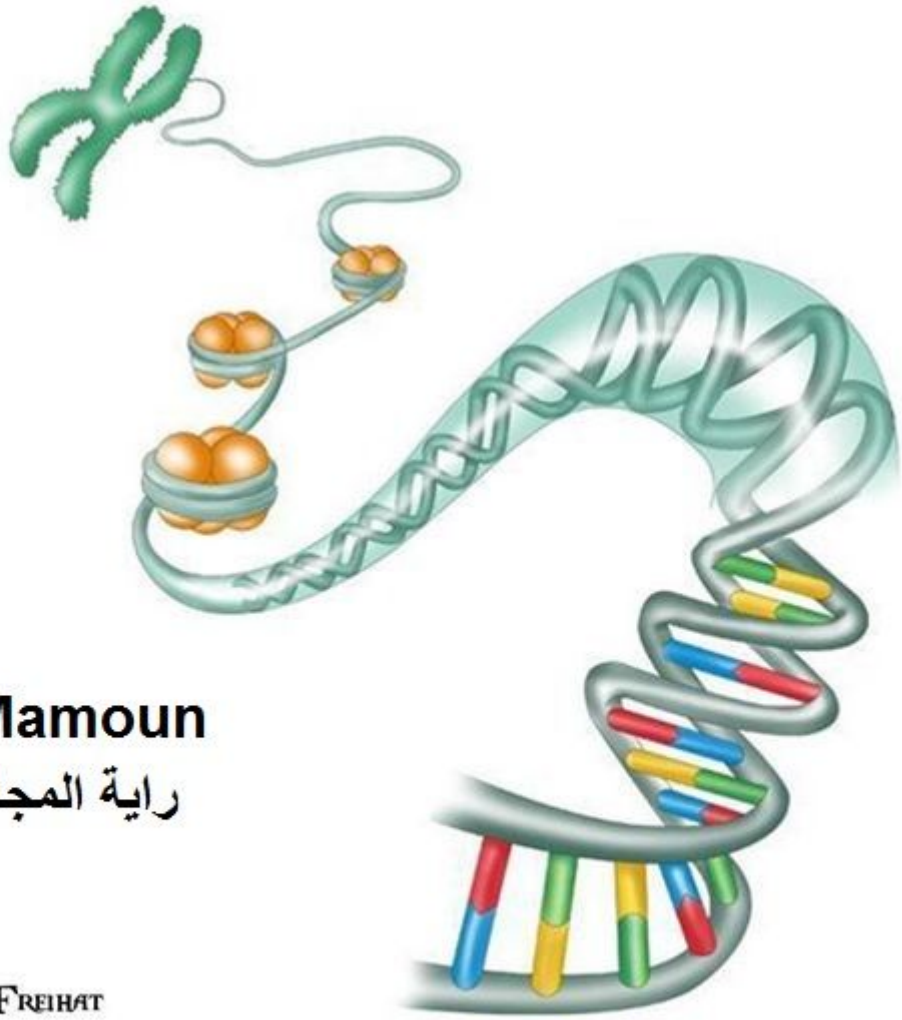
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

○ Slides ● Sheet ○ Handout ○ other.....



Sheet#: 11
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Designed by Nadeen Al-Freihat

Ethical or not?

The doctor started this lecture with two topics that he wanted to discuss with us to decide whether they are ethical or not.

I. Form skin cells to babies!

The first topic was about a breakthrough in cell development, when a group of scientists removed skin cells from an individual, and then they were able to convert them to stem cells. After that, they transformed (*or differentiated*) these stem cells into either a sperm or an egg. So, from a skin cells >>into stem cells >>ending up with a sperm and ovum that can be fertilized and turned into a baby!

NOW, think about it: is that ethical or not?

I think it depends. If they took the stem cells from a married couples, why not? Their own baby and their own genes. Right? But, if they were taken from uncouples, of course it's unethical. What if they take the skin cells from two males? So, now married males can have babies! And married women can have babies as well! Their own babies and genes! So, think about it.

