University of Jordan
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GENETICS & Molecular Biology

Number: 21

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Subject: mitochondria

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Date:

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**This sheet was written according to sections (1+3).

So far, we talked about vesicular transport within the cell, movement of different molecules between different compartments inside the cell, How about taking up materials from outside sources?

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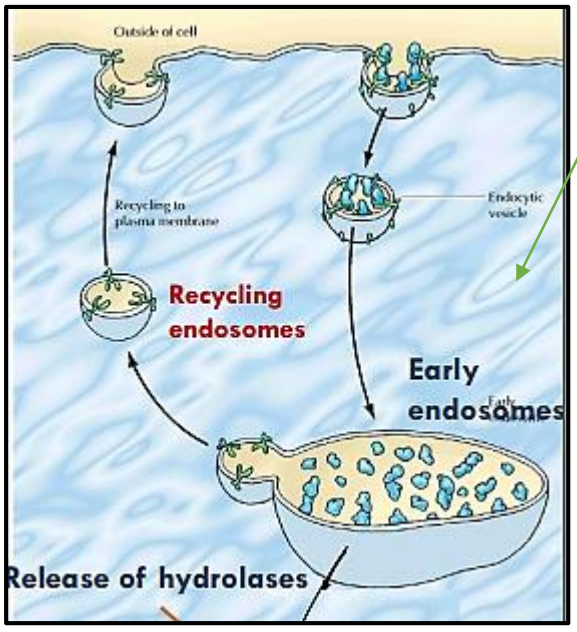
Endocytosis

How endocytosis happen?

Say, this is a target cell for a hormone or a ligand, this ligand is going to bind to its receptor on cell surface (cell membrane), this binding will induce the invagination and budding of the membrane to the inside to form a vesicle, with the ligand inside the lumen of the vesicle and the receptor in its membrane.

Once I have the vesicle inside the cytosol, it is going to be directed to a sorting station "NOT Golgi" , (because Golgi is sorting molecules that moving inside the cell between different compartments) , BUT when we receiving materials from outside sources "exogenous sources" , we need to sort them in another station called : Early endosome".



The vesicle will fuse with the early endosome, I need to have the substrates inside the cell to perform their function , the receptors are just to bring the substrates into the cell , so they must be recycled to bind again another molecules and bring them into the cell.

Example: LDL and its receptor:
LDL→we need to get it inside the cell.

LDL receptor→we need to recycle it on order to bring more LDL.

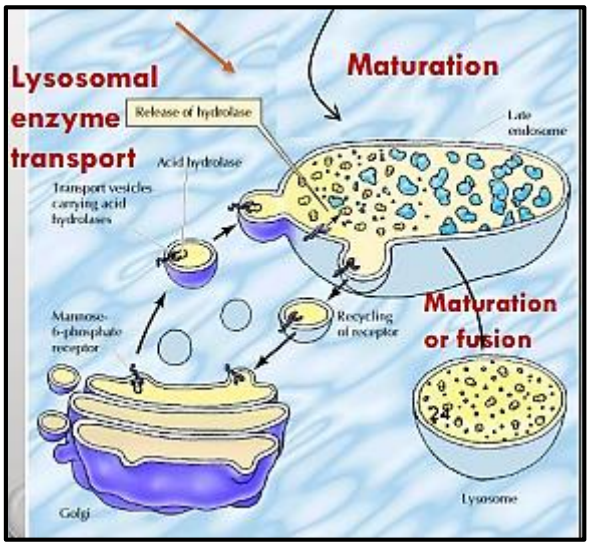
Recycling happen via directing the membrane receptors to another type of endosome called: Recycling Endosome. (After fusion with the early endosome, the empty receptors start to bud and form a vesicle (recycling endosome).

What about the substrate?

It is going to be directed to another type of endosome called: Late Endosome , depending on the type of molecules it could be directed to:

→Late endosome, if it is directed to the late endosome, then it will mature to a lysosome, then degradation happen, (according to section 1: movement of these vesicles from the late endosome to either lysosome or other compartments in the cell like Golgi "depending on the protein").

