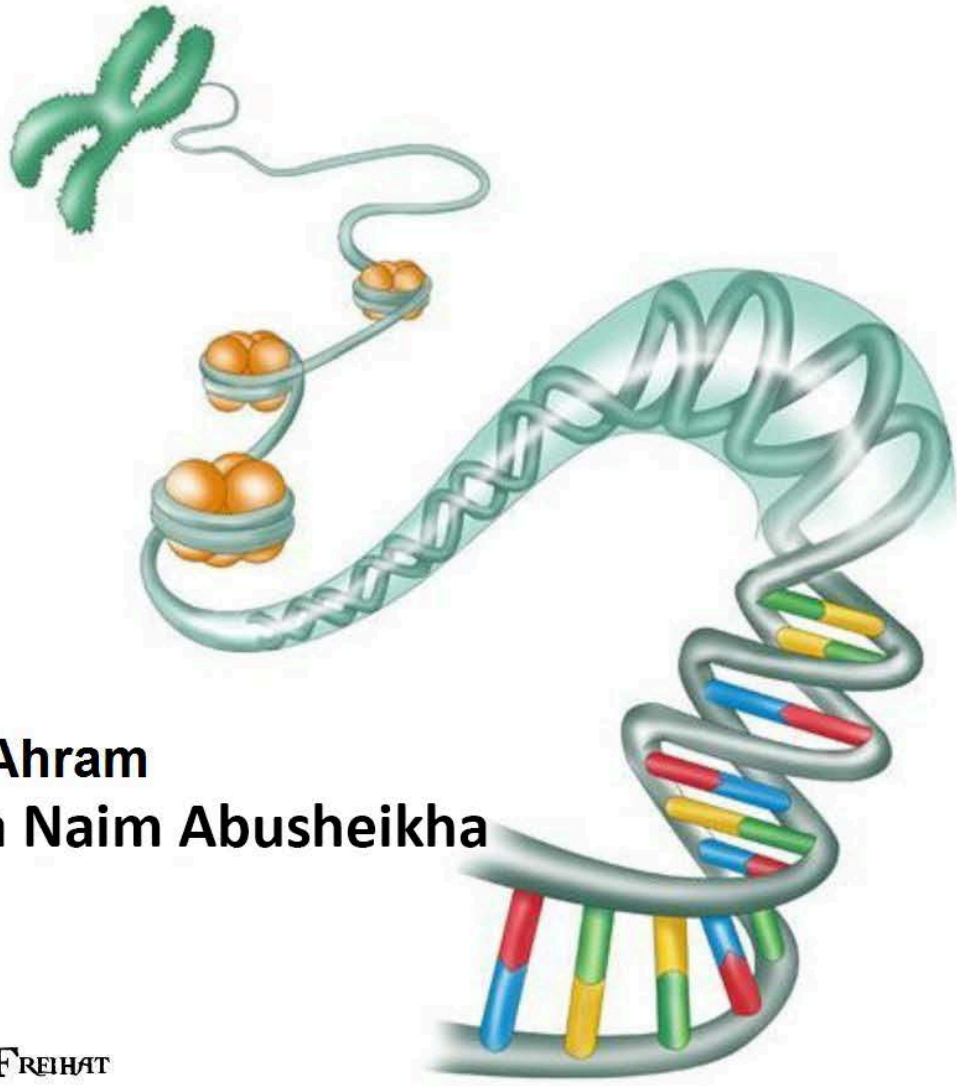
University of Jordan
Faculty of Medicine
Batch 2013-2019

Medical Committee
The University of Jordan

Genetics & Molecular Biology

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



Lecture # 6
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Vesicle fusion and Mitochondria

A week from now you'll wish you had studied this today. Start NOW! It isn't hard.

Vesicle fusion:

There are two mechanisms of fusion of vesicles with the target membrane:

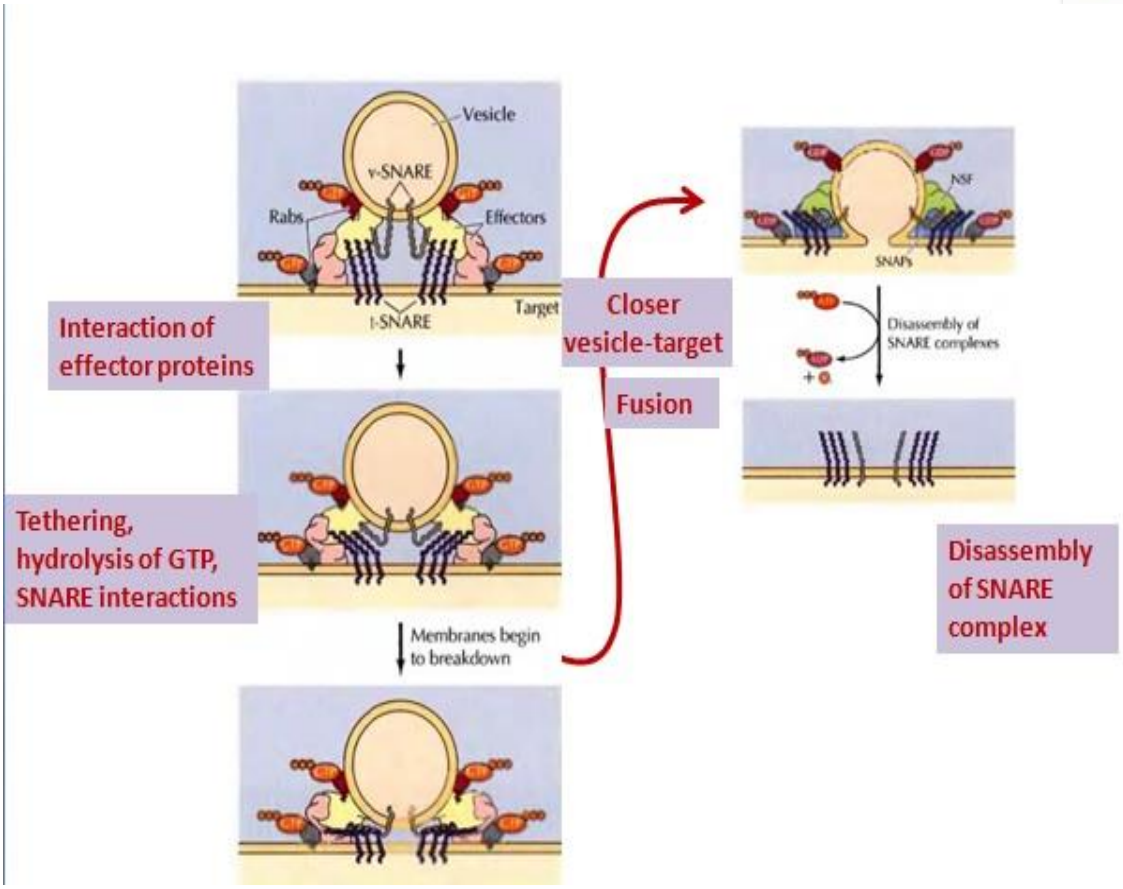
1) **SNARE-mediated membrane fusion**.
The three important components in this mechanism are:

