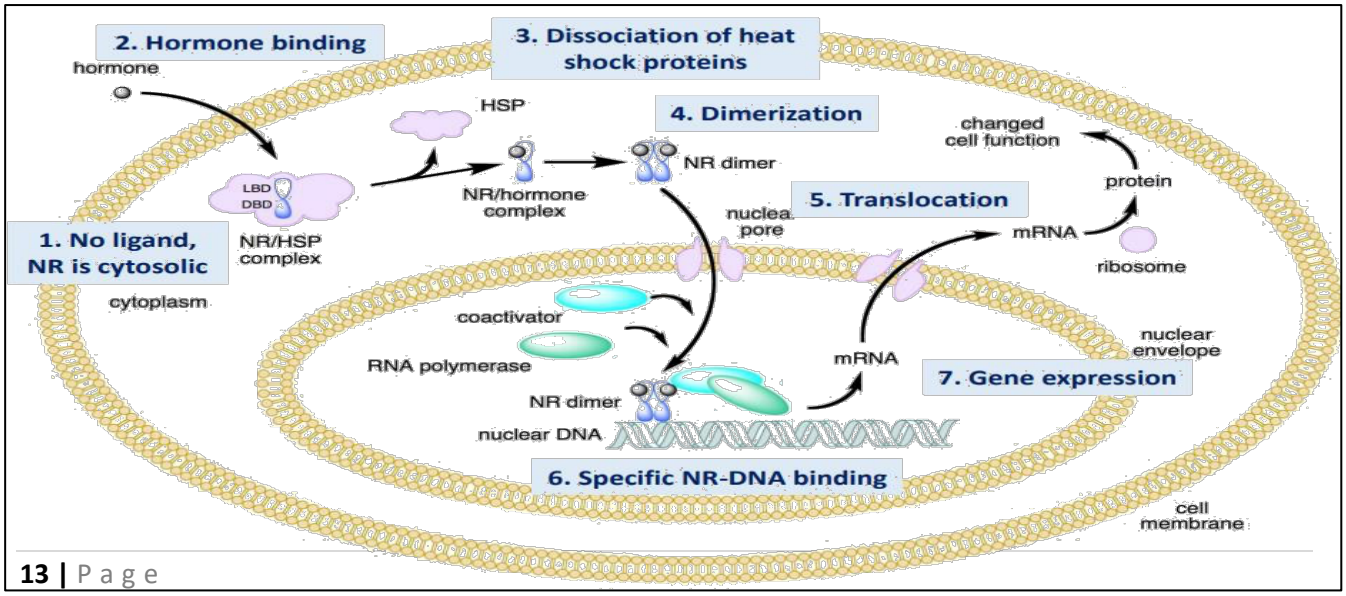
➢ The nuclear receptor is present in the inactive state in the cytosol bound to a protein complex called (HSP- heat shock protein).

➢ Once the hormone enters inside the cell, it is going to bind to the receptor and dissociate the protein complex (HSP) from it.

➢The receptor-hormone complex forms and can dimerize with another receptor-hormone complex forming a nuclear receptor (NR) dimer.

➢ This dimer can enter the nucleus through nuclear pores, and it has the ability to bind certain regions of DNA and activate gene expression. (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:

➢ Are 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.)

