Genetics
& Cell biology

◉ Sheet ○ Slides

Number

11

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Last time we talked about the major components of the ECM: fibrous proteins and their different types, polysaccharides, and adhesion molecules that connect those components.

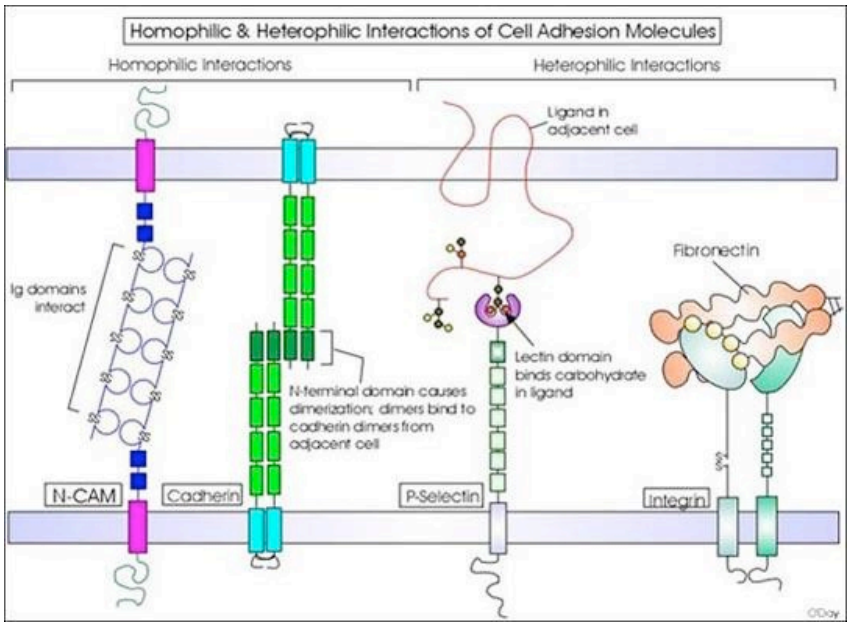
Now the cells need to anchor themselves in the ECM, and this is facilitated by cellular junctions. The junctions mentioned in the previous lecture function in connecting ECM elements to each other and to the cytoskeletal elements of the cell through the plasma membrane. Those cell-cell interactions and cell- ECM interactions are basically interactions between proteins (might be the same or different).

Examples on each:

1. Interactions between proteins of the same kind (***homophilic interactions***): In adherent junctions, the interaction is between two cadherin proteins.
2. Interactions between different types of proteins (***heterophilic interactions***) like desmosomes.

Plasma membrane proteins are going to interaction with the ECM or another membrane protein of another cell. Cellular junctions contain many families of plasma membrane proteins including:

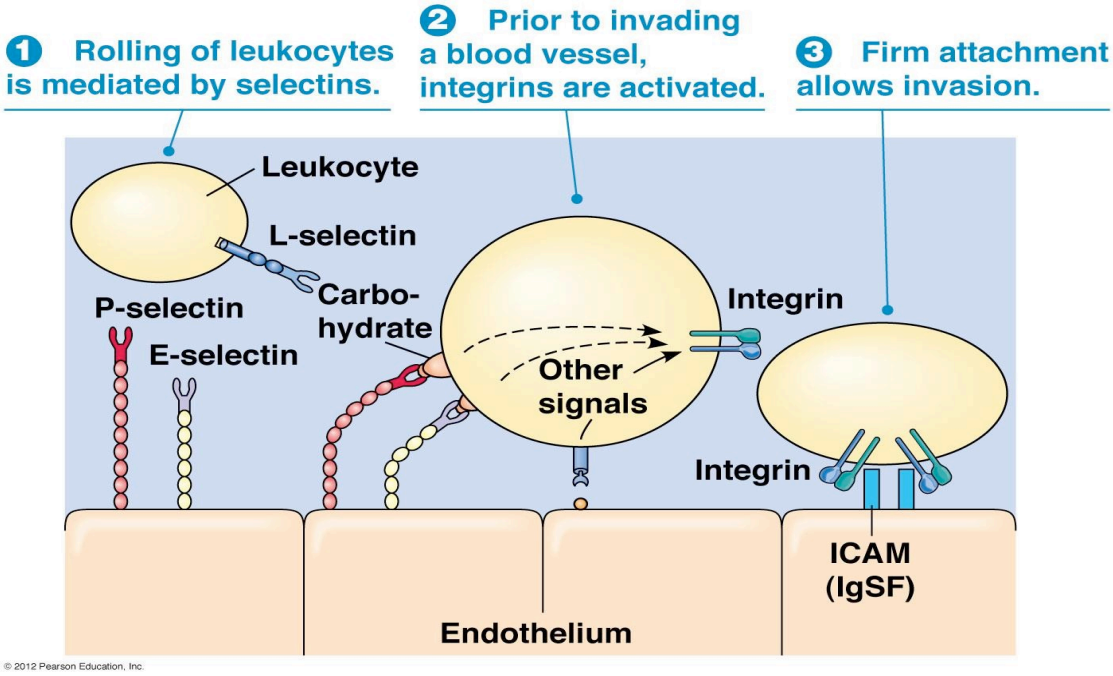
1. **Cadherin**: form homophilic interactions, and plays a role in adherens junctions and desmosomes.
2. Selectins: they interact with sugar molecules that protrude from the cell membrane. (Can't make homophilic interactions)
3. Ig superfamily: form homophilic interactions or interactions with Integrin.
4. **Integrins**: Can interact directly with the extra-cellualr matrix through focal adhesions, or with the Ig superfamily. Plays a role in 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