II. Creation of First Cell!

The second topic is about an application on mobile phones the doctor downloaded years ago ,called "*ted*" .this application contains A series of lectures .one of the popular lectures of it is for one of the genius and crazy scientists , called *"Craig Venter"* (*he has a company now*). What *Craig Venter* is doing, is sampling the ocean. meaning that, he is taking organisms from different layers of the ocean and studying their genomes and genes ,and then comparing these organisms to each other ; how they adapt to the environment that they're in and the kind of photoreceptors that they need (*of course, they should have different photoreceptors*).

Years ago, this *Craig Venter* created the first cell. I had an interview on one of the TV stations *(in another country)* which wanted to ride away and it was the first topic in the news. Actually, what they did was really easy. They

synthesized a DNA, base by base. Then, they eliminated -*from a previous knowledge*- the unnecessary genes. After that, they took a bacterial cell and removed its DNA and then they inserted the DNA that they synthesized inside it. And IT WORKED! This cell survived and started to develop its own lipids and protein and DNA and so on, and it worked.

NOW, think about it: is it ethical or not?

I think it can be both ethical and unethical depending on what kind of cells they are creating. It's ethical when cells you develop in the future can be used to get rid of waist, pollution, trash and synthesis of drugs (*for example, pure insulin*). And you can eliminate the radioactive materials as well. But, it's dangerous and unethical when you create a cell that can do something bad.

It's beautiful but thinks of the bad things that can be done! So you can do a lot with cells.

Cell-Cell Interaction

Cell Adhesion Molecules

Cells don't live with themselves .they interact with each other. And we talked about how cells can interact using receptors on their surface like: selectins ,integrins , imuunoglobulins superfamily and Cadherins.

- These proteins *(receptors)* can interact with each other by :

1) Homophilic interaction:

An interaction of the same exact proteins on different cells.

Like: E-cadherin and E-cadherin coming in together.

2) Heterophilic interaction:

An interaction of two different proteins or receptors.

Like: L-selectins with P-selectins.

❖ **Selectins**

✓ Examples on types of selectins:

There are different types of selectins. You have for example:

- L-selectins : present on the cell surface of the Leukocytes.
- E-selectins : present on Endothelial cells

✓ Role Of Selectins in Leukocyte-Endothelial Cell Interaction:

The mechanism by which leukocytes can get in a tissue from the bloodstream is by using selectins. Selectins of the leukocyte first interact with the selectins of the endothelium to recognize where they should go to. Once selectins interact, they settle down and change their Actin cytoskeleton .then; they squeeze between cells into the tissue.

❖ **Cadherins**

Cadherins are beautiful proteins. They're present on lots of cells. Mediating cell-cell interaction. Mainly, **homophilic** interactions.

✓ Examples on types of Cadherins:

- E-cadherins: for Epithelial cells.(We will focus on it)
- N-cadherins: for Neural cells.
- P-cadherins: for Plasental cells
- desmosomal cadherins

Note:The doctor said that when we say that Epithelial cells for example contain E-cadherins, that means that they are the main cadherins and other types can be found there as well.

Mechanisms of cell-cell interaction

I. Adherenes Junctions:

Have a look at Figure #1 in the next page. It shows two cadherins from two different cells interacting with each other. But, when you look at figure #2 you'll discover that these two

proteins are not by themselves. Rather, these cadherin molecules have interactions with different proteins inside the cell which eventually interact with the actin cytoskeleton .These proteins include: β-catenin(*you have to know this protein because we will talk about it later on*) and α-catenin as well .

This type of complex, which includes the Cadherin,Catenin and Actin is known as "***Adherens Junction***".