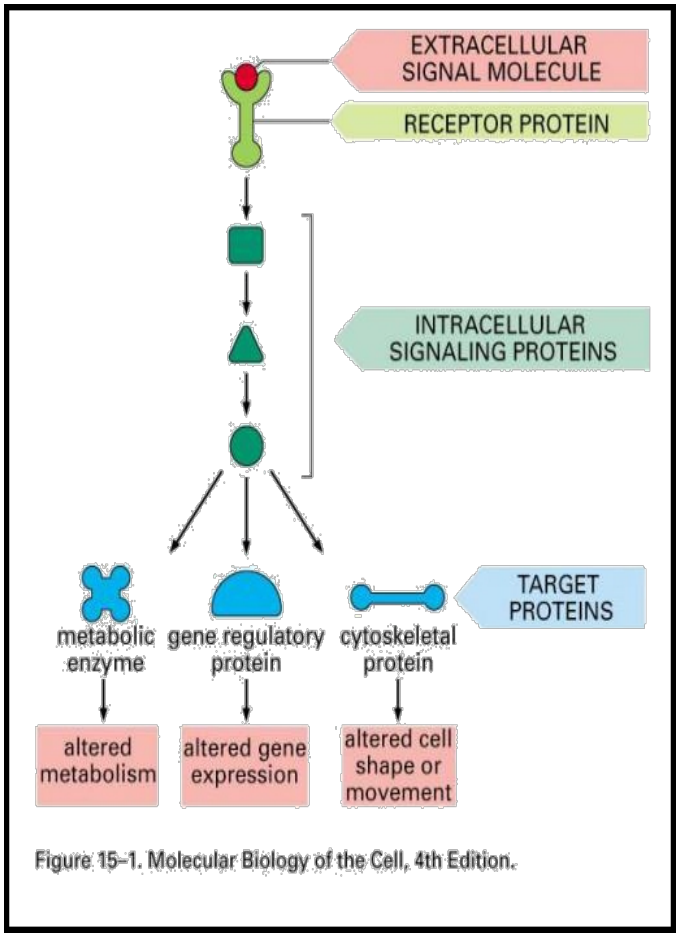


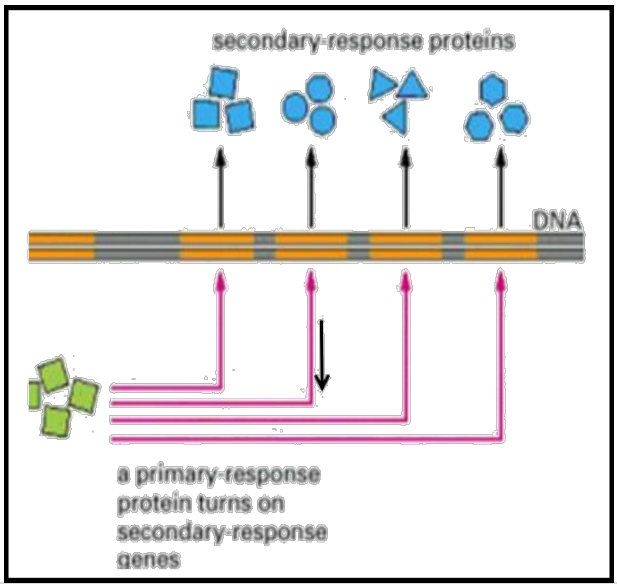
Figure 15–1. Molecular Biology of the Cell, 4th Edition.

Types of Response:

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

1) Primary response:

✓ the ligand binds to the receptor and activates some molecules then activation of gene expression happens which produce a response such as glycogenesis,



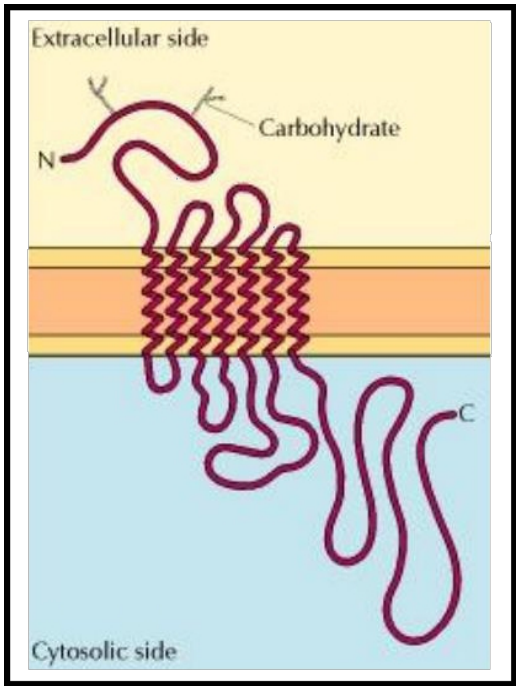
uptake of glucose, movement of the cell or cell division. This response is quick (30 minutes).