- **Effector proteins**, and there are different effector proteins that provide specificity for interaction.
- **SNAREs** and these include **v-SNAREs** and **t-SNAREs**.
- **Rab proteins**, and there are over 60 of them and different combinations of Rab proteins mark different organelles and transport vesicles. The same proteins exist in yeast, and these proteins regulate this interaction and are important in providing specifity for interaction in terms of vesicular trafficking.

For example, Rab proteins say: "This vesicle should go from the Golgi to endosomes and that's it," or "This vesicle should go from endosomes to the plasma membrane ONLY"

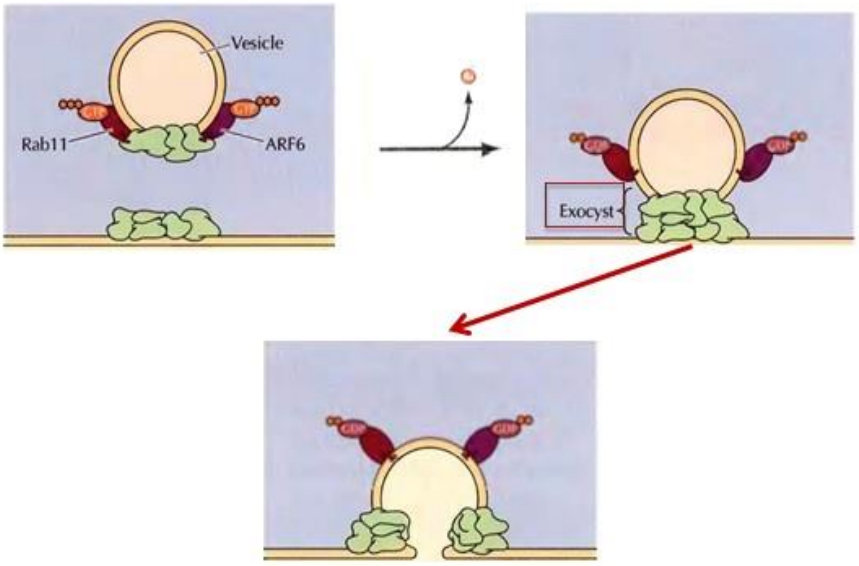
Rab proteins are **GTP-binding proteins** just like ARF and RAS; they are regulated by GTP binding, they have their own GEFs and GAPs.

The doctor says there's a table in your book called *Rab GTP-binding proteins and their sites of action*, which he didn't add in the slides as you do not need to memorize it.



2) A mechanism **specific for exocytosis** only and not any vesicular trafficking process (only for fusion of vesicles with the plasma membrane to release the vesicular content) and is regulated by an **exocyst**, which is basically a complex of 8 proteins.

This mechanism is also regulated by Rabs.



Lysosomes:

In general, Lysosomes degrade molecules using enzymes. We talked about some lysosomal storage diseases and now we're going to talk about Pompe disease and I-cell disease.

Pompe disease (or Glycogen storage disease type III is an inherited disorder caused by the buildup of glycogen in the body's cells. In this disease you have a deficiency of the lysosomal enzyme **α-1,4-glucosidase** which is a carbohydrate-hydrolase that releases alpha-glucose from Glycogen. so if there's a defect in this enzyme the Lysosomes would become enlarged due to the accumulation of glycogen, which impairs their ability to function normally.

I-cell disease is a lysosomal storage disorder due to deficiency of lysosomal enzymes because of the lack of targeting them from Golgi. There are different types of treatments proposed and used to treat this disorder but one of the most promising treatments is **enzyme replacement therapy (ERT)**, which is basically the injection of enzymes into the patient's bloodstream. This method of treatment, however, is extremely expensive so far.

Endocytosis:

Endocytosis is the opposite of exocytosis, and is basically the process by which cells absorb molecules (such as proteins) by engulfing them. It is used by all cells of the body because most substances important to them are large polar molecules that cannot pass through the hydrophobic plasma or cell membrane.

There are four different types of endocytosis:

1) **Endocytosis of intermediate vesicles**.