→Lysosome, then degradation happen.



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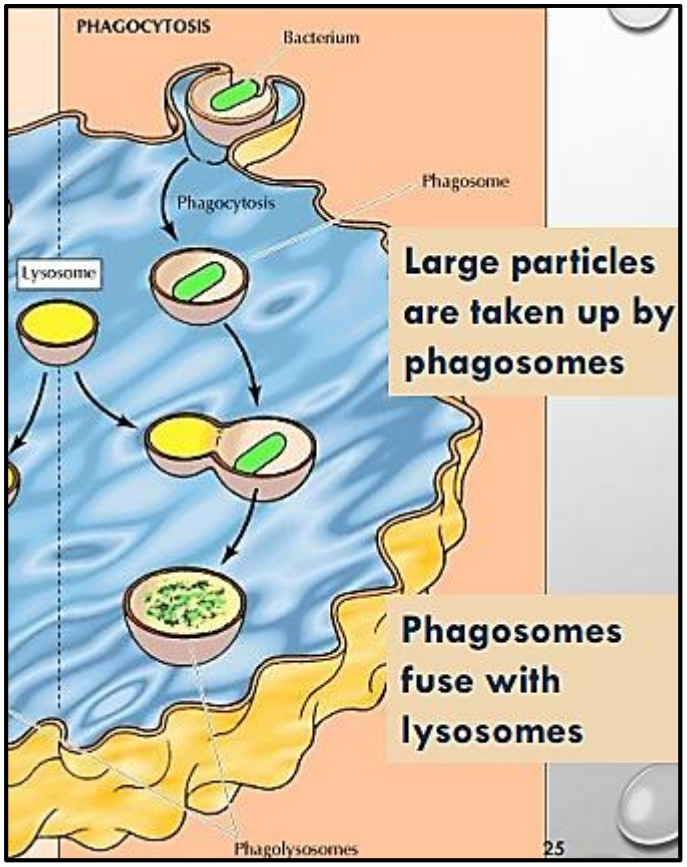
Another process for taking up materials from outside and send them for degradation:

Phagocytosis:

It happen by engulfment some particles like(bacteria , viruses), from outside sources " the cell recognize the foreign materials by certain protein antigens present on its surface" , extending part of the plasma membrane to surround these particles from both sides (podia) , untill these two sides completely encircle the particles , avesicle will be formed "phagocytosis vesicle=***phagosome***".

Now the phagosome is inside the cell , it will fuse with the lysosome(in yellow), to form ***phagolysosome*** , then degradation will happen.



NOTE

Not all cells can do phagocytosis , it has to be a macrophage cell to perform this function , while endocytosis is a feature of all cells , all cells can get the outside materials depending on the receptor type on the the plasma membrane.

