Genetics & molecular biology

◉ Sheet ○ Slide

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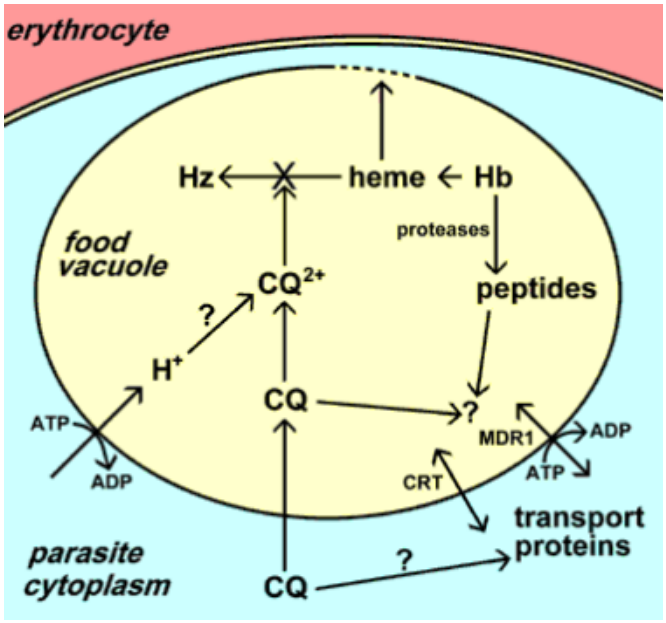
Application: (the role of lysosomal enzymes in the effectiveness of some drugs)

Chloroquine:

- It is an Anti-malarial agent. Malaria is a disease caused by a parasite that targets the red-blood cells (Erythrocytes), specifically the haemoglobin.



Once that parasite is inside the RBC, its vacuole (coloured by yellow) will destruct the haemoglobin by

- separating the protein from the heme
- degrading the heme by (**heme polymerase enzyme**)

- If the parasite isn't able to degrade the heme, it will accumulate becoming a toxic material that kills the parasite.
- Chloroquine is a drug that has the ability to cross the membranes of the parasite reaching the vacuole and inhibiting the heme polymerase enzyme. Thus, the heme will accumulate inside the vacuole inducing a toxic effect on the parasite and killing it.
- But**, What is the role of Lysosome**?

Chloroquine is a weak base that needs to be protonated to perform its function. This protonation takes place in an acidic environment that is found inside the lysosomes. Thus, the lysosomal function is to provide the acidic environment needed to activate the Chloroquine.

ENDOCYTOSIS:

- One of the major functions of lysosomes is the digestion of material taken up from outside the cell or from the plasma membrane itself by a process known as **endocytosis**.

Remember that: protein degradation is of two types:

Either **proteasomal degradation** (degrades the intracellular proteins) or **Lysosomal degradation** (degrades extracellular and membrane proteins). Lysosomal degradation is also called **Receptor mediated endocytosis.**

How does Receptor mediated endocytosis take place?

- Molecules, which are distended for degradation, bind to specific receptors on the plasma membrane. Then, a vesicle is formed carrying these molecules towards the cytosol.
- Early endosomes receive these vesicles and they, then, separate molecules targeted for recycling back to the plasma membrane from those destined for degradation in lysosomes. The molecules to be recycled (which are the membrane receptors) are then passed to recycling endosomes and back to the plasma membrane.
- Early endosomes will mature to become Late endosomes.
- Lysosomal acid hydrolases are, then, transported to late endosomes from the *trans-Golgi* network. How?

  As discussed previously, lysosomal proteins are targeted to late endosomes by mannose-6-phosphate residues, which are recognized by mannose-6-phosphate receptors in the *trans-Golgi* network and packaged into vesicles.
- Following fusion of these vesicles with late endosomes, hydrolases will dissociate from the mannose-6-phosphate receptor, and then they will be released into the lumen of the endosome, while the receptors remain in the membrane and are eventually recycled back to the Golgi.
- Changes like the recycling of the receptors back to the Golgi and the activation of protons pumps that provide the acidic environment needed for hydrolase enzyme activation will induce the maturation of the late endosome into lysosome.