SO, one difference between desmosomes and hemidesmosomes is the type of plasma membrane protein they attach to. The first is attached to cadherin while the latter is attached to integrin.

Selectin- mediated interaction between leukocytes and endothelial cells:



Leukocytes are present in the blood circulation, but in case of inflammation, they need to enter the tissue (extravasate). So, they are going to cross many layers including the endothelial layer of the blood until they reach the targeted site.
This process starts by the interaction between the selectins present on the leukocyte surface (L-selectins) with a sugar molecule present on the surface of endothelial cells, and the selectins on the endothelium cell surface of blood vessels (E and P-selectins) interacting with sugar molecules on the leukocytes surfaces.

Selectins only interact with sugar molecules they don't interact with each other[1]. Such interactions will bring the leukocyte to close proximity to the endothelium, and also to fix its position such that they are at close proximity to the inflammatory site.

The actual extravasation process is mediated by an interaction between the integrins on the leukocytes and ***ICAM*** (part of the IG family).

Once the ICAM and its substrate are in close proximity they bind together[2] pulling the leukocyte inward between the endothelial cells. Then the leukocyte is able to move to the target site.

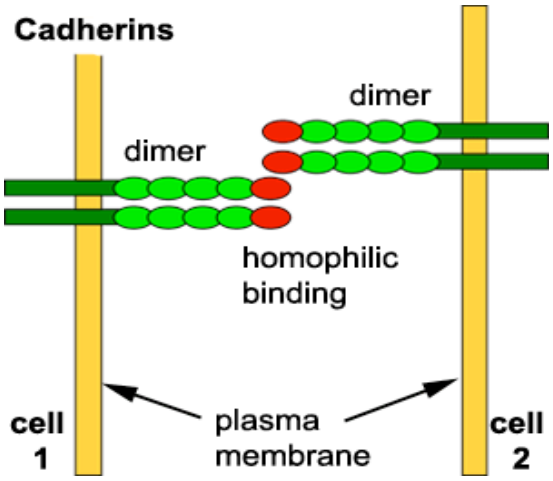
[1] **Please note that for this step to happen, 2 different selectins (one on the leukocyte and one on the endothelial) needs to interact with 2 different sugar molecules. Those selectin protein are not interacting with each other! Because the selectin protein is relatively huge compared to the sugar part, so if the interaction is between 2 selectins, the cells can't get close to each other.**

[2] **ICAM: Intercellular adhesion molecule**

Membrane proteins in depth:

Cadherins

- Function: **Selective** adhesion between embryonic cells and formation of stable junctions between cells in tissues.
- It is a big family divided into:
  A. Classical Cadherins:



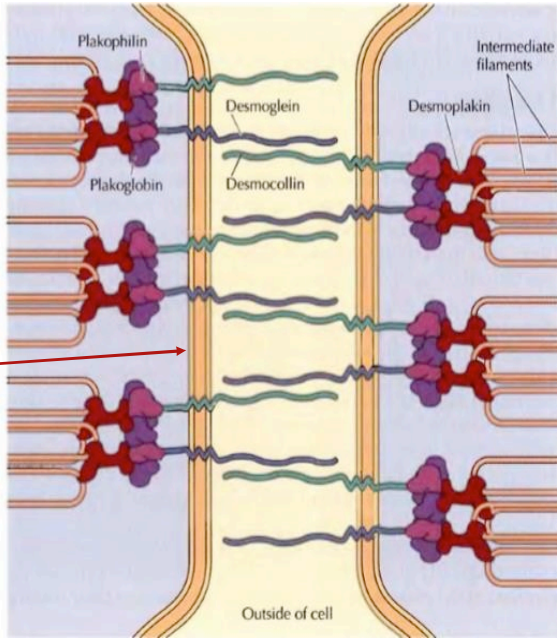
- E-cadherin: epithelial cells
- N-cadherin: neural cells
- P-cadherin: placental cells

They participate in the formation of adherens junctions. (hemophilic interactions, linked to actin filaments).

Subfamilies:

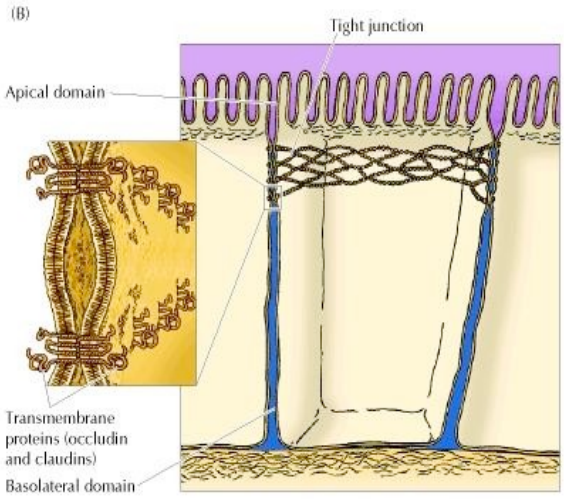
- Classic, Desmosomal, fat-like and 7-Transmembrane cadherins.

- *Non-classical* Cadherins also play a role in those junctions, like the one in desmosomes. The interaction is **heterophilic interaction** because it is made of two ***TYPES*** of cadherin. Also notice the *desmoplakin* that binds to the intermediate filaments from one side and to the cadherin from the other side.



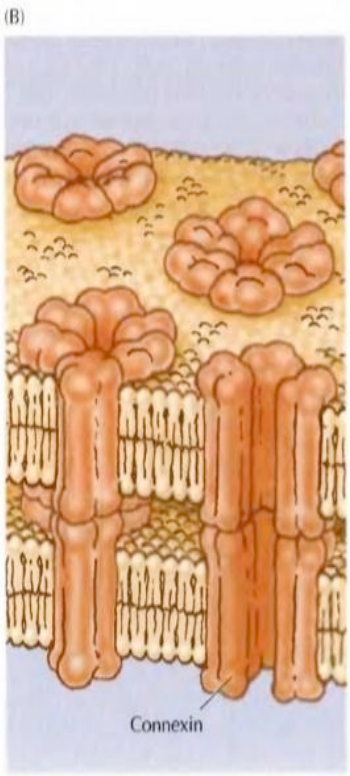
Types of Junctions (a continuation from last lecture):

1. ***Tight junctions*** *(zonula occludens):*
   From its name, it links the cells to each other in a way that makes it hard for the molecules to pass between those cells. It's a network of protein strands that continues around the entire circumference of the cell (epithelial 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.



   As we can notice the main membrane protein of the tight junction can be from different families like mentioned above.
   The protein, on the other hand, that links the tight junctions to the cytoskeleton is zonula occluden (ZO), and it has different subtypes. The subtypes of ZO are what make the tight junction of one cell type different than that of another.
   Tight junctions also separate the apical part from the basolateral part of membranes.