2) Secondary response:

✓ If the **primary response products** bind 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. (gene expression occurs due to two steps here)

➢ *Cell Surface Receptors:*

A) G protein-coupled receptors (GPCR):



✓ A family of proteins composed of seven membrane-spanning α helices.

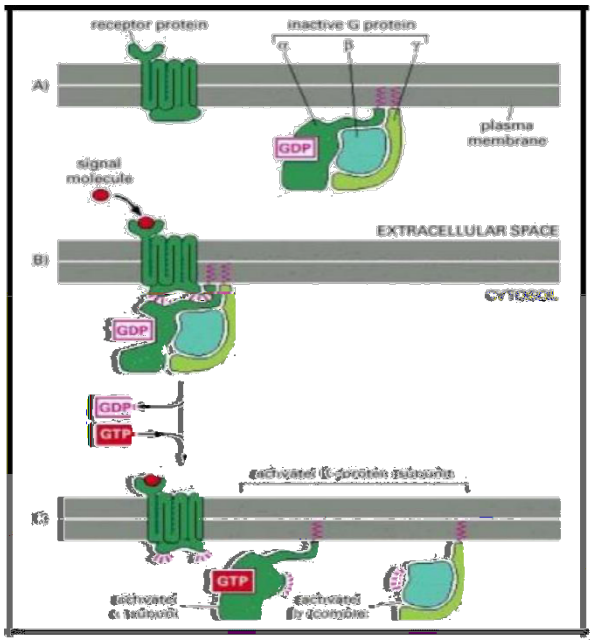
✓ Most abundant type of cell surface receptors

✓ Contains: N-terminus, sugar components, and a **ligand binding site** which are all 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**.

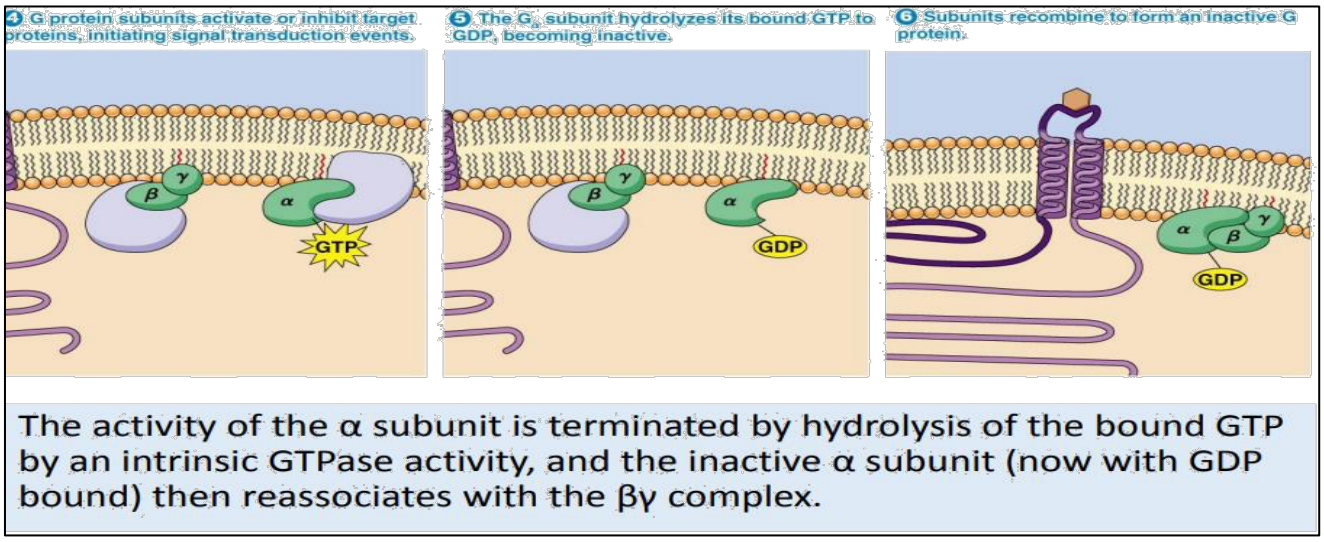
❖ **G-protein activation:**

✓ 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 at the **α subunit (GTP binding protein).**

This exchange causes the G-protein trimer to dissociate into active components:

α subunit alone and a βγ complex.