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Autophagy

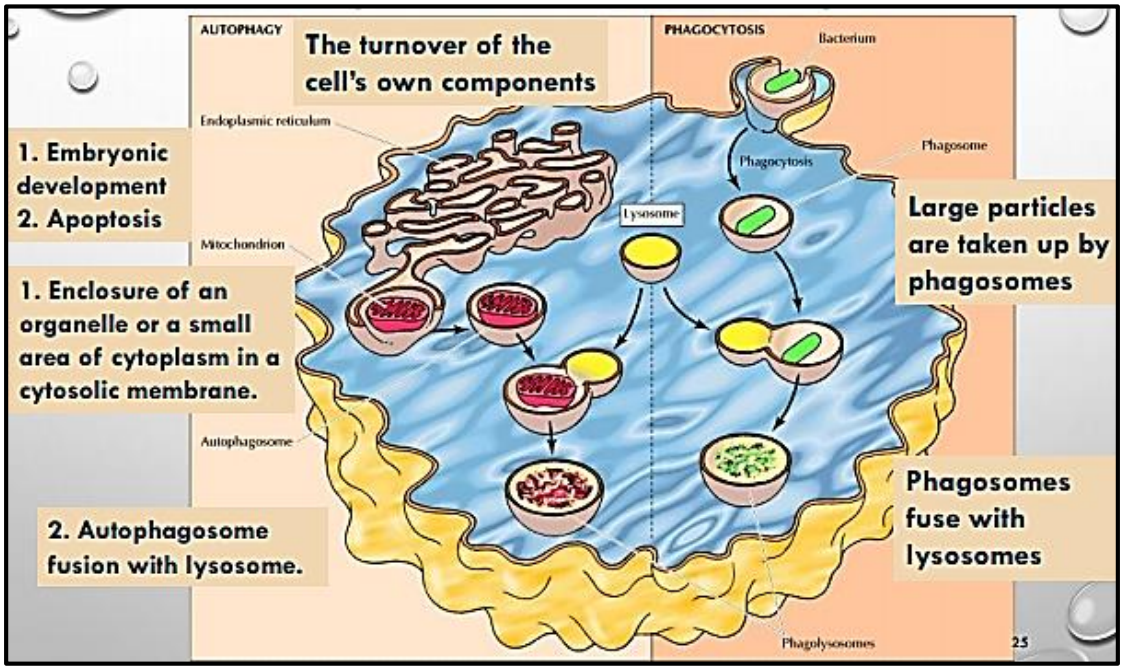
→it is a similar process to phagocytosis , but it starts to digest itself (rather than exogeneous material) , starting with mitochondria.

It is activated in certain conditions:

** apoptosis→when the cell decide to commit apoptosis "there is so much unfolded proteins and the cell cannot fix it using Unfolded Protein Response.

**during embryonic development: at the beginning there is only one cell →it will divide to generate stem cells →these stem cells will differentiate to different types (one cell type is different from another

cell type , one cell type contains more mitochondria than the other). So autophagy is also activated during embryonic development because I need to specialize and differentiate these cells into different and variable cells "in term of function", I may need to ingest and get rid of some components of one cell type but not another.



remember: →most of the cellular organelles are membrane bounded organelles , the membrane will extend to surround the organelle (ex: mitochondria) until it forms a vesicle "***Autophagosome***" that fuse with the lysosome to form" ***Autophagolysosome***" , then degradation happen (in this case degradation of mitochondria).

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Mitochondria

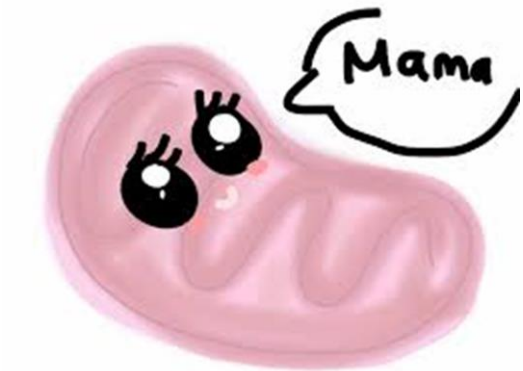
- Function: →generation of metabolic energy in eukaryotic cells. generation of ATP from the breakdown of carbohydrates and fatty acids, so it is important for the metabolism of carbohydrates and fatty acids.

**It has two membranes.

**it has its own DNA

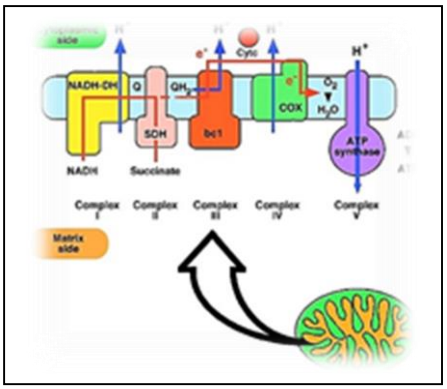
**it has cristae "projection of the inner mitochondrial membrane to the matrix to increase the surface area for more efficient energy production".