PHAGOCYTOSIS:

In **phagocytosis,** specialized cells, such as macrophages, take up and degrade large particles, including bacteria, cell debris, or any "solid" foreign bodies that need to be eliminated from the body. Once Such large particles are encountered, invagination of the cell membrane and formation of pseudopodia will take place in order to take them up in phagocytic vacuoles (phagosomes), which then fuse with lysosomes, resulting in digestion of their contents.

Autophagy: is the turnover of the cell's own components. In contrast to phagocytosis, autophagy is a function of all cells and results in the gradual degradation of long-lived proteins. The first step in autophagy appears to be the enclosure of a small area of cytoplasm or a cytoplasmic organelle (e.g., a mitochondrion) in a membrane derived from the endoplasmic reticulum. The resulting vesicle (An autophagosome) then fuses with a lysosome, and its contents are digested.

Autophagy is activated in many cases or reasons such as:

1- Embryonic development.
2- Autophagy is activated when cells are deprived of nutrients or under stressful conditions (e.g., hypoxia)

Before activating Apoptosis: Autophagy usually a process that precedes apoptosis. Before going through apoptosis, the cell activates autophagy so it can decrease its load. In specific, it decreases the number of mitochondria.

Mitochondria

1- Mitochondria are the **energy factory** inside the cell, they are responsible for most of the energy derived from the breakdown of carbohydrates and fatty acids.
2- It has its own DNA (circular) which encodes **tRNAs, rRNAs**, and some **mitochondrial proteins**, but most mitochondrial proteins are translated on free cytosolic ribosomes and imported into the organelle (I.e., Mitochondrial proteins are encoded by their own genomes and nuclear genome). **It is the only organelle that has its own DNA other than the nucleus.**
3- Mitochondria are Double-membrane structures with an outer and inner membrane in order to isolate their content from the cytosolic environment.

- The inner membrane forms folds called (cristae) to increase its surface area for more efficient energy production.
- The inner membrane contains high percentage (Greater than 70°/o) of proteins, which are involved in oxidative phosphorylation, ATP generation and transport of metabolites (pyruvate and fatty acids).
- The inner membrane is Impermeable to most ions and small molecules, thus maintains H+ gradient that drives oxidative phosphorylation.(from slides)
- The outer mitochondrial membrane is highly permeable to small molecules. It allows a free diffusion of molecules with sizes up to 1000 Daltons and this is because it contains proteins called porins, which form channels. The outer membrane has much lower protein content than the inner membrane or than the matrix.
- Intermembrane space: similar in composition to the cytosol
- Inside the inner space (matrix), we have a lot of enzymes proteins that are required for the function of Mitochondria

- Matrix Contains the mitochondrial genetic system and the enzymes responsible for Krebs cycle.(from slides)

Mitochondrial fusion VS fission:

- The mitochondrion is a dynamic organelle, it may increase in size by **fusion**, or may divide into more than one mitochondrion by **fission. Both processes are in equilibrium**

1- Fusion:

Fusion processes happen in order to:

A- Increase the mitochondrial oxidative *capacity*:

∗ Two mitochondria are fused ⟶ Large mitochondrion The amount of proteins and enzymes increases ⟶ The capacity to perform the reactions increases.

B- Repair the reversibly damaged mitochondria:

- A fusion between a ***reversibly*** damaged mitochondrion and a healthy one resulting in repair of the damage.

C- Limit the mtDNA mutations during aging:

∗∗ With aging, more ***acquired*** mutations are built and accumulated. Fusion process enables the mutated mitochondria to fuse with intact others to overcome these mutations. Thus, mitochondrial DNA mutations that do create diseases are mostly Germline mutations (***acquired*** mutations are repaired through the Fusion process).