This complex dissociates from the receptor. The activated components (specifically the α subunit) will cause downstream events and binds to other effectors allowing a response to be produced.



❖ G-protein inactivation:

✓ At some point, there is need for the inactivation of the GPCR pathway. This is accomplished by hydrolyzing GTP to GDP. This allows the subunits (α, β, γ) to assemble again and stray further away from the GPCR.

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.

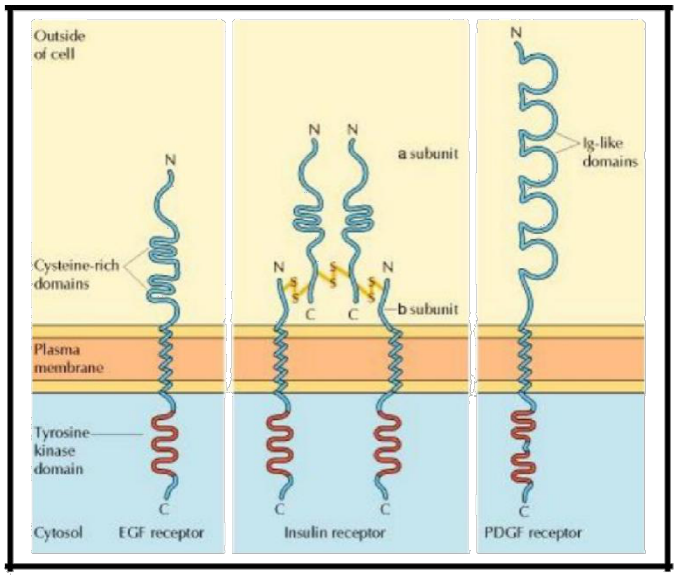
✓ RTKs are also enzymes (they have enzymatic activity as part of the protein itself).

✓ There are several subtypes of RTKs:

1) Epidermal Growth Factor (EGF) receptor

2) Insulin receptor (Insulin-like growth factor 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 to the ligand)** varies among the different receptors, because different receptors have different ligands.

➔ *Binding of the ligands extracellularly activates the cytosolic (intracellular) kinase domains, resulting in the phosphorylation of both the receptors themselves and intracellular target proteins.*

❖ Mechanism of activation of RTKs:

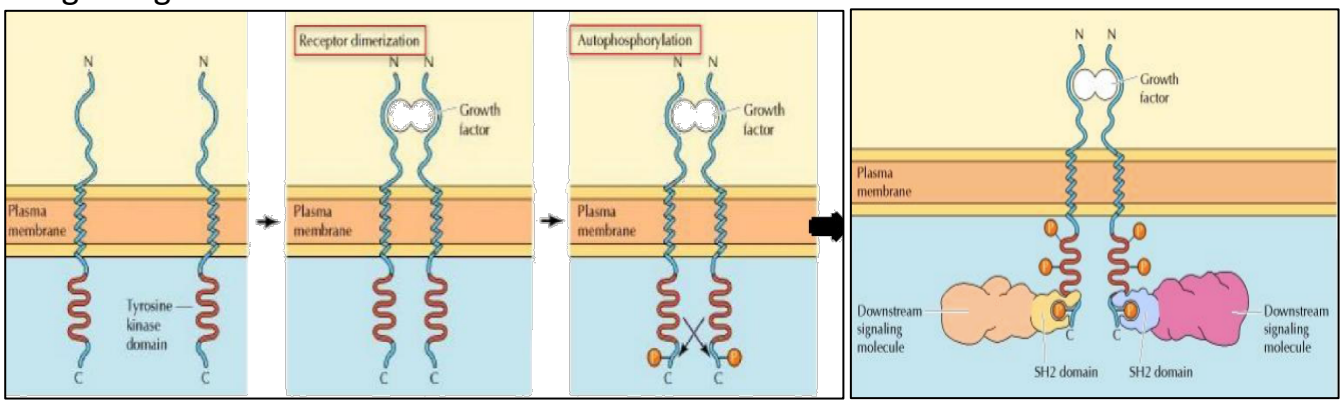
✓ The RTKs are present as single molecules in the plasma membrane found in the inactive state.