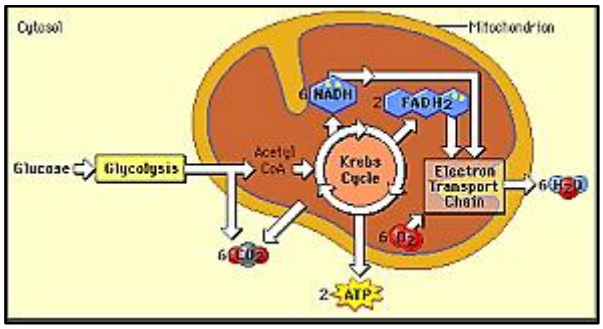
** it comes from mother→because during fertilization only the nucleus of the sperm fuses with the ovum , so all other organelles are part of maternal cell (ovum) , there is no relation with X-linked diseases , but diseases related to mitochondrial genome(mutations) are just inherited from the mother.

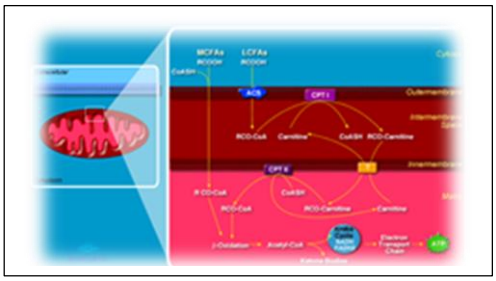


- It has high protein content 75%; (either in the inner mitochondrial membrane, outer mitochondrial membrane, or in the matrix) most mitochondrial proteins are translated on free cytosolic ribosomes and imported into the organelle

Cribs cycle, β oxidation of fatty acids, oxidative phosphorylation), all these processes occur in the mitochondria, so I expect high protein content inside the mitochondria "as membrane proteins or as soluble proteins in the matrix.







It contains its own DNA; mitochondrial DNA encodes 22 types of tRNAs, 2types of rRNAs and some mitochondrial proteins. Mitochondrial proteins are encoded by their own genomes and nuclear genome.

→it is surrounded by two membranes ; inner and outer :

Inner mitochondrial membrane IMM

→Forms folds "cristae" to increase the surface area.

→impermeable " highly selective" , any molecule that have to cross it , has to move through a carrier or transporter , safety measure inside the mitochondria to maintain H+ gradient that drives oxidative phosphorylation.

** Example of molecules need to cross IMM: pyruvate, fatty acids.

Outer mitochondrial membrane OMM

→A flat membrane isolate the mitochondria from the cytosol and other compartments.

→Highly permeable to small molecules (~100 Da) because of porins.

Distinguish

Cristae: projections, folds.

Cisternae: sacs (spaces).

- Intermembrane space : similar in composition to the cytosol.
- Matrix: large space contains the mitochondrial genetic system,and contains many enzymes and proteins either soluble or membrane proteins (IMM proteins).

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Mitochondrial fission versus fusion

Located in cells requiring high energy (like cardiac muscle cells, skeletal muscle cells, the brain, neurons "at the synapse").

→Highly dynamic organelle:

It can divide (fission).