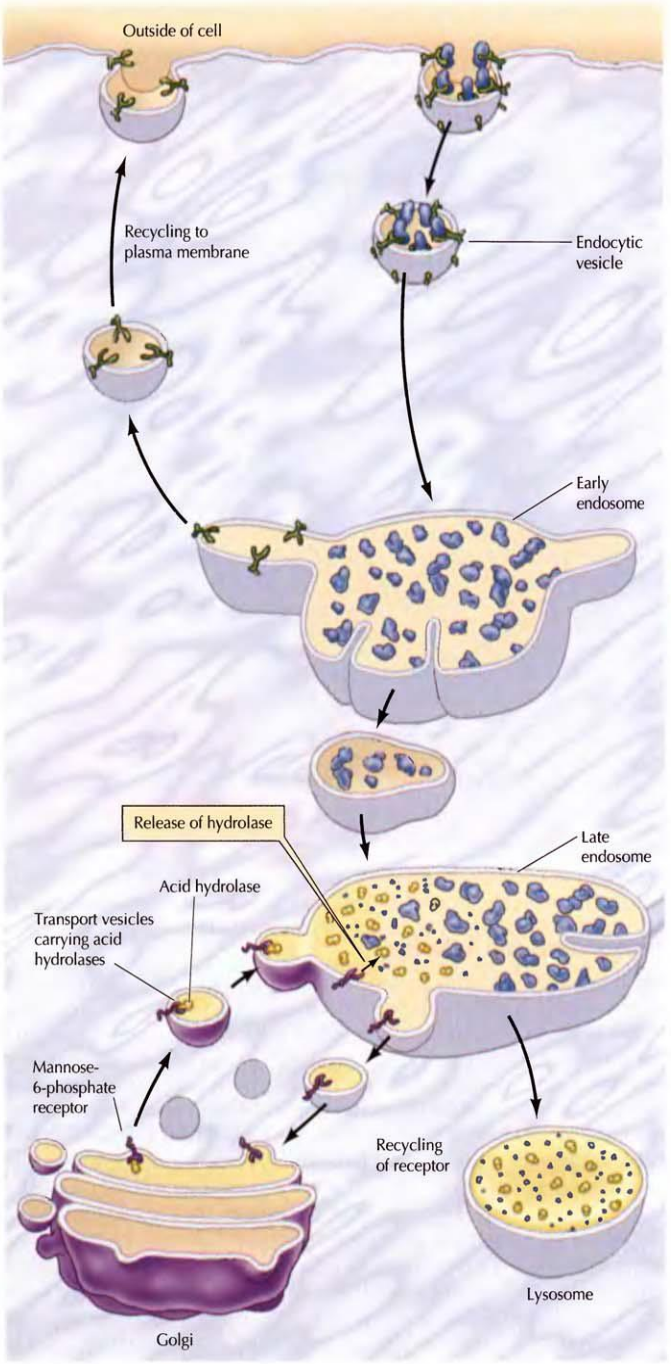
2) **Pinocytosis** (cell drinking), which is a mode of endocytosis in which small particles are brought into the cell, forming an invagination, and then suspended within small vesicles.
3) **Phagocytosis** (cell eating), which is the formation of very large vesicles that can contain organisms as big as bacteria.
4) **Receptor-mediated endocytosis** also called **clathrin-dependent endocytosis** is a process by which cells absorb molecules by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being absorbed. The role of receptor-mediated endocytosis is also well recognized in the downregulation of transmembrane signal transduction so this is one mechanism by which cells can regulate how much receptor there is on the cell surface.
So let's say there's a lot of signaling that reaches the cell, and the cell wants to control the level of signaling that gets into the cell and is affecting the cell's behavior, one mechanism by which the cell can downregulate the signaling is by reducing the amount of receptor that exists on the cell's surface, and it does so by receptor-mediated endocytosis.
Note: As the name indicates, these vesicles are Clathrin-coated vesicles and this is one of the differences between Receptor mediated endocytosis and Phagocytosis.

This mechanism involves several steps:

- The ligand binds the receptor, sending a signal through the membrane, leading to membrane coating and formation of an invagination.
- After that the receptor and the ligand are endocytosed forming an *endocytic vesicle* which fuses with *early endosomes* which will later mature into *late endosomes.*
- During this maturation process the pH in the early and late endosomes is below 7 (it's about 6.5 in early endosomes and 5.5

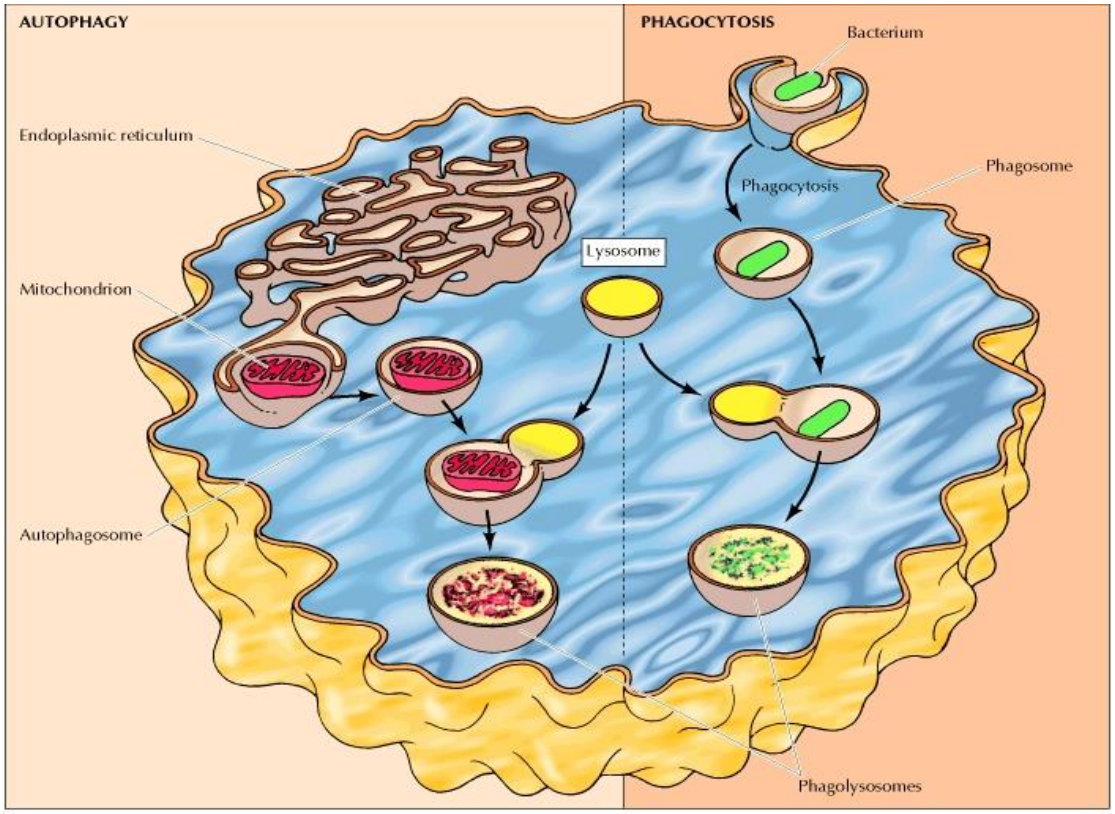
in late endosomes) it's higher than lysosomal pH, but lower than cytosolic pH.