✓ Once the growth factor (ligand) binds to one receptor, it induces the dimerization with another receptor. [Present now is a dimer with a growth factor/ ligand (a signaling molecule) bound to it].

✓ The receptors are activated now, and each receptor phosphorylates the tyrosine of the other receptor in the dimer (autophosphorylation occurs) which includes:

1) Phosphorylation of tyrosine ***within*** the **kinase domain** (the red intracellular part of the RTK in the diagram below) which **increases the kinase activity and facilitates the activation of proteins** (these have no association with any downstream effectors or signaling molecules)

2) Phosphorylation of tyrosine ***outside*** the **kinase domain** (the blue intracellular part of the RTK in the diagram below) which creates **high affinity binding sites (attractive sites)** for the binding of other signaling molecules, effectors, and downstream signaling molecules.



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

➢ Tyrosine kinase is not part of some receptors like cytokine receptors (which binds with TNF for ex). So, the enzymatic activity (phosphorylation) 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 (cytokine) binds, dimerization occurs (dimers of receptors are formed).

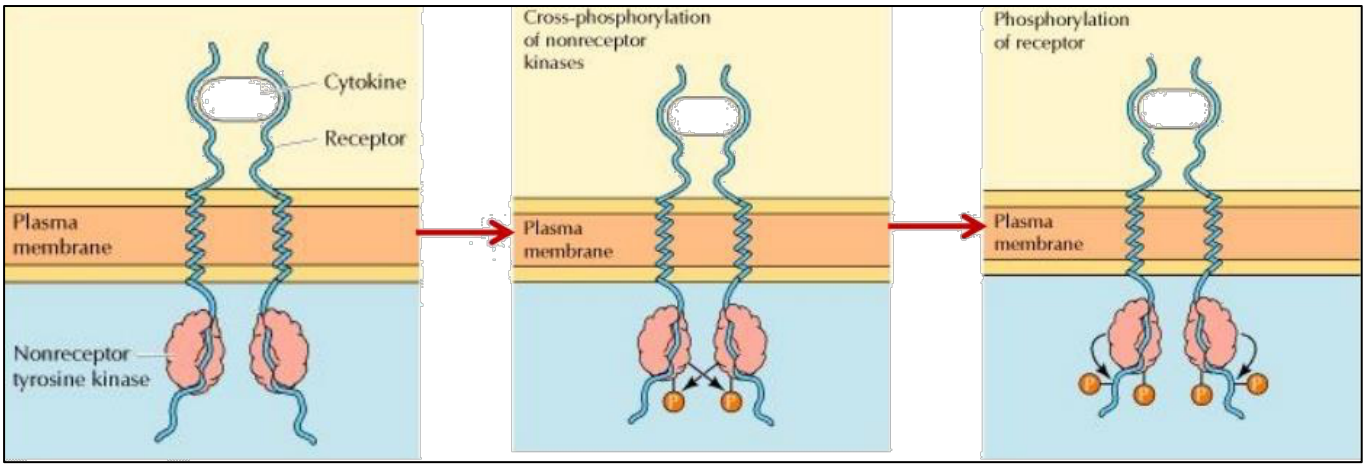
✓ This brings in intracellular molecules called **nonreceptor protein tyrosine kinases**. These molecules will carry out phosphorylation.

✓ 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 tyrosine on the receptor to which it is bound.