2- Fission: (mitochondrial division)

Fission processes happen in order to:

A- Increase in resistance to oxidative stress:

Note that this point is different from the first point we mentioned in fusion.

(Oxidative stress ⟶ increase the presence of the ROS in the cell that occurs as a result in the metabolic processes)

Fission ⟶ Dividing the large mitochondrion into small mitochondria⟶ The total surface area will increase⟶ Increase the capacity of mitochondria to resist the oxidative stress.

(Imagine that a cell has 20 mitochondria that undergo division to get 40 mitochondria, these 40 mitochondria will distribute and occupy larger area within the cell than that of the 20 ones, increasing the probability to interact with more ROS and preventing them from getting access to other organs and proteins and disrupting them).

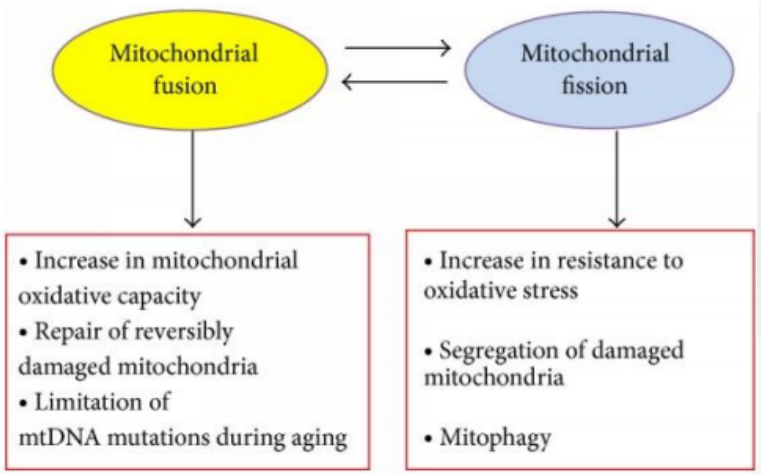
B- Segregate the damaged mitochondria:

∗ If a mitochondrion is NOT totally damaged, there will be segregation between the intact part and the affected part, the cell will get rid of the affected one by Mitophagy (will be discussed soon), and it will keep the intact part.

C- Mitophagy: (autophagy of mitochondria)

The damaged parts of mitochondria get separated, the autophagy happens.

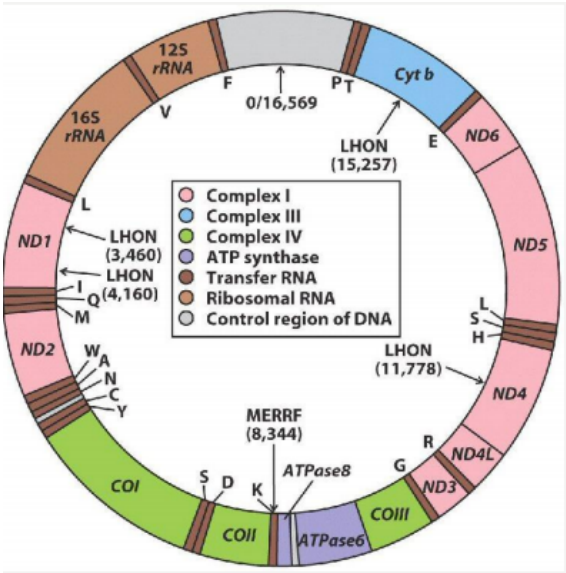
Fusion and fission processes are at equilibrium.



The genetic system of Mitochondria:

- Circular
- Thought to have evolved from bacteria in a symbiotic relationship living in larger cells (endosymbiosis).
- Mitochondrial DNA length is (~16 kb= 16,000 base pair in human); while nuclear DNA is composed of 3 Billion base pairs.
- Multiple copies per organelle (from slides).