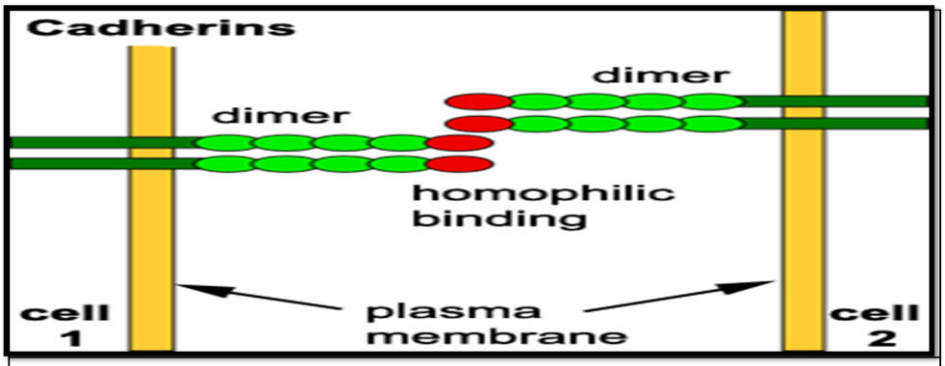

Figure 1: a cell cadherin that interact with a cadherin of another cell.

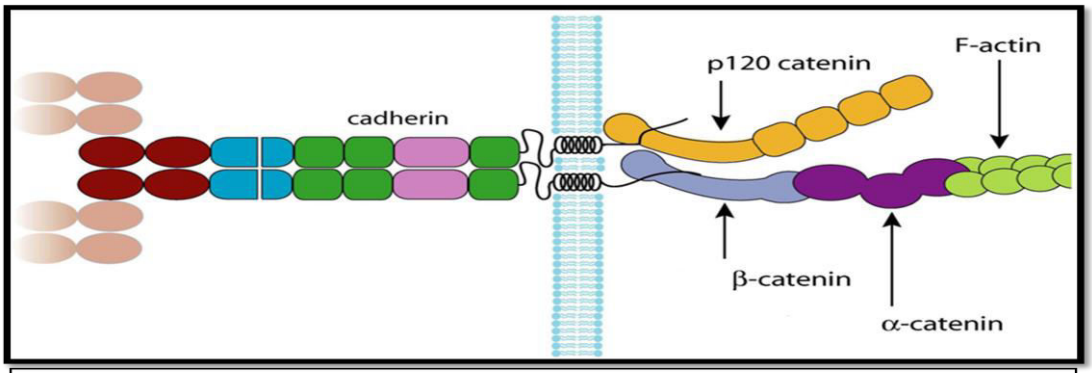

Figure 2: Adherenes Junctions

so,when these two cadherins from two different cells interact with each other they will lead to an interaction between the **actin cytoskeletons** of the two cells. So, an actin of one cell is interacting with an actin of a neighbouring cell by the mediation of the protein-protein interaction.

II. Desmosomes: (Figure 3 next page)

This is another type of interaction that we talked about when we mentioned the Intermediate Filaments .it has different proteins (*receptors*) including **desmosomal cadherins**. These desmosomal cadherins can interact with each other and eventually they interact with the Intermediate Filament. So, when a desmosomal cadherin of one cell is interacting with the desmosomal cadherin of another cell this will lead to the interaction of the **intermediate filament** of one cell with intermediate filament of a neighbouring cell as well.

These are desmosomes which interact via **heterophilic interaction.**

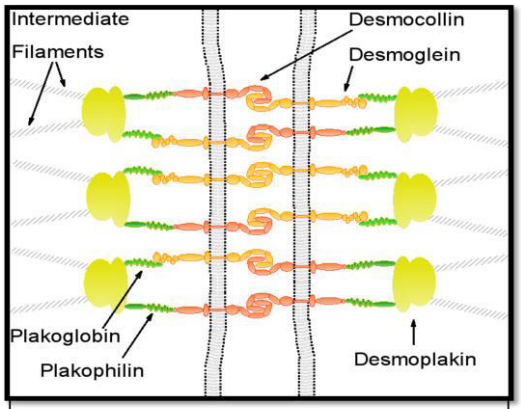

Figure 3:Desmosomes

III. Tight Junctions

tight junctions don't exist by themselves. Rather, you have a combination of different types of cell-cell interaction. Meaning that, they are usually associated with adherens junctions and desmosomes in a junctional complex as shown in Figure 4.

