◉Sheet ○Slides

Subject :	Glycolysis
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Number :	10

In the previous lecture, we talked about the digestion of carbohydrates. We learnt that it ends at the surface of the cells of the intestinal mucosa; where disaccharidases cleave disaccharides into monosaccharides.

Note that: Only monosaccharides can be absorbed, which means that disaccharides can't be absorbed if they are not digested.

So, how does absorption occur?

- ✓ Sugars in general including glucose which is the major sugar in the human body are polar molecules that can't diffuse; because they have five or six hydroxyl groups, so they are prevented from going through the plasma membrane which is non-polar. So, glucose is transported through the plasma membrane by carrier molecules.

❖ The metabolic pathways can be divided into:

1. Catabolic pathways:

- Convert large molecules into their building blocks, so they serve to capture chemical energy in the form of ATP from degradation of energy-rich fuel molecules and also generate NADH, so they can be used in the anabolic pathways.
- Catabolic reactions end by producing energy-poor products (CO_2, H_2O, NH_3).
- Exergonic.
- A convergent process (many molecules are transformed into few end products)

➢ Stages of catabolism:

1) Hydrolysis of complex molecules to their component building blocks:

Proteins, carbohydrates and fats can't be metabolized as such, so these complex molecules are broken down into their component building blocks (amino acids, monosaccharides, glycerol and fatty acids) respectively.

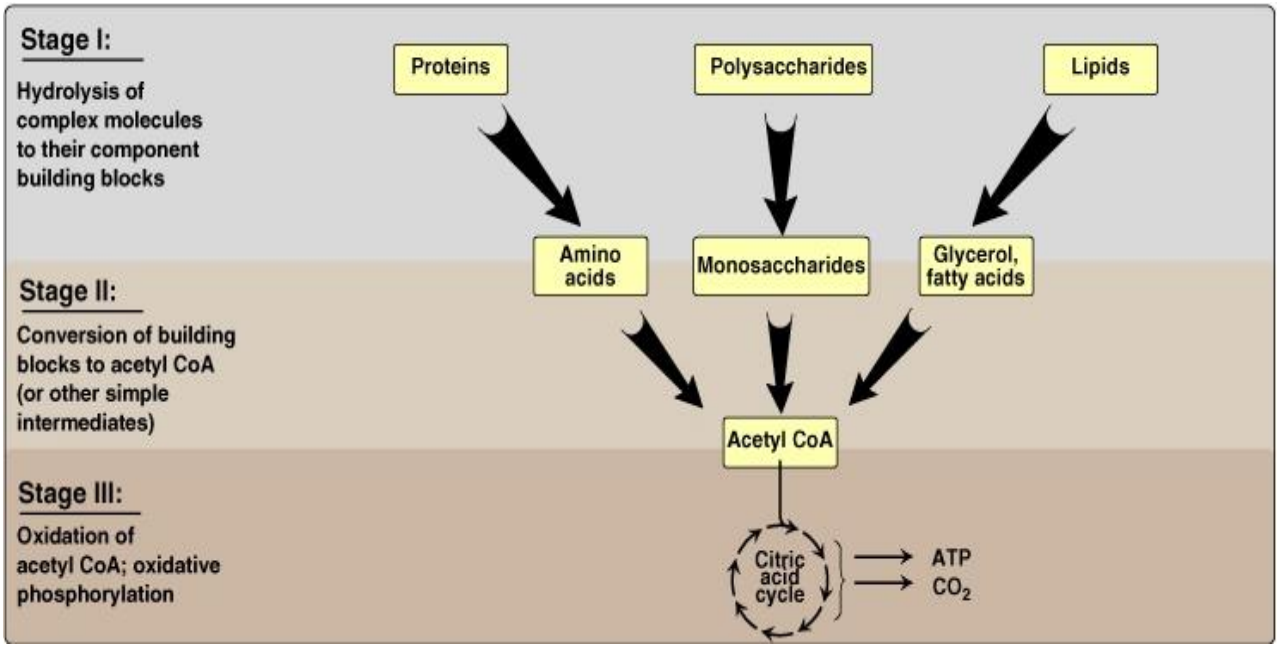
2) Conversion of building blocks to simpler intermediates:

These diverse building blocks are further degraded to acetyl CoA.

Remember that the acetyl CoA is a two-carbon molecule, which is formed by acetic acid that is linked to Coenzyme A, which makes it a high-energy compound.