  Google: whereas nuclear chromosomes are present as either one copy per cell (haploid) or two copies (diploid), organelle chromosomes are present in many copies per cell, including mitochondria)



- Encodes 13 proteins involved in electron transport and oxidative Phosphorylation, two rRNAs ***(***16S+ 12S of ***mitochondrial ribosomes)*** and 22 tRNAs (20 tRNAs for the 20 types of amino acids, and 2 tRNAs have anticodons for two stop codons)

  *(The mitochondrial tRNA is different from the cytosolic tRNA. In mitochondria, each amino acid has only one tRNA, while in the cytosol we have 64 codons (61 for amino acids and 3 for stop codons).

Mitochondrial genetic code and mutations:

- Notice the differences between the universal (nuclear) and mitochondrial genetic codes:
  The same codon in mitochondria and the nucleus may encode for amino acids in each of them.
  For instance, AGG codes for arginine in the nucleus and leads to stop the translation in mitochondria.

TABLE 11.1 Differences between the Universal and Mitochondrial Genetic Codes

Codon	Universal code	Human mitochondrial code
UGA	Stop	Trp
AGA	Arg	Stop
AGG	Arg	Stop
AUA	Ile	Met

Other codons vary from the universal code in yeast and plant mitochondria.

Mitochondrial proteins:

- The mitochondrial proteins and enzymes are distributed in IMM, OMM, intermembranous space and matrix → the **IMM** contains most of the mitochondrial proteins IMM proteins, specifically the involved in Metabolism Any mutations affecting them will lead to metabolic diseases (e.g., diabetes, obesity, mutations in mitochondrial genes of electron transport chain result in Leber's hereditary optic neuropathy disease as well (will be discussed later in this sheet).
- **Proteins encoded by nuclear genes:**
  - Proteins required for DNA replication, transcription, translation, ribosomal proteins, oxidative phosphorylation, and enzymes for mitochondrial metabolism (TCA cycle). (From slides)
  - The proteins encoded by nuclear genes (~99% of mitochondrial proteins) are synthesized on free cytosolic ribosomes and then imported into mitochondria as completed polypeptide chains.
  - Mitochondria from different tissues contain different proteins.

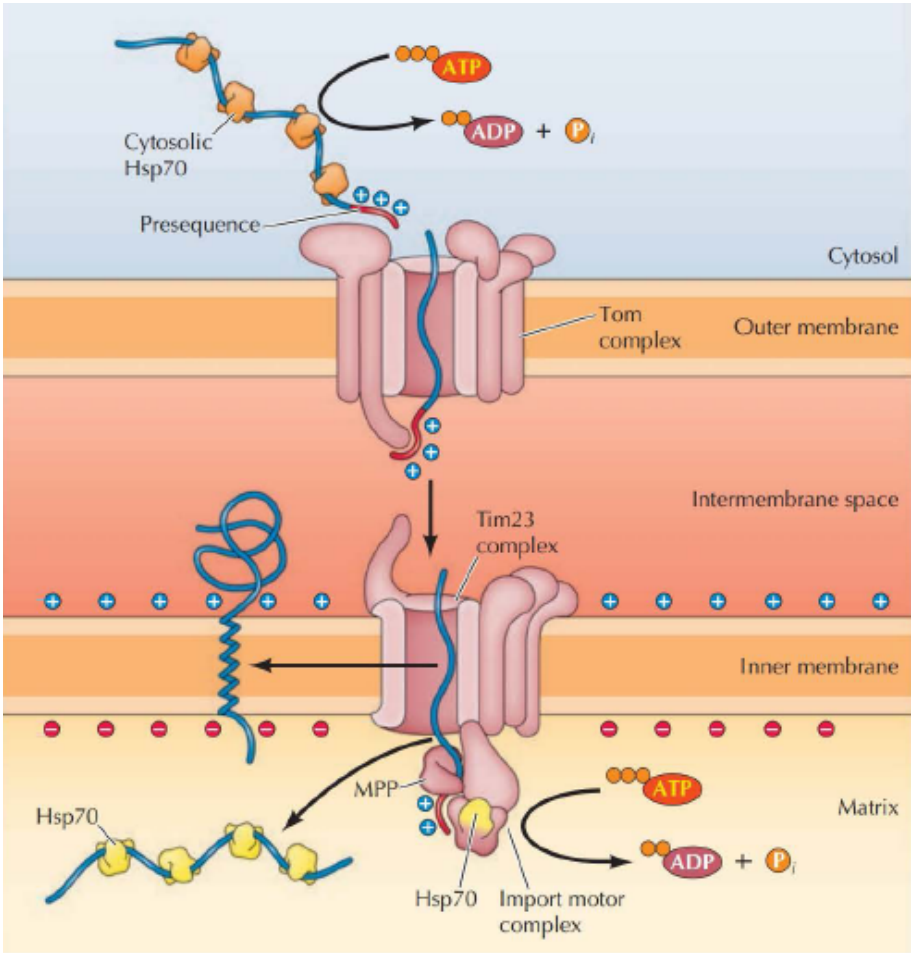
  (< 50 % of proteins are common to all tissues).