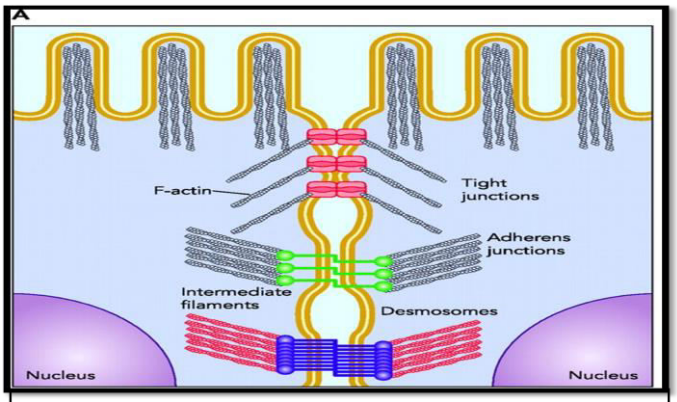

Figure 4: tight junctions. you can have desmosomes and you can have adherens junctions as well.

- <u>the function of these tight junctions is</u>:
  1. They separate the two sides of cell; the apical side and the basolateral side. For example, they separate the lumen from the basal lamina (*underlying connective tissue*) in intestinal cells. So, this side of the cell is completely different from the other side of the cell.
  2. They block interactions and the movement of molecules (*especially small molecules. E.g. calcium and other nutrients*) from a lumen to the basal lamina and vice versa.
  3. They prevent the movment of other molecules (*lipids and proteins*) from the apical surface to the basolateral surface. Because once they get in, they hit these molecules and they can't pass through them .they stay at top.
     Same thing is in the bottom. You have tight junctions also at the basolateral surface so the proteins and molecules can't move upward.
     In other words, they differentiate the cell surfaces from each other.

- Molecular composition of tight junctions:

Tight junctions are mainly made of a combination of proteins: claudins, occludin, and JAMs (*don't memorize these names*).these proteins create a belt surrounding the cell circumference, and then they interact with similar proteins from adjacent cells in a **homophilic** interaction (Figure 5) forming this tight belt junction.
You have adaptor proteins which eventually interact with the Actin cytoskeleton. So, tight junctions cause the interaction of the actin cytoskeletons of cells via these different proteins.

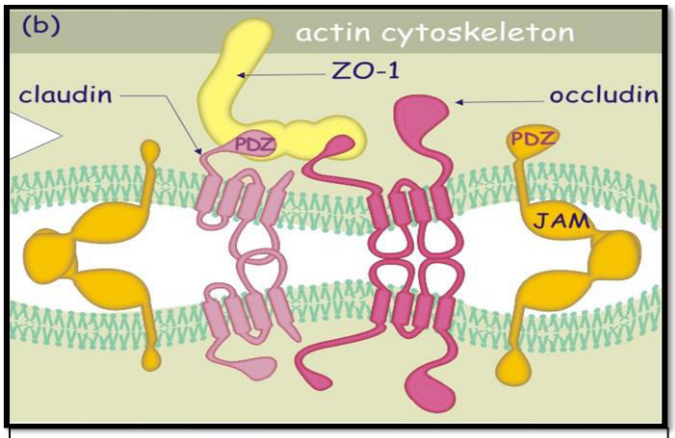

Figure 5 : The 3 proteins in tight junction interacting with a similar proteins in adjacent cells.

IV. Gap junctions:

These are channels that are present on neighbouring cells by which cells can transfer molecules from one cell to another and they interact with each other .these channels are not large enough for proteins and large molecules to pass through, it's only for small nutrients like calcium ions, cAMP, and other things that we will mention later on.

- Molecular composition of gap junctions:

These gap junctions are made of proteins known as connexins . it is a protein that form a channel called connexon.(*connexins*

form connexons) .the connexon of one cell interact with the connexon of another cell and they form one large unit (*one large channel*). Allowing for movement of small molecules between cells.

As an example of how important these connexons are, is the communication of nerve cells with each other. One of the ways they interact or communicate with each other is by releasing neurotransmitters that bind to a receptor of a mother cell to induce a signal (*we will talk about signals later on in five minutes*). Another way by which nerve cells can communicate with each other is by forming these connexon between them.