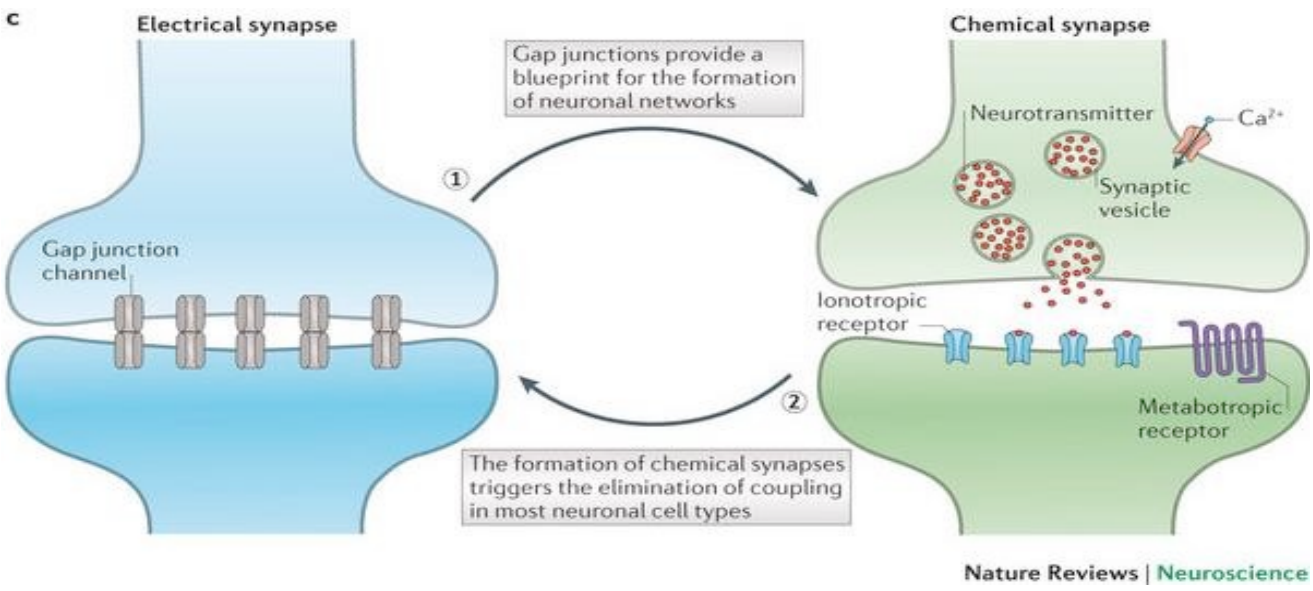
2. Gap junctions:
   - They provide **direct connections** between the cytoplasms of adjacent cells as **open channels** allowing **limited movement of molecules** like ions and small molecules (<1000 Da) including signaling molecules between neighboring cells, but **preventing the passage of proteins and nucleic acids.**



- Present in cells like epithelial cells, endothelia cells, cardiac cells and smooth muscle cells.
- The two proteins that make up the gap junctions have extracellular domains. Those domains get in close proximity with each other, yet the plasma membrane of both cells are still not close to each other. This forms a gap/ space between the two cells instead of them being close to each other. This is why they're called gap junctions.
- Gap junctions are made of transmembrane proteins called **connexions.**
- Six connexins assemble to form a ***connexon*** (a cylinder with an open aqueous pore in its center).
- Two connexons (functional unit) on adjacent cells make a gap junction.

The role of gap junctions in the development of neurons:

During development and also in adulthood the synapses between neurons will develop. They start first by forming gap junctions that will act as blueprints that determine the future location of receptors on the post synaptic membrane of neuron. The pre-synaptic neuron on the other hand releases



substances through vesicles and not through the gap junctions such that an interaction or stimulus is required for the stimulation of neuron rather than the continuous release of chemicals through gap junctions. Thus, the blueprinting of synapses is done by gap junctions. By changing the gap junctions, we are reducing the speed at which the signal is transmitted.

In adulthood when we study “regularly” for example, we ONLY make new synapses between existing neurons. This is how long memory is formed.

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

a) **Charcot-Marie-Tooth disease** (degeneration of peripheral myelinated nerves)
b) **Deafness**: inability to rapidly exchange K^+
c) **Cataracts**: inability to obtain nutrients from the lens epithelial cells, the eye turns into an opaque structure which is unable to pass the light to structures such as the optic nerve.
d) Skin disease

The End of Topic

Cell Signaling:

Cells communicate with each other through signals. One cell produces a molecule that binds to certain receptor. This receptor gives intracellular signal for the cell to do the required action. How? It activates the required gene for the action. For example, if the cell needs to move, it will activate the gene responsible for they synthesis of actin because it is needed in high amount for the movement of the cell.
Any signaling pathway at the end will affect the gene expression (activation or inhibition).
Each signaling pathway has certain targeted genes.