- Because of this lowering of pH, the interaction between the ligand and the receptor is weakened and the **ligand is separated and dissociated from the receptor**.
- The ligand is now in early endosomes which mature into late endosomes. On the other hand, the receptor is recycled back to the plasma membrane via other types of endosomes known as *recycling endosomes*.
- Late endosomes receive lysosomal enzymes (acid hydrolases) from the *trans* Golgi network and either mature into or fuse with Lysosomes .
- The acid hydrolases dissociate from their receptors when the transport vesicles fuse with late endosomes, and theses receptors are recycled back to Golgi apparatus.



To sum up:
The ligand binds to the receptor → receptor-mediated endocytosis→ the vesicle fuses with early endosomes → dissociation of ligand from the receptor due to low pH → receptor is recycled back to the plasma membrane via recycling endosomes, the ligand is now in early endosomes which mature into late endosomes → late endosomes either mature or fuse with Lysosomes

There is a drug known as ***Chloroquine*** which is anti-malarial. Inside red blood cells, the malaria parasite degrades hemoglobin and modifies heme (to acquire essential amino acids, which the parasite requires to construct its own protein and for energy metabolism). Digestion is carried out in a vacuole of the parasitic cell. Chloroquine is a weak base that becomes charged at acidic pH and owing to this property; Chloroquine is selectively accumulated inside Lysosomes. The uncharged compound rapidly diffuses through the plasma and lysosomal membranes, while once charged the compound becomes trapped inside the acidic lysosomal compartment of the parasite. **This drug inhibits the enzyme that modifies heme (heme polymerase) which is part of the parasitic organism, and if heme is not modified it is toxic to the parasite**.



Phagocytosis is the process by which a cell engulfs a solid particle to form an internal vesicle known as a *phagosome*, which later fuses with a lysosome forming a *phagolysosome*. Phagolysosomes contain the acid hydrolases that degrade the bacterial cells or any particle that gets into the cell.

Autophagy (self-eating), the turnover of the cell's own components, a mechanism involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes, so it allows the degradation and recycling of cellular components and also plays an important role in programmed cell death (Apoptosis). The first step is the enclosure of a small area of cytoplasm or a cytoplasmic organelle (ex. Mitochondria) in a cytosolic membrane, forming vesicle known as *astophagosome* which fuses with a lysosome, and its contents are digested.

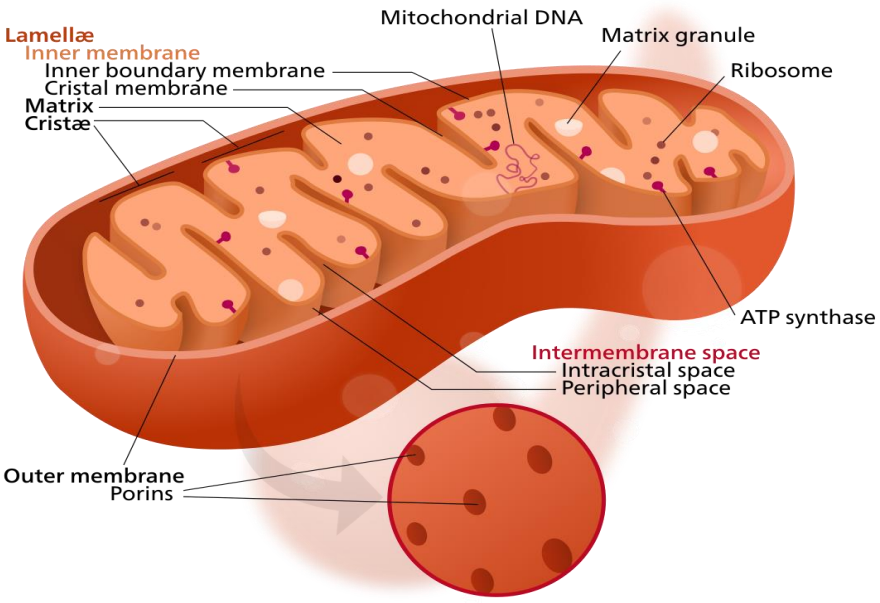
Mitochondria:

Mitochondria are thought to have evolved from bacteria via a process known as **endosymbiosis,** which explains the origin of eukaryotic cell **organelles** from prokaryotes**.** Notice that mitochondria contain some bacterial features (in terms of structure and proteins for exmaple).

The main function of mitochondria is the generation of energy and they are also involved in apoptosis; so some of the enzymes that are found in mitochondria can be activated during apoptosis.

Most mitochondrial proteins come from **free cytosolic ribosomes** so they are not transported from the Golgi, these proteins are synthesized in the cytosol and then they are transported to the mitochondria.

Mitochondria have their own DNA and it's known as mitochondrial DNA, and it is **circular** not linear like eukaryotic DNA. This is one of the things that are common between mitochondria and bacteria. The genome is also small and it encodes some important molecules like tRNA, rRNA as well as some proteins that are important in respiratory chain reactions, but most of the mitochondrial proteins are coded by the nuclear DNA.



Mitochondria are composed of an outer membrane, an inner membrane and an intermembrane space between them. The inner membrane surrounds the matrix.

The outer membrane is permeable as it's composed of openings that are large and are known as *Porins*. Porins are large enough to allow the entry of molecules as large as 1000 Daltons and that's why the composition and environment of the intermembrane space resembles the cytoplasm – all molecules that exist in the cytoplasm can go into the intermembrane space **freely**.