✓ The phosphorylated tyrosine on the receptors now acts as attractive sites for the binding of effectors and downstream molecules.

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



Other types of receptors include:

- Protein-tyrosine phosphatases: which remove the phosphates and lead to inactivation. (activation and inhibition roles)
- Protein-serine/ threonine kinase: which phosphorylates serine/threonine associated with transforming growth factor β receptors (TGF – β)
- Receptor guanylyl cyclase
- Protease-associated receptor: tumor necrosis factor (TNF)

Second Messengers

Why are second messengers important?

➔ *As long as (for e.g.) the G-protein is active and bound to the ligand, the receptor will be active and will bind to second messengers as well as downstream molecules, activating them and later on detaching from them. Further second messengers and downstream molecules will continue binding onto the receptor and so on. Thus, a single receptor, as long as it's active, will have a large amount of second messengers and downstream elements*

constantly binding, one after the other, to it, leading to signal amplification. This is <u>not</u> a one to one relationship because when the ligand binds to the receptor it doesn't activate only one second messenger molecule, it activates multiple second messengers and downstream molecules, not simultaneously, but rather one after the other.

(**Signal amplification** by the generation of second messengers)

➔ *Membrane proteins also cannot enter the cell (they are attached to the cell membrane) and that's why second messengers are required to transfer the messages inside the cells.*

(They are often free to **diffuse to other compartments** of the cell)

➔ *To allow cross- talk (side conversation)/ interaction between pathways. For example, cross-talk between the pathway for G-protein coupled receptors and the pathway for receptor tyrosine kinase. Since there is a large amount of second messengers and molecules in both pathways there is a chance for interaction between these two pathways either through processes of activation or inhibition.*

(Common second messengers in multiple signaling pathways often result in **cross-talk** between different signaling pathways)

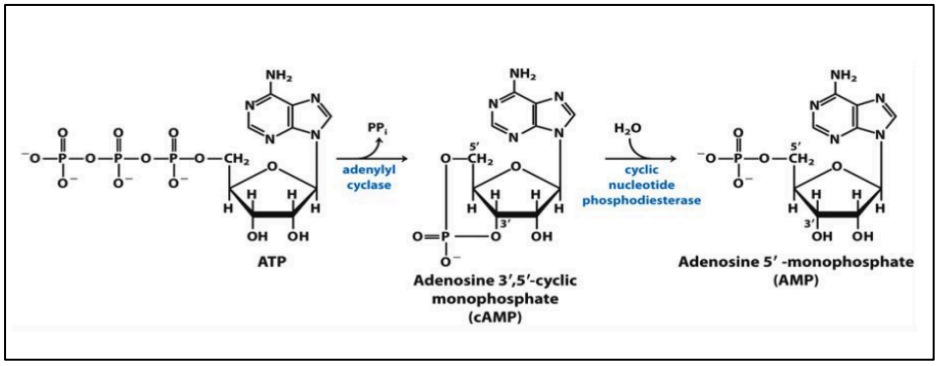
A) <u>cAMP (an e.g. on second messenger)</u>

~Formation of cAMP~

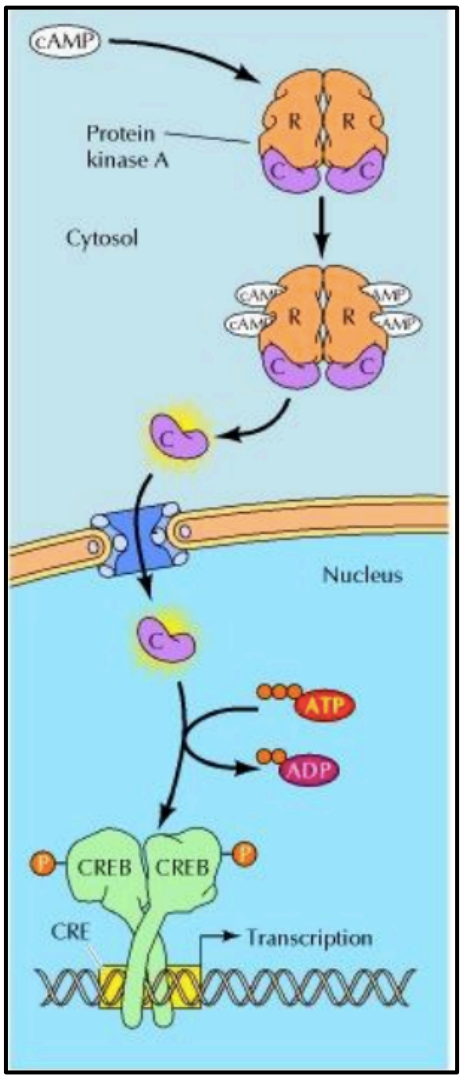
-cAMP is formed from ATP by the action of *adenyl cyclase*. cAMP contains a phosphate group attached in the shape of a ring (cyclic).