  Mitochondrial proteins that are synthesized on both free cytosolic ribosomes and mitochondrial ribosomes will be imported to different destinations within the mitochondria (IMM, OMM, intermembranous space and matrix).
  How do targeting and importing take place?

Protein import and mitochondria assembly:

1) Import of mitochondrial proteins with presequences (synthesized on free cytosolic ribosomes):

- These proteins contain:
  - A hydrophobic AAs part that will form the helix within the IMM.
  - A signal sequence at the N-terminus that contains positively charged amino acids (this sequence is called Pre-sequence)
- These presequence amino acids will be recognized by a protein complex lies in the OMM called **TOM** (Translocase Of Outer membrane), **TOM** complex directs translocation of the protein across the outer membrane.
- The presence of large amounts of such a protein with positively charged presequence in the intermembrane space will create an electrochemical gradient (electrochemical potential) that pushes the proteins through a complex called **TIM 23**(Translocase Of Inner membrane) complex found in the IMM.
- In the matrix, an **import motor complex** containing a group of proteins such as **matrix processing peptidase (MPP)** that will CLEAVE the presequence AND **hsp70** chaperons (and other chaperons) that are responsible for the FOLDING of the proteins.

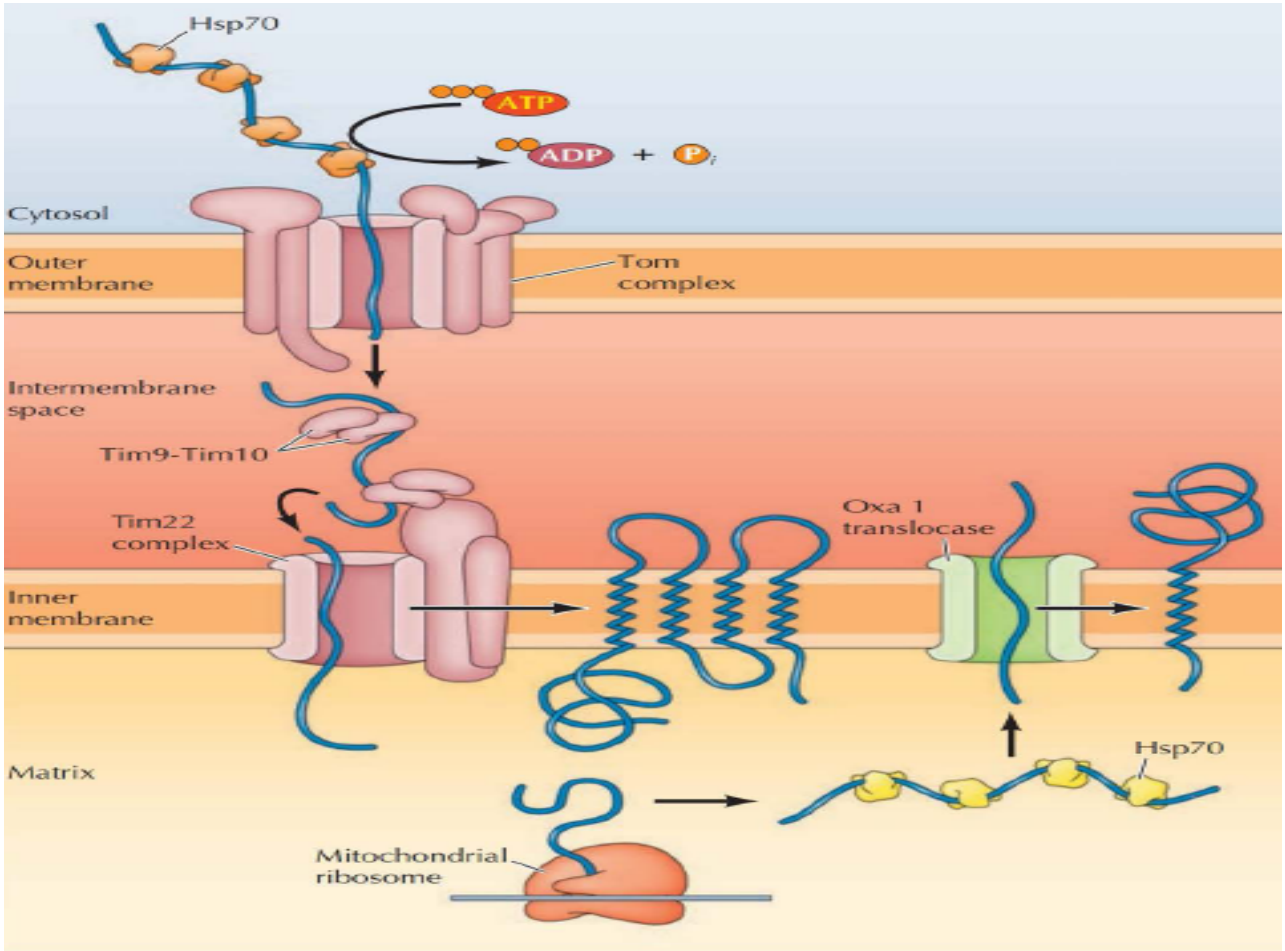


- Following the folding of the proteins, they will be either inserted into the **IMM** or **soluble matrix proteins**.

2) Import of mitochondrial proteins <u>without presequences</u>:

Proteins can be targeted to the inner membrane not only by presequences, but also by other signals. Many mitochondrial proteins are *multi-pass transmembrane* proteins that do not contain presequences, but have **multiple internal mitochondrial import signals**.

- Proteins marked by these sequences cross the outer membrane through the **TOM** complex. **Hsp70** chaperons, also, participate in guiding the proteins through the **TOM** complex.
- Proteins are recognized by mobile chaperones called (**Tim9** and **Tim10**) chaperones. These chaperons will escort the proteins to the **Tim22** complex**.**
- If this protein is a ***soluble protein***, it will enter the ***matrix*** and undergo folding by the chaperons, AND if it is *an* ***IMM protein***, its hydrophobic stretches will contribute in the ***formation of the helices***, whether one or more helices and it will be ***inserted*** in the IMM.