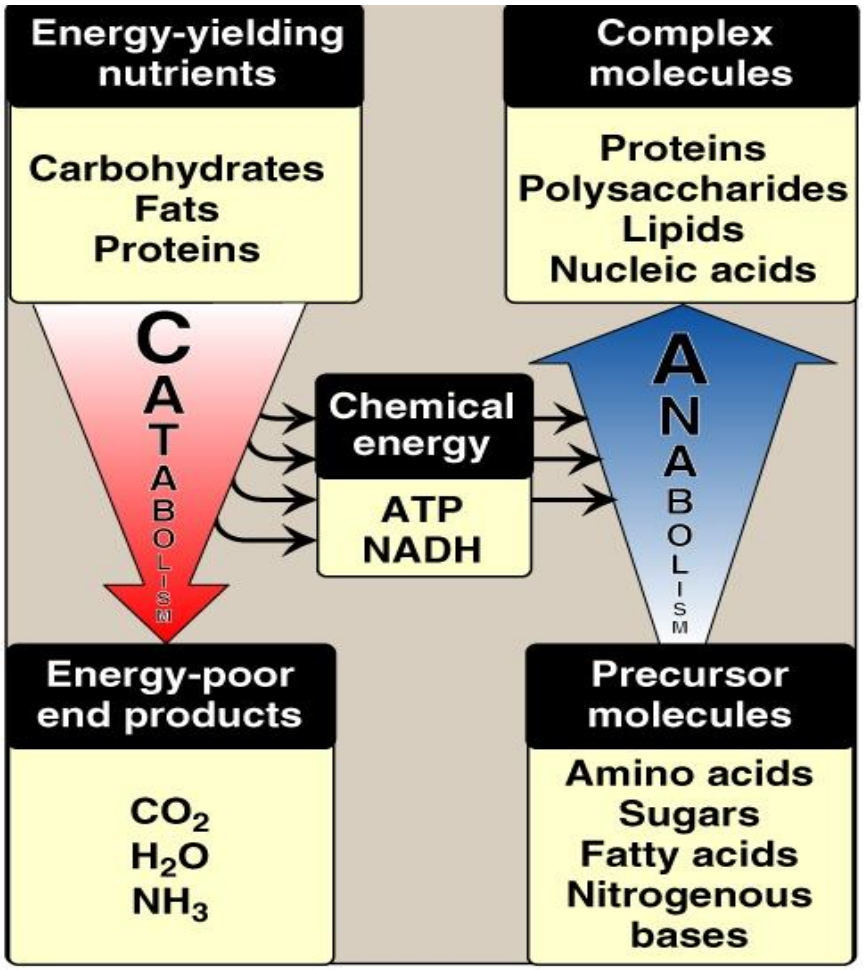
3) Oxidation of acetyl CoA:

Acetyl CoA is the fuel for the citric acid cycle and it's oxidized completely in the cycle to produce (CO2 and ATP).