~Inactivation of cAMP~

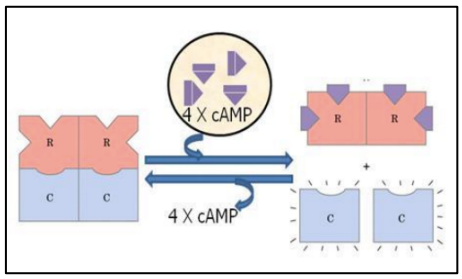
-The phosphate ring gets degraded from one side only by the action of *cyclic nucleotide phosphodiesterase* (requires water) and forms AMP.



Mechanism of Action of cAMP:

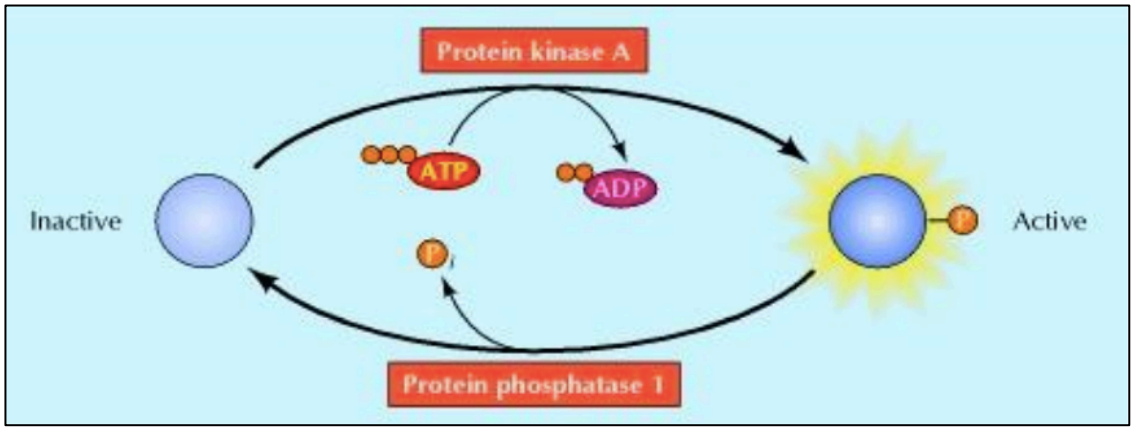


-cAMP binds to protein kinase A (PKA). This kinase has four regions, 2 regulatory domains and 2 catalytic domains. The regulatory domains (shown in orange in the diagram) control the activation or the inactivation of the kinase. The catalytic domains (shown in purple in the diagram) are responsible for the phosphorylation. When the (**4**) cAMPs bind to the protein kinase A, they bind to its regulatory domains. This leads to the dissociation of the catalytic domain. The free catalytic subunit later translocates into the nucleus through the nuclear pore complex, takes the phosphate from ATP, and phosphorylates the **transcription factor CREB** thus activating it (CRE-binding protein). This CREB binds to a region in the DNA called **CRE** region leading to the expression of cAMP-inducible genes.