➔ Modes of cell signaling:

1. Endocrine: signaling molecules are secreted by endocrine cells and carried through the circulation to act on target cells at distant body sites.
2. Autocrine: cells respond to signaling molecules that they themselves produce. (most common in cancer cells)
3. Paracrine: a molecule released by a cell acts on neighboring target cells. (Local hormones; they don’t travel through the blood stream)
4. Direct interaction of a cell with its neighbor through junctions.

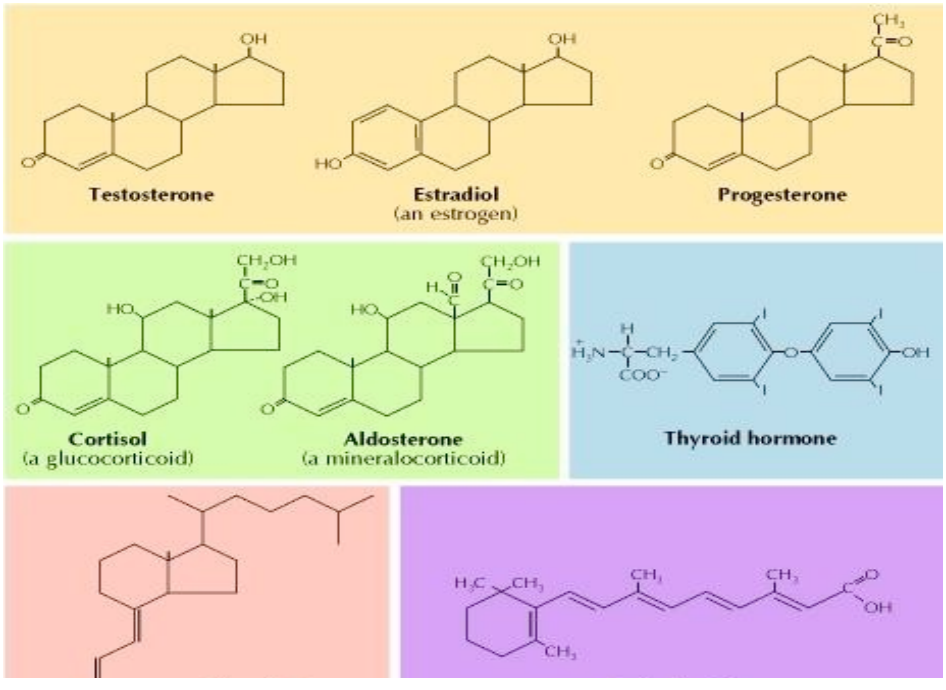
The molecules that get secreted, despite the mood of signaling, are classified chemically to several categories:

1. Protein: contain more than 50 amino acids.
2. Peptides: 40-50 amino acids like the parathyroid hormone, growth factors (EGF), peptide hormones (insulin, glucagon), or neuropeptides (oxytocin, enkephalins)
3. Small molecule neurotransmitters: derived from amino acids like epinephrine and thyroid hormone (tyrosine), serotonin (tryptophan).
4. Steroids: derived from cholesterol like estradiol, cortisol, calciferol (Vitamin D), testosterone and vitamin A.
5. Eicosanoids: derivatives of arachidonic acid including prostaglandins, leukotrienes, and thromboxanes B.
6. Gasses: Nitric oxide (NO, in oxidative stress) and carbon monoxide (CO). (VERY RARE)

Signaling molecules also are classified into:

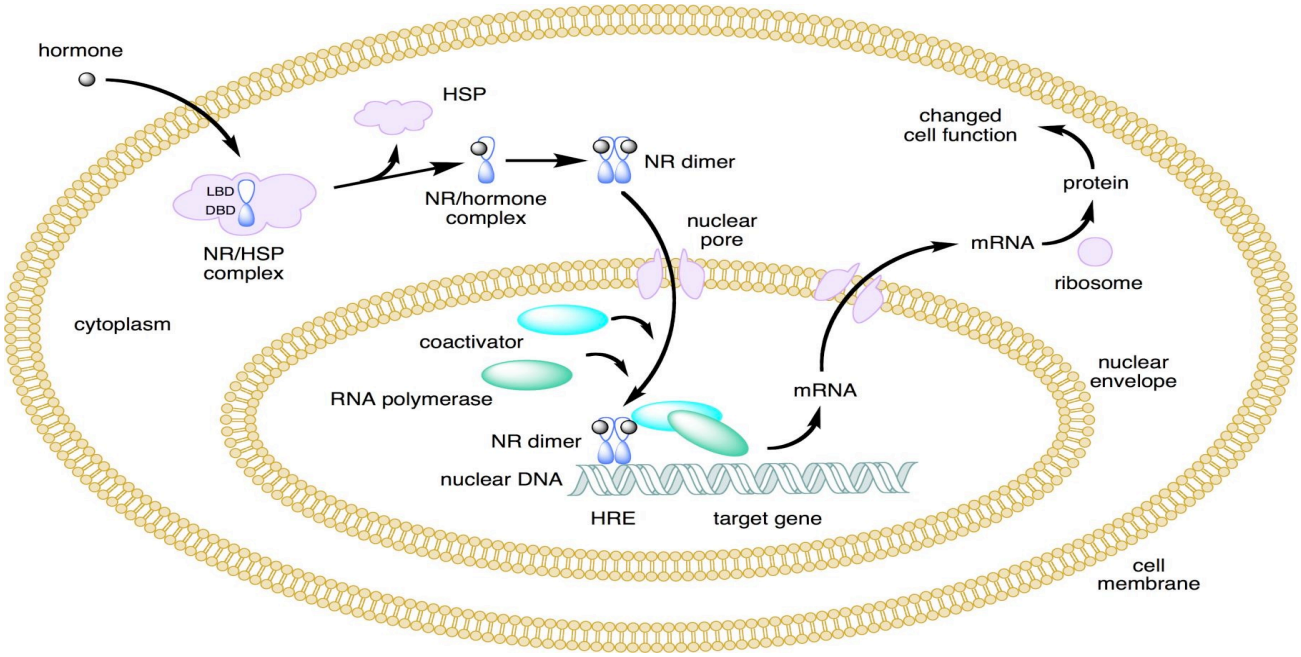
1. Fat soluble: their receptor is inside the cell. Thus, they need to cross the membrane to enter and interact with their receptor.
2. Water soluble: their receptor is a plasma membrane protein.

➔Lipophilic hormones:



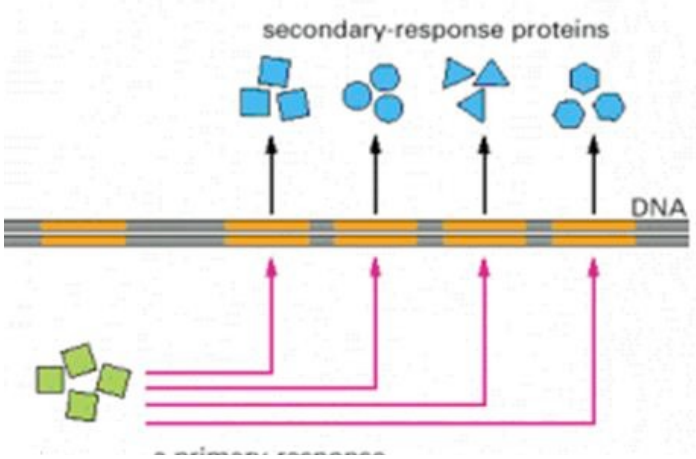
Steroids:

Mechanism of action of hydrophobic molecules:



1. The hormone crosses the membrane in its inactive form. The receptor also is in a protein-complex form to stay in the inactive state.
2. The hormone binds to its receptor: once the hormone gets inside the cell, it is going to bind and induces conformational changes ➔ the complex is no longer there and the ligand is bound to the receptor. Then the ligand-receptor complex is going to dimerize (2 receptors with 2 ligands).
3. The dimer is transported to the nucleus through the pores.
4. It bind to a certain part of the DNA
5. It activates the expression of the targeted gene ➔ mRNA ➔ translated to proteins.

In this mechanism we can notice that the receptor and its ligand work as transcription factors.



Types of responses we get from these signals:

- Primary response: direct activation of a small number of specific genes (30 minutes).
- Secondary response: the protein products of the primary response activate other processes in the cell.