These Porins are channel proteins and these channel proteins are **composed of B-sheets** rather than Alpha helices. (Remember that Alpha-helices make up the transmembrane domains of eukaryotic membrane proteins while Beta-sheets make up the membrane proteins of bacteria.) Porins resemble a bacterial protein with the same name "Porin".

The inner membrane contains a high percentage of proteins. Remember the first lecture when the doctor talked about the composition of membranes and how it's different from one cell to another and among organelles. Recall that one of the features of the mitochondrial membrane is that it's **composed of a large amount of protein relative to lipids**. These proteins are important in oxidative phosphorylation and production of energy.

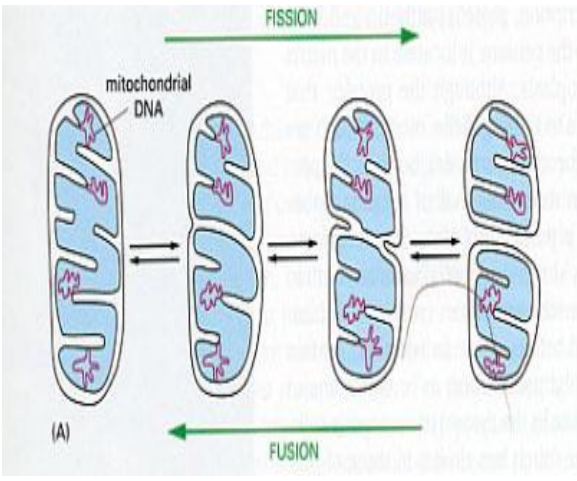
The inner membrane is impermeable to most ions and small molecules so **it's the inner membrane that actually protects and gives those special features to the mitochondrion matrix not the outer membrane.**

The matrix, which is internal, contains all the enzymes necessary for the Krebs cycle.

These mitochondria are present in almost every cell except in RBCs, but they are abundant in cells that require the production of

energy like muscle cells and neurons. They are also clustered in certain areas depending on the needs of those cells.

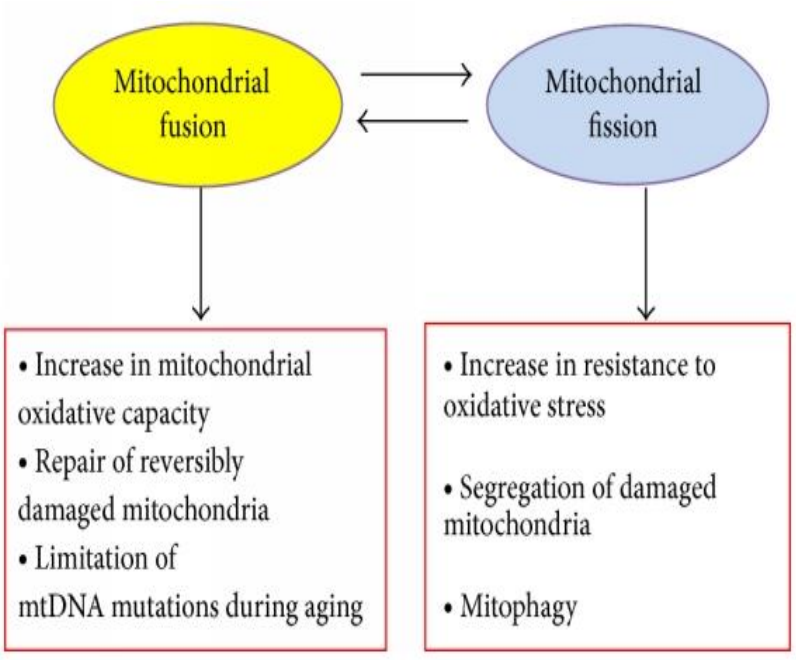
Mitochondria can exchange genetic material, meaning that they are **dynamic** because of reversible processes known as **fusion and fission (division).**



So you could have two mitochondria coming in together forming one mitochondrion and you can have one mitochondrion that can divide to two mitochondria.

So the purpose of fusion and fission is:

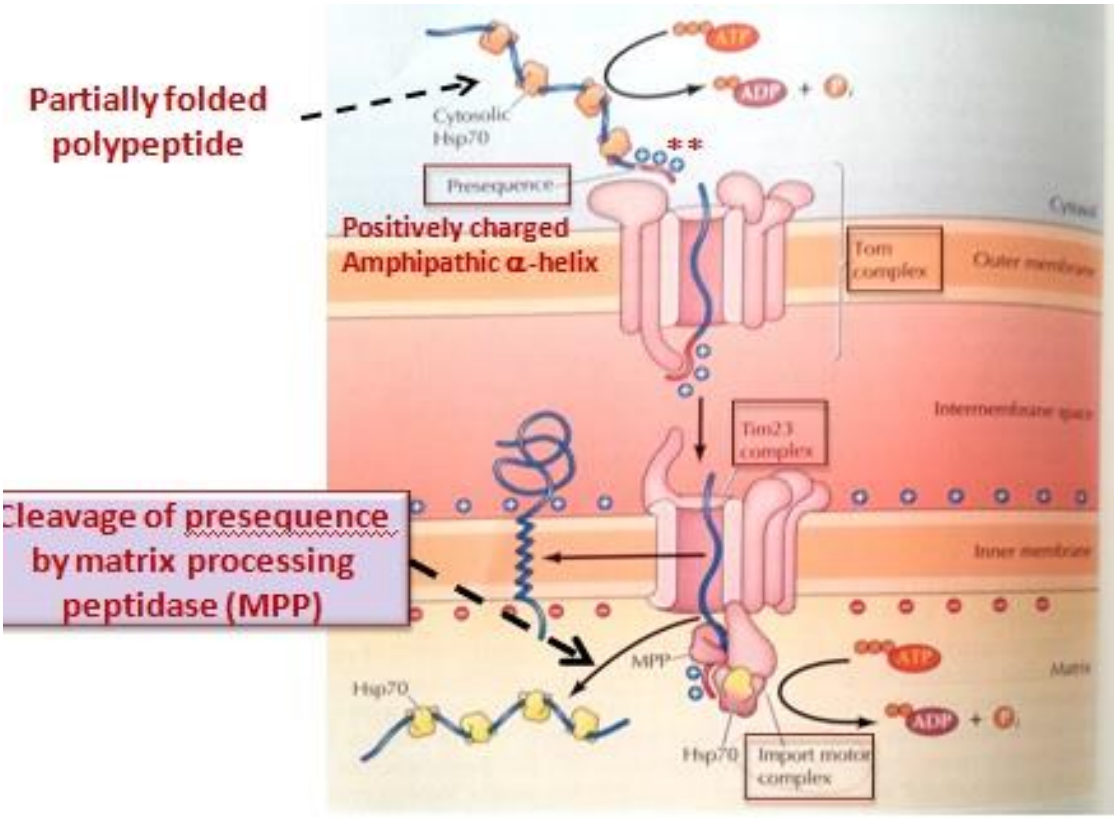
- Exchange of genetic material
- Regulation of autophagy
- Cell survival