2. **<u>Anabolic pathways:</u>**

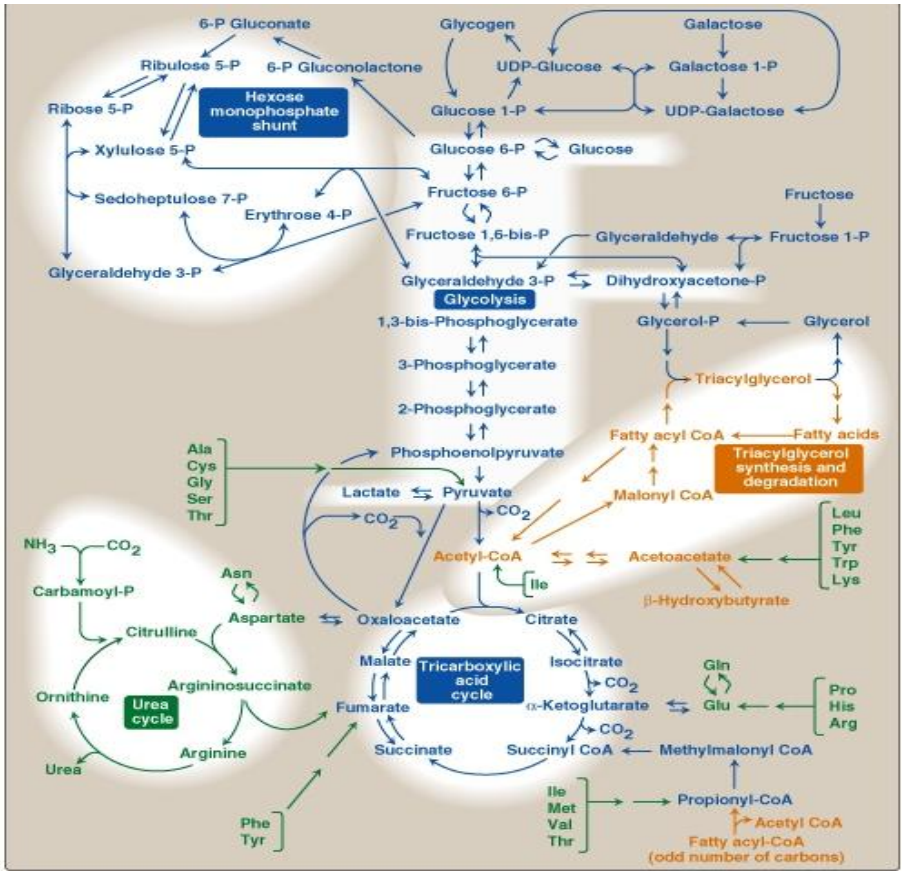
- Combine small molecules such as amino acids to form complex molecules such as proteins.
- Anabolic reactions require energy which is generally provided by the hydrolysis of ATP into ADP and inorganic phosphate (Pi).
- Endergonic.
- Our cells or even mono-cellular organisms such as bacteria and parasites, don't always have the same conditions (for example: Glucose won't be available for the cell all the time), so how can we adapt to continuously changing environment? By regulation of metabolism using **signals** therefore, we can meet the variable demands of the cell.
- A divergent process (few precursors are transformed into various complex products)



Glycolysis

- Gly: Refers to sugar or carbohydrate.
- Lysis: Breaking down.
- It is a universal pathway in all cell types.
- The aim of glycolysis is to generate ATP.
- It can act with the presence or absence of oxygen.
- It is an anabolic pathway; because some of the intermediates of glycolysis are used for the synthesis of large molecules.

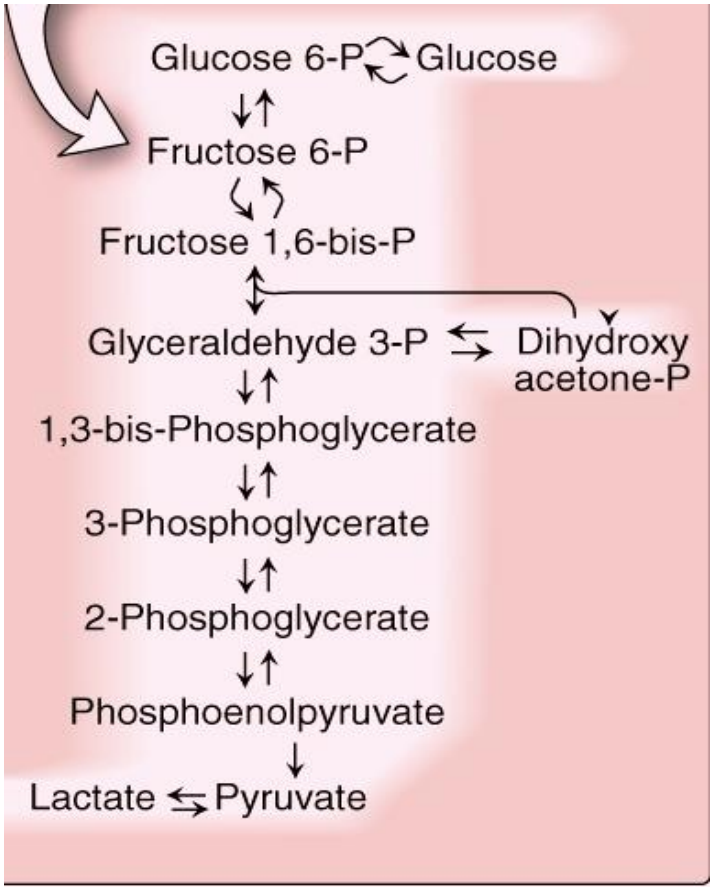
❖ Overview of glycolysis:

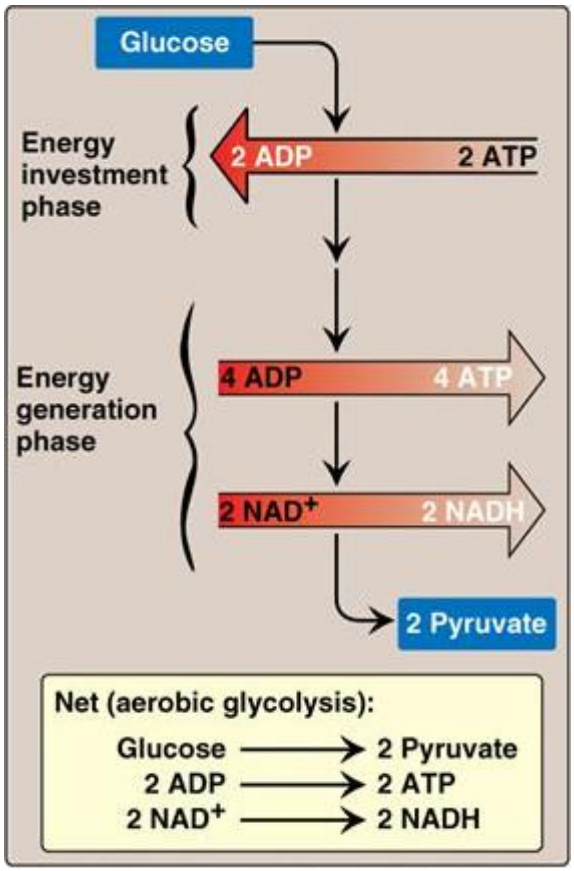


(Don't panic we'll take this map step by step, so it'll be easy at the end hopefully).

- It starts with glucose and ends with pyruvate or lactate passing by many intermediates
- Glucose is converted to lactate by 10 steps.

- The product of one reaction is the substrate for the next reaction.
- It's an example of a metabolic pathway.
- The metabolic pathways are like any road; they can intersect, so there might be intersection between two or more metabolic pathways.
- Notice that when metabolic pathways intersect, they form a complicated network (the metabolic map) which contains all the metabolic pathways forming a network of chemical reactions.
- These chemical reactions are well controlled and don’t happen in isolation of each other.
- If these chemical reactions were not well controlled, all these chemical reactions will end with the product that has the lowest energy and that's not what we want.





❖ Transport of glucose into cells:

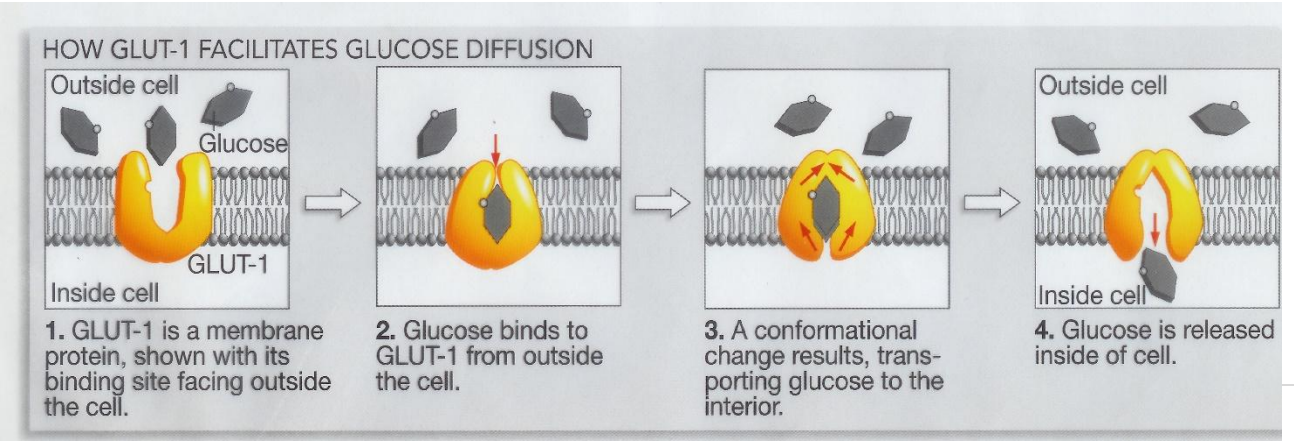
The transport of glucose through the plasma membrane can occur by two major mechanisms:

1. Sodium-independent facilitated diffusion.

- Recall that facilitated diffusion occurs by the facilitating activity of a transport protein.
- This system is mediated by a family of 14 glucose transporters: GLUT-1 to GLUT-14 (glucose transporter isoforms 1-14).
- Transport proteins: Proteins that span the cell membrane and they have two conformations.
- The movement of glucose occurs with the concentration gradient (from high concentration to low concentration).
- Extracellular glucose binds to the transporter which then alters its conformation; transporting glucose across the cell membrane.
- The most common glucose transporters are the first five (GLUT-1 to GLUT-5), and they follow this mechanism.