- <u>defects in gap junctions:</u>
  a) Charcot-Marie-Toth disease :
     We talked about it before. Just work a bit hard and gather all the information we took before in one piece.
  b) Deafness:
     The way the hair cells of the auditory system of the ear interact with each other is not only by releasing molecules but also by gap junctions. So, if you have a defect in these gap junctions and formation of these channels between cells, then these cells will not be able to function.
     A number of studies have been done around the world including Jordan about the role of gap junctions in deafness. They noticed that deafness can be caused genetically by number of genetic mutations one of them is gap junctions.
     Actually, there are families in Almafreq ,Jordan that have hereditary deafness. Children there are born fine and then they develop deafness! Studies were done before and mutations in gap junctions were

identified. But, gap junctions don't tell the whole story, there are other proteins that are important.

c) Cataracts:
When the retinal cells of the eye can't transfer molecules and nutrients between each other because of defections in Gap junctions, this will lead to cataracts.

d) Skin diseases.

Cell-Cell Interactions and Cancer

(We will have a whole lecture about it)

One of the first mechanisms cells can become aggressive is when they dissociate from each other. As an example, when you have epithelial cells becoming tumour or fibroblast-like. in order for them to become mesenchymal or fibroblast-like cells, they have to dissociate from each other and this is done by reducing the expression of E-cadherin and no more cell-cell interaction by e-cadherin.

Astudent asked (Aseil el habla): do mesenchymal cells have other types of cadherins?

The doctor answered: No, but even if they do they don't mediate strong cell-cell interaction.

This's what Cell-Cell interaction is! Let's revise :

- Cell Adhesion Molecules are: **selectins ,integrins , imuunoglobulins superfamily** and **Cadherins.**
  - These proteins can interact with each other by **Homophilic** interaction (same exact proteins on different cells) or **Heterophilic** interaction (An interaction of two different proteins).

- **Selectins** can be L-selectins (on the cell surface of the Leukocytes), E-selectins (on endothelial cells) and so on. Their interaction helps the leukocytes to move from the bloodstream to the tissue.
- **Cadherins** are included mainly in the **homophilic** interactions .they can be E-cadherins (for Epithelial cells), N-cadherins (for Neural cells), P-cadherins (for Plasental cells) and desmosomal cadherins.

Mechanisms of cell-cell interaction:

a. Adherenes Junctions:
   - Proteins (*β-catenin and α-catenin*) interact with the cadherin molecule itself and eventually interact with the actin cytoskeleton of the cell.
   - Interaction of two cadherin molecules in different cells will mediate interaction of their **actin cytoskeletons**.
   - Complex of Cadherin,Catenin and Actin is known as "***Adherens Junction***".

b. Desmosomes:
   - **Heterophilic** interactions
   - *desmosomal cadherins* interact with each other and eventually they interact with the Intermediate Filament.
   - Interaction of two desmosomal cadherins of two different cells will lead to the interaction of their **intermediate filaments.**

c. Tight Junctions:
   - **Hemophilic** interactions
   - Usually found with combination of adherens junctions and desmosomes in a junctional complex.
   - Their function is to **separate the two sides** of the cell and block the movement of **small molecules**

from the two sides. Also, it prevents the movement of **large molecules** from the **apical surface to the basolateral** surface and vice versa.

- They are mainly made of a combination of proteins that create a belt surrounding the cell circumference and interact with similar proteins on other cells. **Adaptor proteins** of this junction interact with the actin cytoskeleton.
- Tight junctions mediate the interaction of the **actin cytoskeletons of cells.**

d. Gap junctions:

- **Connexins** form connexons which are channels to transfer **small molecules**.
- Have a role in Nerve cells interaction.
- Defects in gap junctions can cause Charcot-Marie-Toth disease, Deafness,Cataracts as well as Skin diseases.

- Loss of cell-cell interaction (Like loss of E-cadherins) helps cells to be cancerous and aggressive.

Cell Signaling

Cell signaling is one of the mechanisms that cells can use to communicate with each other.