*** Remember that: All the above-mentioned proteins are synthesized on free ribosomes.**

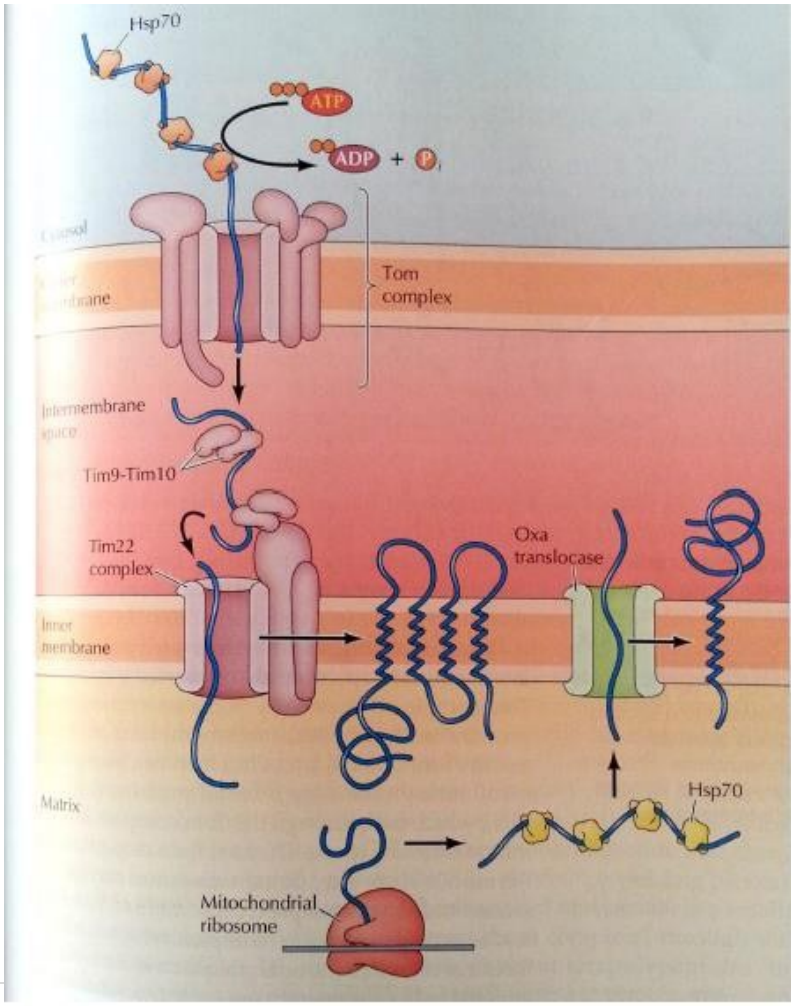
3) So, what about the proteins synthesized on the mitochondrial ribosomes, which are encoded by the mitochondrial DNA, How are they targeted and transported?

After these proteins are synthesized on ribosomes within the mitochondrial matrix, they will be held by chaperones to prevent them from folding (because they need to be inserted in the inner membrane); a protein complex located in IMM that is called **Oxa translocase** does insert them. (notice the last Figure)

4) Targeting of outer membrane proteins:

Proteins destined for the outer membrane or the intermembrane space are also imported through the **Tom** complex.

- Outer membrane proteins with **α-helical** transmembrane domains enter through the **Tom** complex and when the hydrophobic stretches are encountered, they induce the formation of helix proteins are then inserted into the membrane.
- If it is a soluble protein (an intermembranous space protein), will enter through the **Tom** complex to the intermembranous space and then is folded by chaperons.
- Many outer membrane proteins (e.g., porins) are **β -barrel** proteins. They