➢ Mechanism:

Glucose will get rid of the hydrogen bonds with water, then new hydrogen bonds are made between glucose and the transport protein. Once glucose binds the transport protein, the protein can change its conformation, so glucose will be facing the inside of the cell, then it can leave. (Remember the concentration inside is lower).



2. Sodium-monosaccharide co-transport system (Na^+ dependent):

- An energy requiring process that transports glucose against its concentration gradient (from low glucose concentration outside the cell to higher concentration within the cell).
- This system is a transporter-mediated process in which the movement of glucose is coupled to the concentration gradient of Na^+, which is transported into the cell at the same time. So, once sodium is bound to the co-transporter, the glucose will bind too.

  ✓ Remember that sodium is the major ion outside the cell and potassium is the major ion inside.

- The transporter is a sodium dependent glucose transporter (SGLT).
- In the small intestine, there is a rapid uptake of glucose from the lumen of the intestine into the cells. So, at some point, the concentration of glucose in the lumen will be very low, definitely lower than the cells, which means that we can't depend only on the concentration of glucose. In order to take the very last molecules, glucose has to be transported against its concentration gradient by the help of sodium, which is found in high concentration outside the cell.

As sodium is moving from high concentration to low concentration, at some point, sodium concentration might become very low and not adequate to transport glucose, is that true?

➢ No, because the sodium-potassium pump uses energy allowing sodium to be pumped out from the cell, and that maintains the sodium concentration to be low inside the cell.

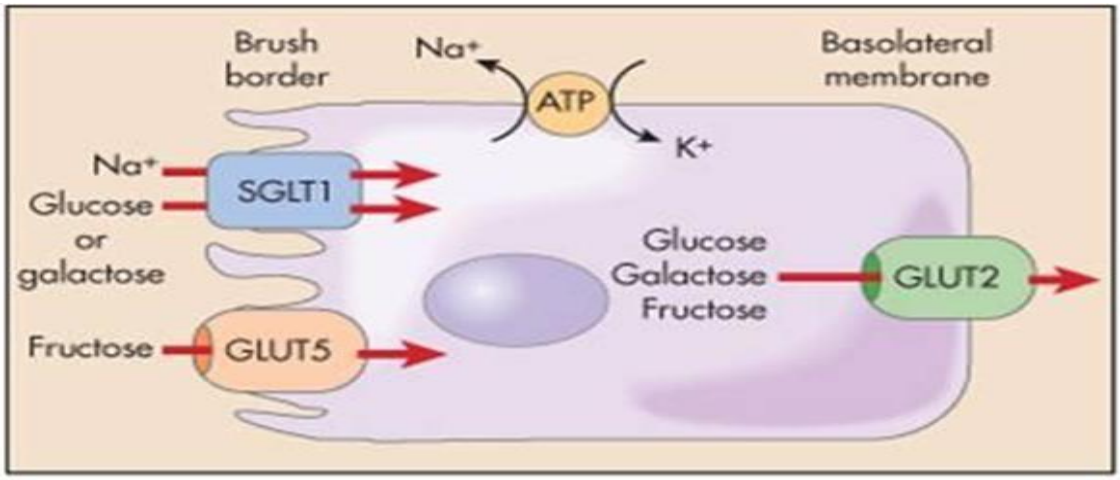
- On the serosal side of the intestinal cell, glucose is transported by facilitated diffusion to the capillaries (from high concentration to low concentration) - This transporter can bind galactose too.

Oral rehydration solution: it's found in pharmacies, and simply consists of sodium and glucose, so it will allow the glucose, sodium and water to be absorbed, and that will help treating diarrhea.

Note that giving sodium alone would not be useful, because it can't be transported without glucose.

- ✓ What about fructose?

In contrast with glucose and galactose, it totally depends on facilitated diffusion, because the concentration of fructose in the blood is very low, so it's continuously transported to the blood. Therefore, even if the fructose concentration is low in the small intestine, it can still be transported from high concentration→low concentration.