Protein Kinase A Regulation by dephosphorylation:

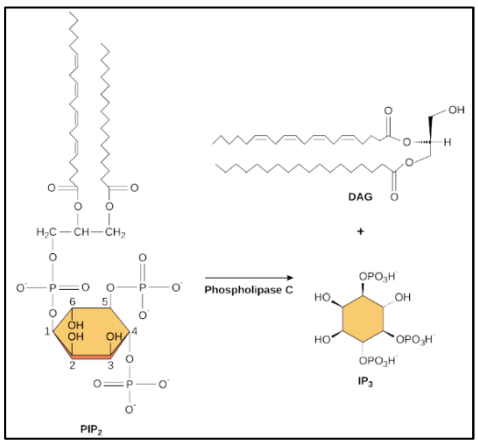
-The phosphorylation of target proteins by protein kinase A is reversed by the action of *protein phosphatase 1.*



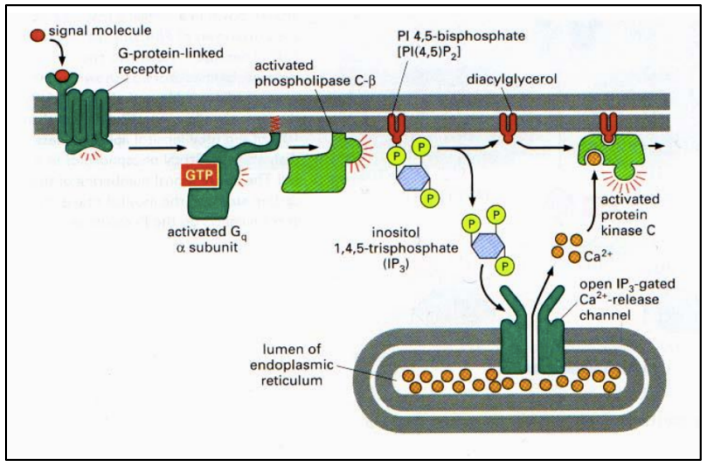
B)Ca2+

➔ *Phospholipids and calcium ions* ~ associated with G-protein coupled receptor.

*STEPS:



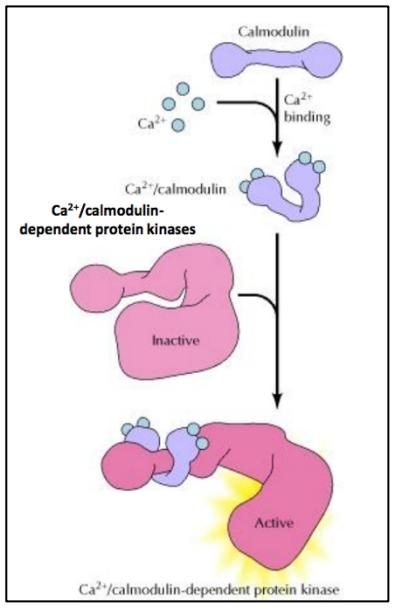
1- A signaling molecule binds to the G-protein linked receptor and this activates the alpha subunit of the receptor
2- Exchange occurs (GDP is exchanged with GTP -> active conformation)
3- The active alpha subunit activates phospholipase C
4- The phospholipase C degrades a type of phospholipid called **PIP2** (contains 3 phosphates) -> forming diacylglycerol (**DAG**) which remains attached to the membrane and **IP3** (contains the three phosphates and is considered a polar head so doesn't bind to the cell membrane)



5- This IP3 now can bind to an IP3 gated calcium channel found on the ER membrane inside the cell
6- Calcium is later on released from the ER
7- Calcium binds to protein kinase C thus activating it
8- Phosphorylation of target molecules

➔ *Calmodulin and Calcium ions*

~ Cal means it attaches to calcium, once **calmodulin** binds to **calcium** its conformation changes and it can bind to **calcium/calmodulin dependent protein kinases**. Once this binding happens, the calcium calmodulin dependent protein kinase is now active and can regulate the synthesis and release of neurotransmitters.



The end of this sheet… finally ☺