Modes of cell signaling

There are many types of signaling pathways or mechanisms. You can have:

- Cell-cell interaction (*the direct interaction which we talked about*).
- paracrine signaling :

It is communication between two different types of cells. It is basically a cell (*like fibroblast cell*) releasing a molecule that can interact with a receptor on the cell surface of (*epithelial cell for example*).so that's a way by which a fibroblast and epithelial cell can communicate with each other.

- Autocrine signaling : (*auto meaning self*)
  The cell secretes a molecule and it binds to a receptor on the same cell (*or the same type of cells*) and this is a way by which the cell can stimulate itself and cells of the same type.
- endocrine signaling:
  a molecule is released and goes to the blood (*the circulatory or lymphatic system, but* ***mainly*** *circulatory system*) and then travels along the way where it can bind to its receptor (*just like hormones for example*).
- intracrine signaling:
  Synthesis of a molecule inside the cell which then acts inside the cell itself (*and not the same cell type*).

Mechanism of Action *(in general)*:

As we learned in physiology, the Ligand or the hormone or the molecule is found in vesicles. It gets secreted outside and then binds to its receptor to produce an action. After that, signal is terminated.

Players of signaling by cell surface receptors:

When a ligand binds to a receptor, the cell needs these players or different molecules to transfer the signal from outside to inside:

- **A Ligand**: which can be a hormone or a growth factor.

- **Receptor**: the ligand will bind to it.and then its shape becomes different and binds to a transducer.
  Example on receptors: G-protein coupled receptor (GPCR) and receptor tyrosine kinases(RTK).
- **Transducers:** they are proteins that are modified. They can send signals to effector molecules.
  Transducers include**:** G protein, Ras (*really important.*).
- **effector molecules**: what these molecules do is that they change (*or manipulate, modulate*) the levels of second messengers.
  Transducers include: adenylate cyclase, MAPK .
- **Second messengers**: small molecules that can eventually activate more effective proteins (*the final target molecules*).
  Second messengers include: Ca^{2+}, cAMP,cGMP
- **Final target molecules**: the final molecule that is going to give us a response.
  They can be:
  - a DNA *(gene expression)*:
    There are different levels of responses.if you have a target molecule that is transcription factor, what it will do is to activate gene expression. This is known as the *"primary response"*, because it is the first thing to happen. Then, the molecule that was produced from the primary response may act as a transcription factor and regulate the expression of other protein and this is what e call *"secondary response"*.
    Now, primary response takes around thirty minutes (*slow mechanism*) and the secondary response take about two hours.

Tertiary response can also be done the same way (*from the products of secondary response*) and so on. (*Continuing process).*

- a channel (*where it opens up or closes down*)
- Or anything else.

Signaling Molecules (Also called, *Ligands*)

There are different molecules that the cells can secrete and use to interact with each other. You can have:

- peptides,
- small molecule neurotransmitters: Molecules derived from Amino Acids or Amino Acids themselves act as signaling molecules.
- steroids
- Eicosinoids
- gases like nitric oxide

lipophilic hormones:

they include the estrogens, androgens, the progesterons ,the cortisoles, aldosterone, vitamin D, Retinoic Acid and thyroid hormones .they're all almost derived from cholesterol (*except Retinoic acid*).

they are small and lipophilic .so, they can diffuse through the plasma membrane without the need of plasma channel protein or a carrier protein or anything like that .they can just pass through the plasma membrane because they are small and they are lipophilic.

☆ Mechanism of action of steroid *receptors*:

We talked about this before.the lipophilic hormones diffuse inside. then, they bind to a receptor that is in the cytosol which

is bind to the receptor that is released from the heat shock protein and then dimerize forming a receptor-receptor complex (each receptor of them is bound to a ligand).Afetr that, the dimer goes into the nucleus and binds to the DNA and control genes expression.