❖ Glucose transporters:

1. GLUT-1:

- It is found in the human erythrocyte, blood brain barrier and blood retinal barrier.
- The common thing about these tissues is that they depend totally on glucose, which means that **GLUT-1 has high affinity to glucose**, so it can transport glucose even if its concentration is very low.

2. **GLUT-2:**

- It's found in the **liver**.
- Has high capacity but low affinity to glucose, which means that if the concentration of glucose is low in the blood it will not be transported into the liver. (Remember that the liver functions to deliver glucose to the blood not to take it from the blood).

3. **GLUT-4:**

- It's found in adipose tissue, skeletal muscle and heart muscle.
- These tissues have the option to use glucose or fatty acids as a source of energy. Glucose should be taken as a source of energy when blood glucose is high (remember that when glucose rises, insulin will rise too), so no need to use fatty acids as a source of energy in this case.
- It's an **insulin sensitive transporter**, which means that in the presence of insulin, the number of GLUT-4 transporters increases on the cell surface.

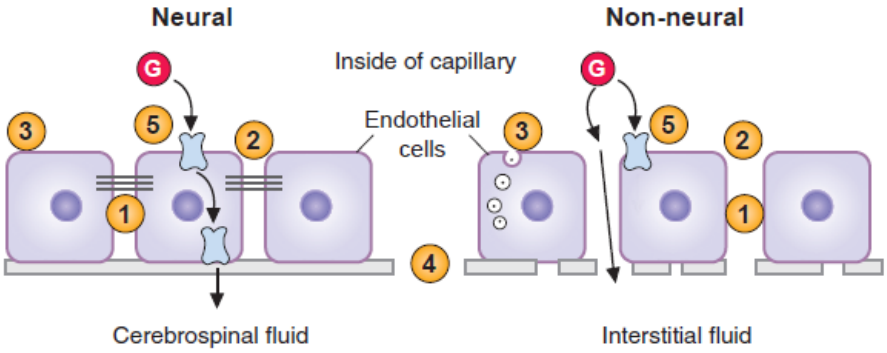
4. **GLUT-5:**

-It's a **fructose** transporter.

❖ The transport of glucose to tissues:

- It differs from one tissue to another, based on the demand of the tissue.
- The tissues that have high demand for glucose should be able to get glucose easily and freely.
- The tissues that have low demand for glucose; for example, the skeletal muscles might take glucose slowly.

	Neural tissues	Non-neural tissues
Dependence on glucose	Absolutely dependent on glucose.	Can depend on other sources.
Presence of tight junctions	There are tight junctions between endothelial cells, which prevent glucose from passing between cells.	No tight junctions.
Continuity of the basement membrane	Continuous basement membrane.	Discontinuous basement membrane.
Intercellular space	Narrow intercellular space.	Sometimes wide intercellular gaps.
Pinocytosis	Lack of pinocytosis.	Glucose is taken by pinocytosis (endocytosis).
Glucose transporters	There should be glucose transporters in both membranes.	Glucose can diffuse between cells and into the interstitial fluid.



Don't pay attention to the numbers drawn on the figure above, unless you want to refer to the slides.

❖ Regulation of metabolism is controlled by:

1. Intracellular communication:

The rate of a metabolic pathway can respond to regulatory signals that arise from within the cell.

Examples:

a) Substrate availability:

For instance: No glucose → No glycolysis.

No ammonia → No amino acids' synthesis.

b) Product inhibition:

When the product of the pathway is high, this pathway should be slowed down (by negative feed-back inhibition).

c) Alteration in the levels of allosteric activators or inhibitors

These are small molecules that regulate the activity of the pathway.

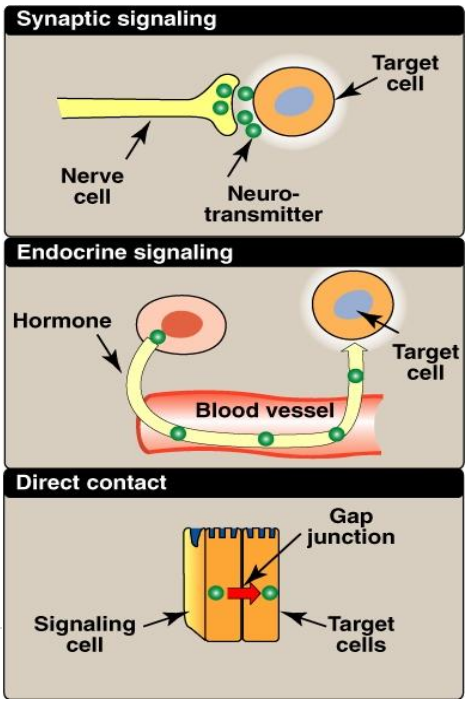
-Intracellular communications typically elicit rapid responses, and are important for the moment-to-moment regulation of metabolism.