So, say, a cell contains thousands of these mitochondria, and let's says 10 were damaged by oxidizing agents, these can either be removed by autophagy or they can be repaired by fusing them with good, functioning mitochondria.

Mitochondrial DNA encodes 13 proteins involved in electron transport and oxidative phosphorylation, two rRNAs, and 22 tRNAs. (Numbers aren't for memorization)

Protein import and mitochondrial assembly



The nucleus contains the genes that encode most of the mitochondrial proteins required for oxidative phosphorylation.

How are mitochondrial proteins transported from the cytosol to the mitochondria?

There are different mechanisms depending on the protein. Some of them contain what is known as a **pre-sequence**. This pre-sequence is a specific sequence that tells cells that this protein is mitochondrial, and is positively charged. Synthesis of that protein occurs in the

cytosol then you have chaperones that cover this polypeptide and take it to the mitochondria. This protein is translocated across the outer mitochondrial membrane via a complex of proteins known as a **Tom complex (Translocase of the outer membrane**), then is transported through the inner mitochondrial membrane into the matrix via another complex known as **Tim23**.

There's a complex that is made of three components:

1) **Import motor complex**
2) **HSP70 (Heat Shock Protein)**, which is a chaperone that allows the entry of this mitochondrial protein.
3) **MPP (Matrix Processing Peptidase)** just like the peptidase in the ER, it **cleaves the pre-sequence**. When it cleaves the pre-sequence it goes into the mitochondria, then it is covered by the heat shock proteins that help in folding the protein.

To sum up:

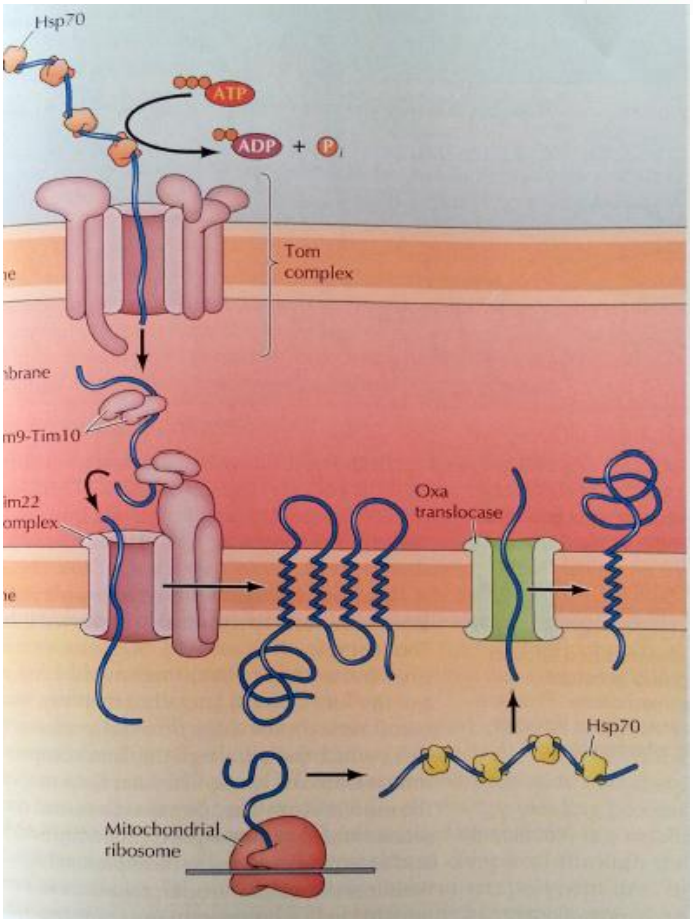
There's a pre-sequence, this sequence is possibly charged, it is recognized by heat shock proteins, which take the mitochondrial protein through the Tom complex and then through the Tim23 complex and as it goes in, the pre-sequence is cleaved off, and the protein is folded with the help of these chaperones inside the mitochondria.

Sometimes if this protein has a transmembrane domain (hydrophobic) then the same Tim complex can recognize it as a transmembrane protein and instead of getting it inside the matrix it pushes it to the side (laterally) and inserts into the inner membrane.

Targeting of inner membrane proteins:

Some proteins are composed of multiple transmembrane domains and they are recognized by another Tim complex known as **Tim22**

rather than Tim23. So Tim22 recognizes this protein, it folds it and it pushed it to the inner membrane.



Sometimes the proteins that are encoded by the mitochondrial genome and are part of the inner mitochondrial membrane are recognized by another complex known as **OXA.** These proteins are synthesized in the mitochondrial matrix, covered by chaperones, and are recognized by the OXA Translocase which translocates these proteins into the inner mitochondrial membrane.