Cell surface receptors

Types of cell Receptors:

We have three types:

1. G- protein coupled receptor (GPCR):
   - ☆ Belongs to a family known as "7 *trans membrane domain receptors*" because they have seven transmembane domains.
   - ☆ It is coupled to G-protein and this is why it was given this name .G-proteins are large proteins and composed of three polypeptides (*trimers*). These polypeptides are known as: alpha, beta and gamma.
     Alpha is a GDP-bound when it is inactive and it is a GTP- bound when it is active.
     Now, when a ligand binds to a receptor (*the outside or the extracellular sideof the receptor*), it causes a changes in the receptor's shape allowing for the G-protein to bind to it (*the receptor*). The GDP that is present on the alpha subunit is released, allowing for the GTP to bind to it.when the alpha subunit binds to the GTP instead of GDP, it is released from the beta and gamma subunits and it is free. Alpha now is an active transducer. Beta and gamma can also be active transducers. So, you can have alpha binding to an effector protein, and you can have beta and gamma binding to an effective protein, also.and eventually what you need is to send a signal from outside to inside.

- ☆ You can't have the g protein active all the time. It is catastrophic to the cell! You have to terminate the signal and get back the inactive form of the G-protein. G-protein is inactivated by an enzymatic activity know as *"GTPase activity"*. This enzymatic activity hydrolyzes GTP to GDP allowing the alpha subunit to bind to the GDP and then the GDP-alpha complex can then bind to beta and gamma and the signal is finally terminated.

2. <u>Receptor Tyrosine Kinases</u> (RTK):
   - ☆ A receptors which their cytoplasmic side have a kinase activity.
   - ☆ There are different types of it: monomers, dimers, trimers and so on.
   - ☆ when a ligand binds to it ,you'll have receptor dimerization (*meaning that you have a monomer coming in with another monomer becoming one dimer*) .now, the kinase domains of these two receptors are very close to each other,so they phosphorylate each other and this is known as "*autophosphorylation*" , a receptor that phosphorylate itself (*since they both became one dimer*). The phosphorylated domain allows the interaction with other proteins.
     Now, other proteins can interact with the phosphorylated domain, so you can bring a collection of proteins at the cytosolic surface of the receptor.
     So,The autophosphorylation increased the kinase activity (*it was active a bit, but once its phosphorylated it's really active and can phosphorylate other proteins as well*).Also, it brings in different proteins to the same site since the cytosolic part of the receptor can't binds to other proteins when it's not phosphorylated , but when you have the phosphorylated part of the receptor it can bind to other proteins.

3. nonreceptor tyrosine kinases :
   - ☆ They are receptors that don't have a Kinase activity (*kinase domain)* .rather; it can be bound to normal kinases that are found in the extracellular when a ligand binds to the receptor.
   - ☆ These kinases phosphorylate the receptor and other proteins.
   - ☆ Examples on these enzymes : JAK and Src .(*you need to know it*)
4. Phosphatases receptors:
   - ☆ Instead of adding phosphate they remove Phosphate.
5. serine/threonine kinases:
   - ☆ They phosphorylate threonine and Serine.
6. Receptor guanylyl cyclases :
   - ☆ Converts GTP to cGMP.
7. Protease-associated receptor:
   - ☆ Such as tumor necrosis factor (TNF) ,we'll talk about it in apoptosis because it determines cell survival .

Second messengers

❖ AMP:

- cAMP is synthesized from ATP by an enzyme known as "*adenelyl cyclase*".
- You can have the same thing with cGMP. so you have guanyly cyclase it converts GTP to cGMP.
- cAMP can be inactivated by converting it into AMP. *(*Mechanism *of terminating the signal).*
- Once cAMP is synthesized it can bind to a kinase called "Protein *Kinase A*". This protein kinase A is really important in metabolism. Because when you need energy you need to hydrolyze or break down glycogen to release glucose,the body secretes hormones and growth factors like epinephrine and glucagon which then bind to a

receptor. The receptor then activates adenylyl cyclase which in turn increases the level of cAMP. cAMP binds to protein kinase A which then phosphorylates other proteins. Eventually, this will lead to an increasing in glycogen degradation and stop glycogen synthesis because this is what you want to generate energy .this is controlled by cAMP and protein kinase A.