2. Intercellular communication:

This type of communication commonly uses these mechanisms:

a) Synaptic signaling*: (by neurons)*

Remember that the nerve endings release neurotransmitters that bind to the target cell and affect its activity.



For example: this signal can target a cell to secrete or not to secrete hormones.

b) Endocrine signaling:

The hormone is transferred from one cell to another, through the blood and affects the activity of the target cell.

c) Surface-to-surface contact (direct contact between two adjacent cells).

One cell sends a signal for the adjacent cell to modify its activity. In some tissues, this happens by formation of gap junctions allowing direct communication between cytoplasm of adjacent cells.

3. Second messenger systems:

The most widely recognized second messenger systems are:

a) Adenylyl cyclase system
b) *B-Phosphatidylinositol system*

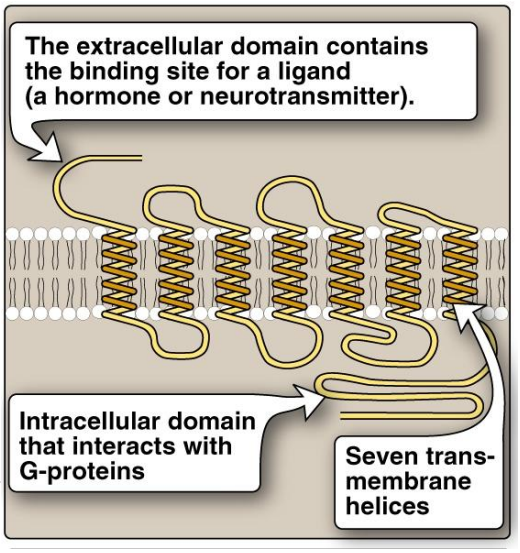
*We'll only discuss the adenylyl cyclase system in this sheet.

➢ Adenylyl cyclase system:

How can the cell recognize the signal when it reaches the cell?

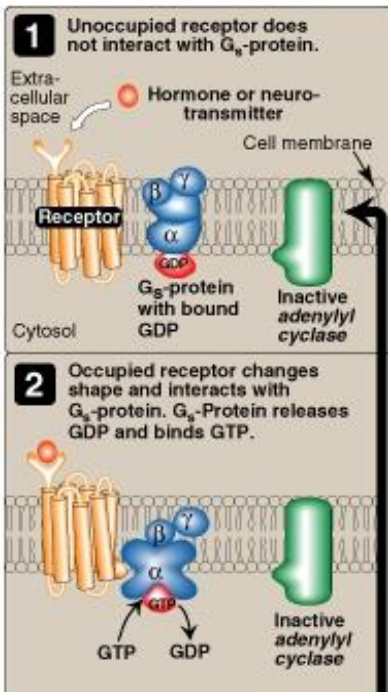
- ✓ By receptors.

- The most common type of receptors is called **G-protein coupled receptors (GPCRs)** also known as **seven-transmembrane domain receptors**.
- These receptors are formed form **three** domains:



1. Extracellular domain: which contains the binding site for the ligand (a hormone or a neurotransmitter)
2. Transmemrane domain: which is formed by seven transmembrane alpha helices.
3. Intracellular domain: which interacts with G-proteins.

Steps:



1. The hormone or the neurotransmitter will bind specifically to the receptor.
2. Once the ligand binds to it, the conformation of the receptor will change, therefore the **G-protein** will become closer to the receptor, and will be able to bind to it.