Players of signaling by cell surface receptors:

1. ***Ligand*** (hormone, growth factor)
2. ***Receptor*** (GPCR, RTK):
3. ***Transducers*** (G protein, Ras)
4. ***Effector molecules*** (adenylate cyclase, MAPK)
5. ***Second messengers*** (cAMP, cGMP, Ca2+)
6. ***Final target molecules*** (e.g., DNA, channel)

➔ Response

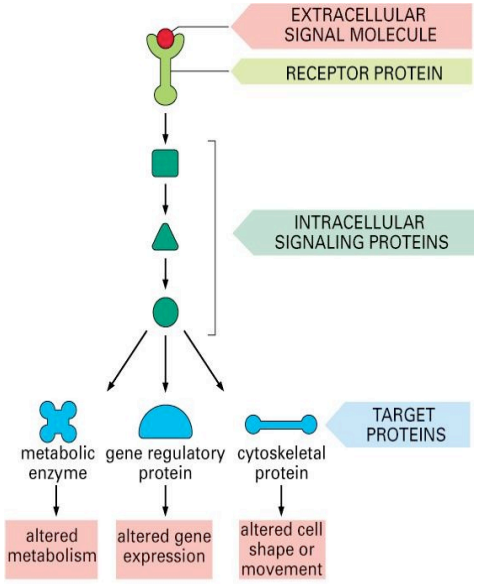


Figure 15–1. Molecular Biology of the Cell, 4th Edition.

We already talked about the ligands so we'll talk start by the types of ***receptors:***

1. *G protein-coupled receptors (GPCR):*

- A family of receptors composed of **seven** membrane-spanning α helices.
- Ligand binding to the *extracellular domain* of GPCRs induces a conformational change that allows the *cytosolic domain* of the receptor to bind a G protein.
- G proteins are composed of three protein subunits—α, β, and γ. (Heterotrimeric G proteins)
- 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 exchange causes the trimer to dissociate into active components: α subunit and a βγ complex.
- 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

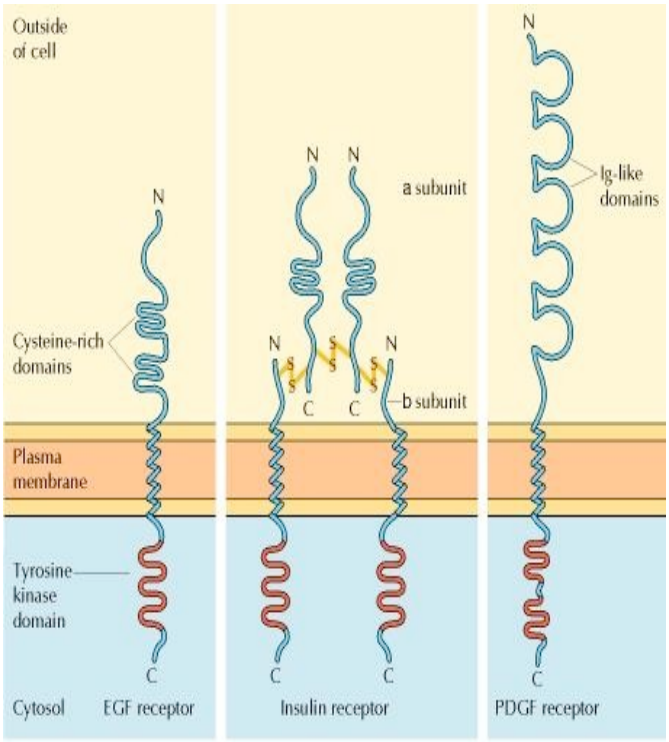
GDP bound) then associates with the βγ complex forming the trimer again.

2. *Tyrosine kinase (TK):*

Action of TK: phosphorylation on the tyrosine and tyrosine usually phosphorylate plasma enzymes.
There are two types of TK:

1. *Receptor protein tyrosine kinase (RTK)*
➔ Some receptors are directly linked to intracellular enzymes. RTKs have the enzymatic activity as part of the protein itself.
➔ Binding of ligands extraellularly activates the cytosolic kinase domains, resulting in phosphorylation of both the receptors themselves and intracellular target proteins.

The figure on the side shows us 3 types of RTK. Those types differ in their extracellular parts indicating that they respond to different types of ligands. Also, their intracellular domains are very similar indicating that they might trigger/ activate the same pathway inside the cell.
However there is a slight difference in their cytoplasmic domains thus they attract different secondary messengers and result in different responses.