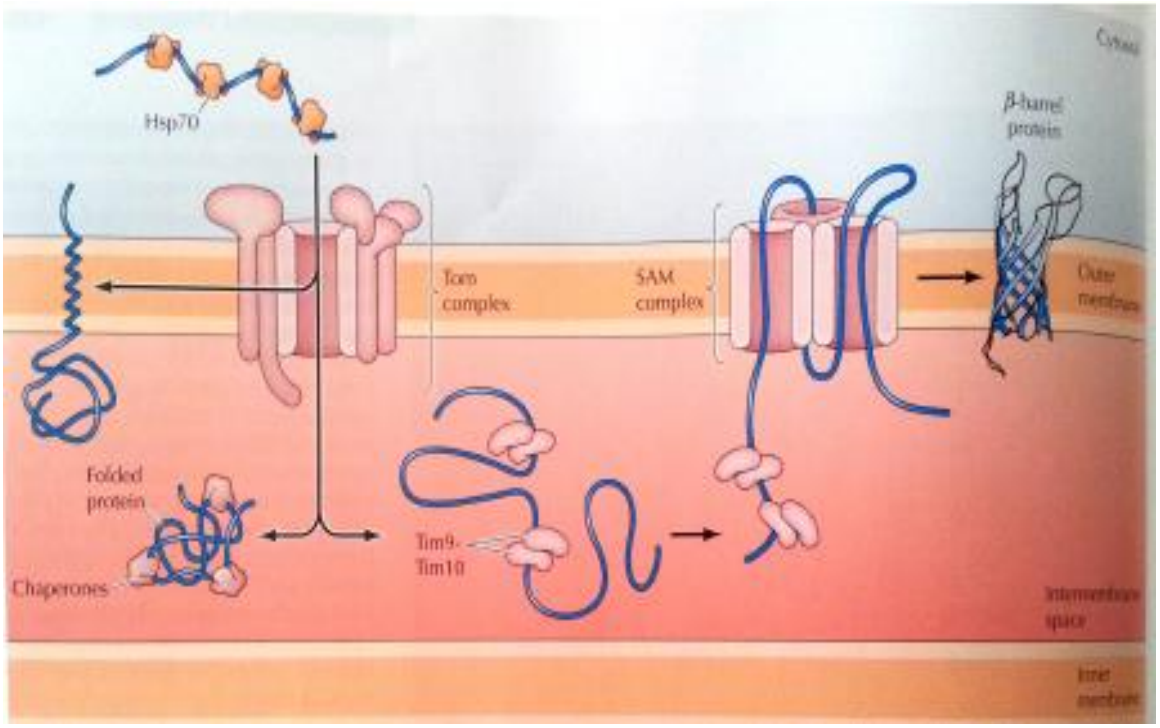
Remember that proteins with multiple transmembrane domains do not have pre-sequences but they have other sequences/signals (multiple internal mitochondrial import signals).

What is the mechanism of getting the protein out of the translocon into the membrane? How is it pushed? In the ER we said that what happens is that the channel closes, does the same mechanism occur in mitochondria? Maybe it does maybe it doesn't, the doctor says he doesn't think scientists know.

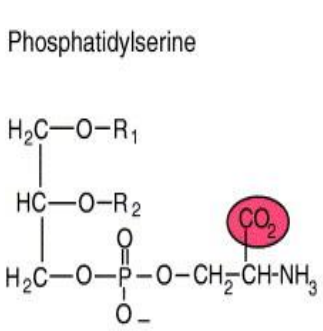
Targeting of outer membrane proteins:

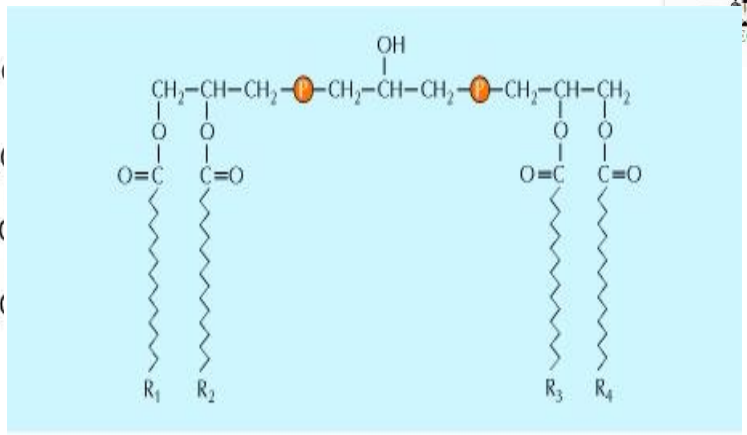
There are two translocons. Again, the protein is synthesized in the cytosol; it is recognized by the Tom complex which can tell that this protein has a transmembrane domain and pushes it laterally into the outer membrane.

The Tom complex **is specific for proteins that have a transmembrane domain that is alpha-helical**, but proteins that are made of B-sheets like the Porins for example, are recognized by another complex known as the **SAM complex (Sorting and Assembly Machinery)**.



The mitochondrial membrane is composed of specific lipids as well. It has **Phosphatidylcholine** and **phosphatidylethanolamine** which are synthesized in the ER and carried to mitochondria by phospholipid transfer proteins. The mitochondria then synthesizes **Phosphatidylserine** from phosphatidylethanolamine (just like in ER).





The unusual phospholipid, **cardiolipin**, which contains four fatty acid chains, is also synthesized in the mitochondria.
This molecule **improves the efficiency of oxidative phosphorylation by restricting proton flow across the membrane**. So what it does is that it prevents the protons from leaving the mitochondria, maintaining the proton gradient across the inner membrane in order to allow the generation of as much ATP as possible.