- The G-protein: is a trimeric protein that is made from three different subunits (alpha, beta and gamma), this G-Protein is bound to the membrane, and it's given that name because it binds GTP and GDP.
- One molecule of a hormone is able to activate 100 or more G-Proteins; this is called **amplification or magnification.**

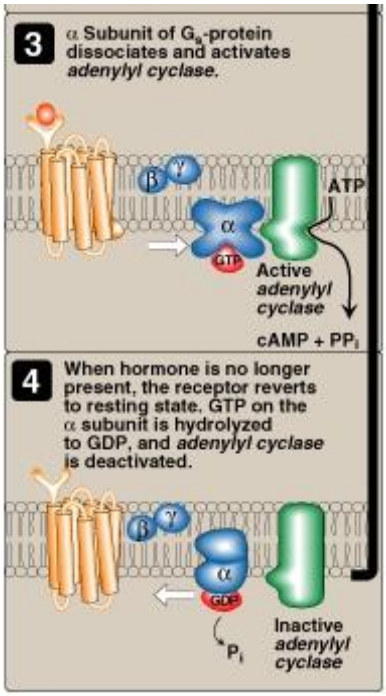
Doctor Faisal gave this example to understand amplification:

(لما الدكتور يحكي لطالب في محاضره اليوم .. و هاد الطالب يحكي لعشر طلاب و العشره يحكوا لكمان عشره .. نفس المبدأ).

3. The GDP that is bound by the alpha subunit is **replaced** by GTP.

- When G-Proteins are bound to GTP they are "on", and when they're bound to GDP they're "off".

4. Once the alpha subunit is bound to GTP, it will dissociate leaving the beta and gamma subunits to form a stable dimeric complex (beta-gamma complex).
5. The alpha subunit that is bound to GTP is now able to bind and activate **adenylyl cyclase**, this enzyme that is found in the cell membrane converts ATP → cAMP + Pyrophosphate (PPi).



- When the hormone is no longer present, because it's concentration has decreased in the blood, the receptor goes back to its resting state, so the GTP that is bound to the alpha subunit is converted to GDP and the alpha subunit regions the beta-gamma complex, and adenylyl cyclase will be deactivated.

<u>How the GTP is converted to GDP?</u>

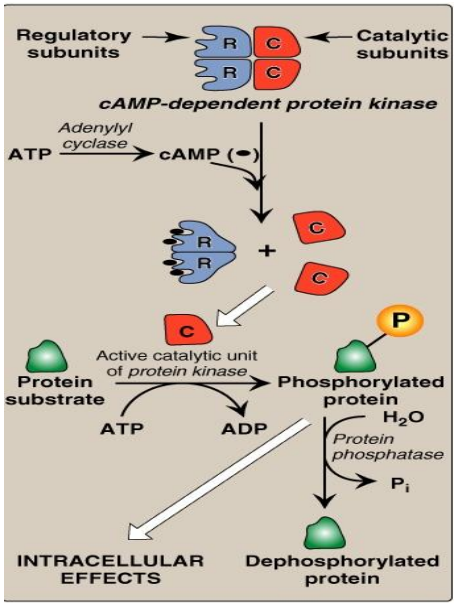
- ✓ The alpha subunit has an intrinsic GTPase activity, which means that it's continuously converting GTP→GDP (hydrolysis reaction).

<u>If the hormone concentration remains the same (isn't increasing or decreasing). Does the activation continue?</u>

- ✓ No, instead, desensitization will happen, which means that the receptor is no longer sensitive to the hormone. For example: if a city is used to smell a certain kind of odor, after a period of time, they'll not be able to smell it anymore.
- ✓ So, the hormone must increase and decrease in order to continuously activate the receptor.

6. The cAMP, which is a second messenger, activates a family of enzymes called cAMP dependent protein kinases, such as protein kinase A.

- <u>Kinases</u>: are enzymes that can add a phosphate group to its substrate, and they take that phosphate group from ATP.
- The protein kinase is formed from four subunits (2 regulatory and 2 catalytic), and that is the inactive form of it.



7. c**A**MP activates protein kinase **A** by binding to its regulatory subunits, causing the release of two active, catalytic subunits. So now the active (catalytic) subunits can add a phosphate group to a protein substrate, making it phosphorylated.

- Remember that phosphorylation **doesn't always mean activation**, it may also cause inhibition of the protein (enzyme). For example: if glucose is available, we have to switch on synthesis of glycogen, and switch of degradation of glycogen).

<u>How can we remove the phosphate group added by protein kinases?</u>

✓ By **phosphatases** which are enzymes that hydrolytically cleave phosphate esters. So, changes in protein activity induced by phosphorylation are not permanent.

Kinases # phosphatases

- cAMP is rapidly hydrolyzed to 5`-AMP by cAMP phosphodiesterase which convert cAMP→AMP.

8. The phosphorylated proteins may act directly on the cell's ion channels, or, if enzymes may become activated or inhibited. Protein Kinase A can also phosphorylate proteins that bind to DNA, causing changes in gene expression.

Good luck everyone