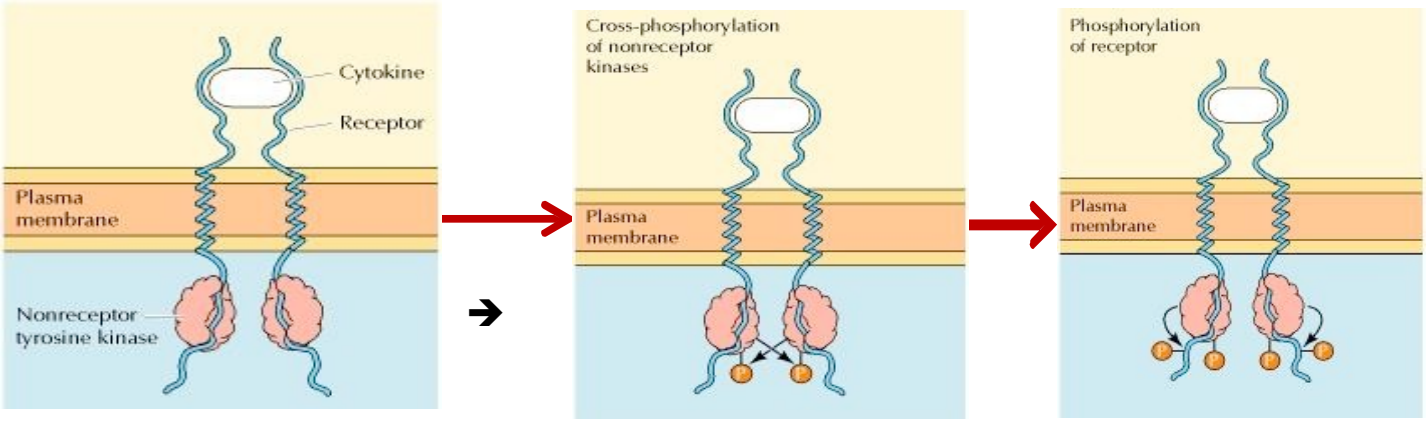


Mechanism of activation of RTKs:

A. *Binding of the ligand to the RTK*
B. *Dimerization (active form) then autophosphorylation* of RTK which activates signaling by:

- First: phosphorylation of tyrosines within the kinase domain **increases the kinase activity.**
- Second: phosphorylation of tyrosines outside the kinase domain **creates high- affinity binding sites (attraction sites) for the binding of other signaling proteins (second messengers, which are themselves kinases)**
- Conformational changes in the second messenger (Activated) ➔ they bind to other receptors and eventually lead to activation of gene expression.

2. *Nonreceptor protein tyrosine kinases (Cytokine receptor superfamily)*



Examples: JAK and Src

Mechanism:

A. Binding of a ligand
B. Dimerization of the receptor
C. This dimerization will attract another protein from the cytosol that will attach to the cytoplasmic domain of the receptor and. This protein is called: nonreceptor tyrosine kinase. (It's called nonreceptor TK because it is not part of the TK itself).
D. Each NRTK protein will phosphorylate the opposite NRTK protein and thus activate each other.
E. Once they are phosphorylated they can phosphorylate their receptors. Each NRTK phosphorylates its own receptor.

Other examples:

Now, we need an enzyme that can stop this process and terminates it. This enzyme is called **protein-tyrosine phosphatases (**usually acts as tumour suppressor genes). Recall that in cancer cells, they have mutation in

phosphatases that inhibits the action of the kinase (which usually acts as oncogene) responsible for the proliferation, thus the kinase is always activated.

In some kinases, they can phosphorylate other molecules beside the tyrosine (2nd messenger) like **serine, threonine kinases**. Thus, the phosphorylation process in general is what transmits the signal to the next molecule.

Receptor guanylyl cyclases ➔ found in the pathway of G-protein coupled receptors. (We'll talk about it later). **Protease-associated receptor**: tumor necrosis factor (TNF) ➔ We'll talk about next in the next lecture.

Second Messengers:

Function: propagation and amplification of the signal as well as in the cross talk between pathways.

How does the amplification happen?

1. When a receptor is activated, it binds to more than one second messenger (but not at the same time). It keeps binding to second messenger as long as the ligand is still present and the receptor is in the active conformation.
2. After the activation of the second messenger, the second messenger itself binds to more than one receptor. Those receptors might activate more than one signaling pathway inside the cell itself. This is called "***cross-talk***" between the signaling pathways.

"Stop acting so small. You are the universe in ecstatic motion."
— Rumi

Good Luck