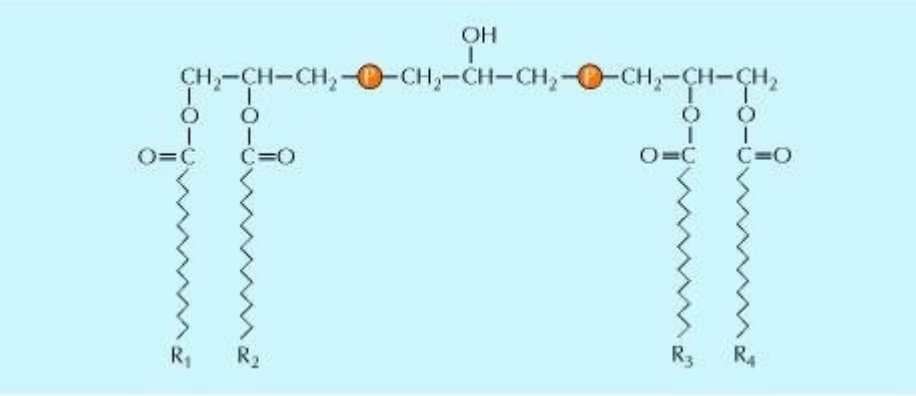
pass through the **Tom** complex into the intermembrane space. They are then recognized by the mobile **Tim9** and **Tim10** chaperones and carried to another complex, called the SAM (sorting and assembly machinery) complex, which mediates their insertion into the outer membrane

*Proteins are targeted to the intermembrane space by cysteine-rich sequences that are recognized by chaperones after the proteins exit the Tom complex.

*The **β -barrel** proteins are found usually in porins, proteins that function as pores, which increase the permeability of the membrane. Thus, they are only present in the outer membrane not the inner one.

Mitochondrial phospholipids:

- Phosphatidylcholine and phosphatidylethanolamine are synthesized in the ER and carried to mitochondria by phospholipid transfer proteins.
- **Phosphatidylserine** is synthesized in the mitochondria through the carboxylation process of the phosphatidylethanolamine.
  (Phosphatidylethanolamine is also synthesized in the ER as discussed previously.)
- A special type of phospholipids is exclusively present in the inner mitochondrial membrane, which is ***cardiolipin.***



- cardiolipin is composed of (2) phospholipid molecules, that are connected by a glycerol molecule, where it, specifically , links the 2 phosphate groups of each phospholipid molecule together.

Remember **:{** the **phospholipid** head **contains** a negatively charged phosphate group and **glycerol**; it is hydrophilic. The **phospholipid** tails consist of 2 long fatty acid chains**}**

Cardiolipin function —it→improves the efficiency of oxidative phosphorylation by restricting proton flow across the membrane.

Mitochondrial Diseases

There are many diseases caused by mitochondrial DNA mutations, we will explain some of them, but you also have to read this paper (here is the link https://cvmkr.com/ar/CV/edit?id=6484253#0 **). You have to memorize the NAMES of the diseases only.**

- These disorders are associated with dysfunction of the respiratory chain (electron transport chain) because all 13 subunits encoded by mtDNA are subunits of respiratory chain complexes ⟶ resulting in metabolic diseases.
- One main syndrome is myoclonic epilepsy and ragged red fiber disease (MERRF), which can be 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. (From slides)
- Other syndromes include

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

- These diseases are caused by mutations that can be single mutation, or multiple mutations affecting more than one gene of the mitochondrial DNA.
- These mutations are **GERMLINE** mutations (point mutations) that are transmitted by **MATERNAL** inheritance, why maternal? ⟶ because during fertilization, the sperm only contributes its nucleus whereas the egg contributes its cytosol with all containing organelles including the mitochondria.

Leber's hereditary optic neuropathy (LHON)

- A rare inherited disease that results in blindness because of degeneration of the optic nerve.

- Vision loss is the only manifestation that occurs between 15-35 years old.
- Females (10%) are affected less frequently than males (50%)
- Males never transmit LHON to their offspring and not all individuals with mutations develop the disease.
- Inheritance is mitochondrial (cytoplasmic) not nuclear.
- The mutations reduce the efficiency of oxidative phosphorylation and ATP generation and this can affect other systems, mainly the eyes (vision) in this disease.

Q: Do we all have the same number of mitochondria in our brain cells or even muscle cells?

Definitely not, persons who practice higher intellectual efforts and activities such as thinking, studying, meditation..., would have larger number of mitochondria. Playing sports increases the number of mitochondria in muscle cells as well.