Mitochondrial diseases: (not mentioned in the book)

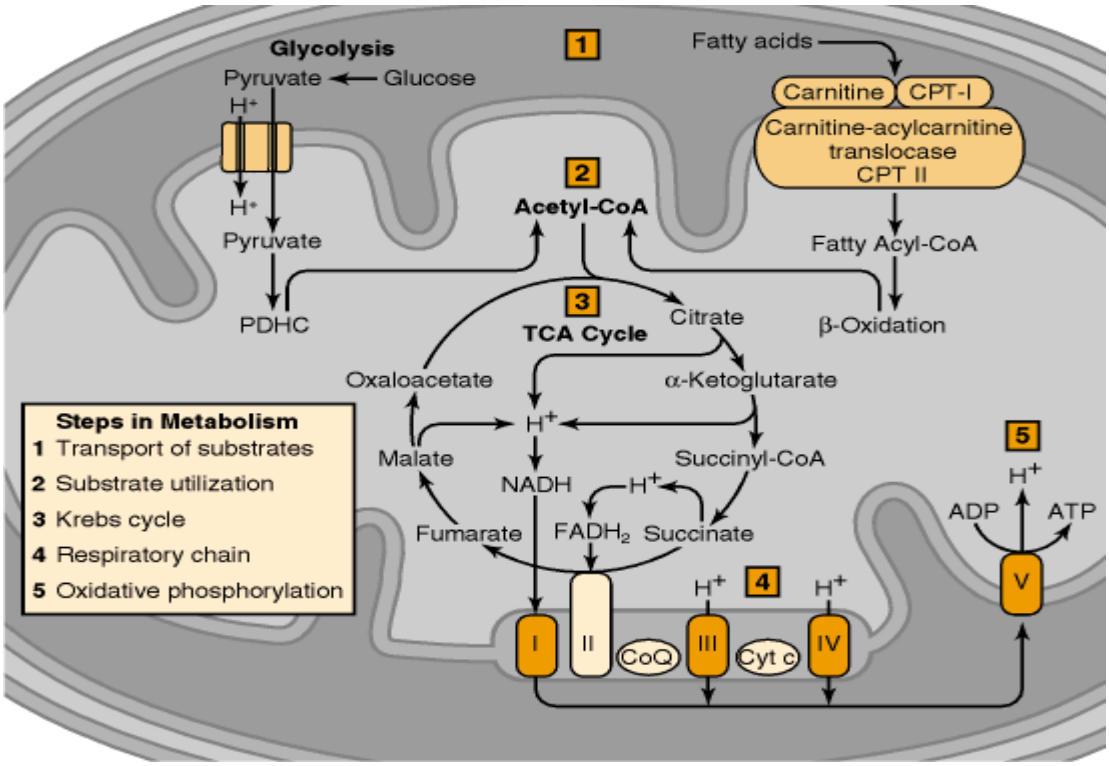
Most of the mitochondria that we get are from our mothers and this is why when scientists wanted to study the human origin and their relationship between world populations, they relied on mitochondrial DNA because it comes from one source so it's easier to compare. They also studied the Y-chromosome because it also comes from one source.

Logically, tissues that are mainly affected by mitochondrial diseases are mainly the muscle and the nervous tissues because these are the two tissues that require mitochondria more than anything else.

Mitochondrial diseases can be classified into: **biochemical or genetic.**

According to the biochemical classification of mitochondria, there are five types of diseases depending on what and where the mutation is:

- **The transport system** that allows for the entry of pyruvate or fatty acids into the mitochondria. There are two transport systems: **Carnitine-acylcarnitine Translocase** specific for fatty acids, and the **Pyruvate dehydrogenase complex**.
- **Substrate utilization.** For example you can have defective pyruvate dehydrogenase so in this case there's **no good utilization for pyruvate** so the main source of energy for this individual is fatty acids and lactic acids as glycolsis cannot continue so there will be accumulation of pyruvate in the cell, there's no conversion of pyruvate to acetyl Co-A so these individuals which are mainly males, suffer from **metabolic acidosis** as a result of the production off a high level of lactate and this lactate would present in high levels in blood as well as the CSF.
- **A mutated enzyme in the Krebs cycle** just like **Fumerase** which converts Fumerate to Malate, so these individuals would have high levels of Fumerate as well as Succinate in their fluids. The main tissues affected are muscles and the liver.
- **A mutation in the respiratory chain reaction**
- **A mutation in the ATPase**, resulting in faulty production of ATP.



The doctor says there's a mistake in slide #21, the second bullet point should say: "respiratory rate" instead of "oxidative phosphorylation." In this case there is burning of metabolites and burning of glucose and pyruvate, movement of protons across the inner membrane so there's energy but **there's defective production of ATP from ADP** so there's continuous burning of metabolites and there's no ATP produced so mainly what the person develops is **hyperthermia** and **hypermetabolism** because energy is lost in the form of heat.

The genetic basis of mitochondrial diseases:

Mitochondria produce different proteins **but most mitochondrial proteins are of nuclear origin**, so you can classify mitochondrial diseases according to the genetic classification (whether the mutation is from the nuclear DNA or mitochondrial DNA.)

MERRF (**myoclonic epilepsy and ragged red fiber disease)** is caused by a mutation in one of the mitochondrial transfer RNA genes required for synthesis of the mitochondrial proteins responsible for electron transport and production of ATP.

Other syndromes include:

- lactic acidosis and stroke-like episodes (MELAS)
- Leber's hereditary optic neuropathy (LHON),
- neurogenic atrophy, ataxia and retinitis pigmentosa (NARP)

Leber's hereditary optic neuropathy (LHON)

Visual nerve is affected, and individuals have problems seeing well. The inheritance is mitochondrial so it comes from the mother because again most mitochondria come from the mother. So in this case **this mutation reduces the efficiency of oxidative phosphorylation and ATP generation.**

- 50% mutation in a subunit of complex I of the electron transport chain (NADH dehydrogenase)
- 30% is due to two mutations in other subunits of complex I or a mutation in cyochrome *b* (a component of complex III)
- Since the central nervous system (including the brain and optic nerve) is most highly dependent on oxidative metabolism, blindness is the main manifestation.
- The low incidence of disease among carriers of LHON mutations is because each cell contains thousands of copies of mitochondrial DNA, which can be present in mixtures of

mutant and normal mitochondria (heterogeneity in terms of severity).

Defects of nuclear DNA are more common than mitochondrial DNA because most of the proteins come from the nucleus.

Trust yourself; you know more than you think you do.

By Aya Naim Abusheikha