- cAMP can do something else with protein kinase A .part of the protein kinase A can go inside the nucleus and phosphorylate a transcription factor CREB which become activated as well as the protein kinase A activate certain genes like cAMP induceable genes.
- The signal must be terminated and the mechanism of termination is phosphatases.
- When protein kinase A phosphorylate a protein it may be activated or inactivated depending on the protein.

This's what Cell signaling is! Let's revise :

- Modes of cell signaling :
  - cell-cell direct interaction
  - paracrine signaling :
    Between two different types of cells
  - Autocrine signaling : on the same cell (or the same type of cells)
  - endocrine signaling:
    A molecule is released and goes to the blood (just like hormones for example).
  - intracrine signaling:
    Synthesis of a molecule inside the cell which then acts inside the cell itself (and not the same cell type).
- Players of signaling by cell surface receptors:

- Signaling Molecules (Ligands): which can be a hormone, growth factor, peptides, small molecule neurotransmitters,steroids,Eicosinoids and gases like nitric oxide.
  - ☆ lipophilic hormones: include the estrogens, androgens, the progesterons ,the cortisoles, aldosterone, vitamin D, Retinoic Acid and thyroid hormones .they're all derived from cholesterol (except Retinoic acid).they are small and lipophilic . They can diffuse through the plasma membrane without the need of plasma channel protein or a carrier protein. Their receptors are in the cytosol bound to a hot shock protein.
- Receptor: Types of cell Receptors:

1. G- protein coupled receptor (GPCR):
   - ☆ 7 trans membrane domain receptors
   - ☆ G-protein:
     - ✓ large ,
     - ✓ trimers.
     - ✓ Alpha, beta and gamma subunits.
     - ✓ Alpha, is a GDP-bound when it is inactive and it is a active (free) when it GTP- bound.
     - ✓ Signal termination: "GTPase activity".
2. Receptor Tyrosine Kinases (RTK):
   - ☆ cytoplasmic side have a kinase activity.
   - ☆ when a ligand binds:
     - ✓ receptor dimerization
     - ✓ autophosphorylation
     - ✓ Interaction with other proteins.
3. nonreceptor tyrosine kinases :
   - ☆ Can be bound to normal kinases when a ligand binds to the receptor.
   - ☆ kinases phosphorylate the receptor and other proteins.
   - ☆ Examples: JAK and Src .

4. Phosphatases receptors:
   - ☆ Remove Phosphate.
5. serine/threonine kinases:
   - ☆ phosphorylate threonine and Serine.
6. Receptor guanylyl cyclases :
   - ☆ Converts GTP to cGMP.
7. Protease-associated receptor:
   - ☆ Tumor necrosis factor (TNF).

- Transducers:
  - ✓ G protein, Ras .
- effector molecules:
  - ✓ Modulate the levels of second messengers.
  - ✓ Examples: adynelate cyclase,MAPK.

- Second messengers:
  - ✓ include: Ca^{2+} ,cAMP,cGMP
  - ✓ cAMP:
    - ➢ synthesised from ATP by an enzyme known as "adenelyl cyclase".
    - ➢ Inactivated by converting it into AMP.
    - ➢ once it is synthesized it can bind to a kinase called " Protein Kinase A".which either affect metabolism pathways and causes degredation of glucagon and releasing of glucose.or part of it goes to the nucleus and phosphrylate transcription factor CREB to be activated and activate cAMP induceable genes.
    - ➢ Signal is terminated by phosphatases.
      - ✓ cGMP:
    - ➢ guanyly cyclase it converts GTP to cGMP.
      - ➢ Final target molecules :
        - ✓ a DNA (gene expression):

        Different levels of responses.

"Primary response": takes around 30 minutes.

"Secondary response" takes around two hours.

- a channel (where it opens up or closes down)

- Note: releasing the ligand can terminate the signal.

"يا طِبُّ .. يا صاحبَ الدّربِ الطويل ..

إنّي مشيتُك خطًى كُتِبَت عليَّ ،، و من كُتِبت عليه خطًى مشاها.."

إلى أولئك الذّين ساروا معيَ الدّرب من أوّله ، والى الآنَ ما سئموا ولا كَلّوا و لا ضَلّوا الطريق ..

راية عبد الحميد مسلم المجالي