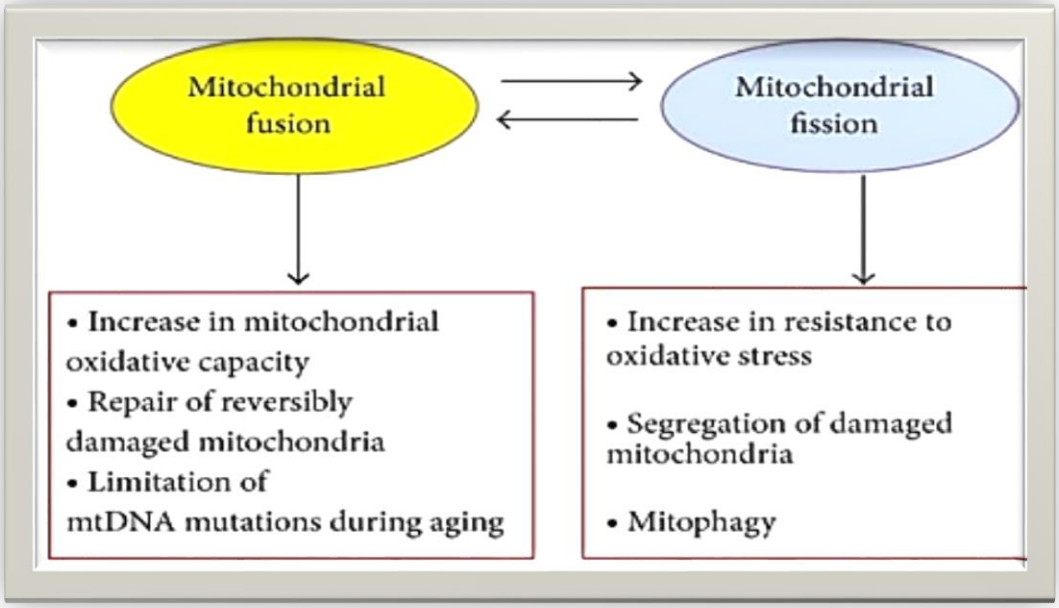
It can fuse with other mitochondria (fusion).

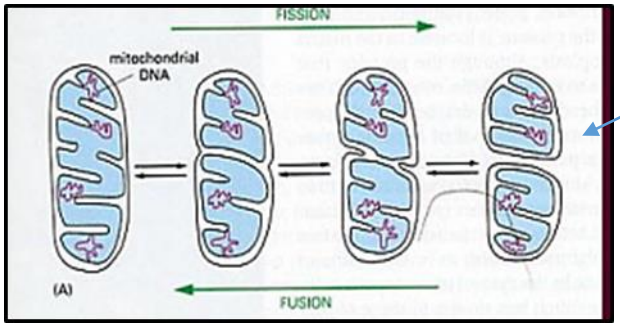
**the purpose and the mechanism of these processes are not well understood.

Mitochondrial fusion.

-increase in mitochondrial oxidative capacity: larger mitochondria→increase the surface area→more enzymes→more reactions→more efficient energy production.

-repair of reversibly damaged mitochondria (say this mitochondria has a problem, it will fuse with normal one, this normal mitochondria starts to recycle the defective components "by segregation of these components followed by fission"→ fusion then fission).
-limitation of mtDNA during aging (diluting the effect of the bod mitochondria.





Note from the figure:

During fusion, only the amount of DNA will increase in the newly fused mitochondria without DNA fusion.

Mitochondrial fission:

-segregation of damaged mitochondria, segregate the good components in one mitochondria and the bad components in another one and send it to autophagy.

-increase in resistance to oxidative stress because of targeting bad mitochondria to autophagy.

**mitochondrial proteins are encoded by their own genomes,
and nuclear genome by free ribosomes in the cytosol.

So we need another type of ribosomes in the mitochondria "mitochondrial ribosomes".

Based on student questions:

- can the cell use the mitochondrial genome for production of cellular proteins?

→remembre there is a spatial organization , mitochondrial DNA is separated from nuclear DNA by mitochondrial membrane , and the IMM is impermeable , so not easy to move the DNA across the IMM.The doctor said:"I am not very sure if in certain conditions the cell can use the mitochondrial DNA , but still not a major mechanism to use mitochondrial DNA".

- About mitochondrial ribosomes?

Any ribosome is made of rRNA and proteins, the protein component is a nuclear gene, expressed in the cytosol and then transferred into the mitochondria, then the assembly will happen in the mitochondria because the rRNA is encoded by mitochondrial DNA. →other factors (like transcription factors, translation initiation factors, elongation factors) are not encoded by mitochondrial genome, they have to be synthsied on the free ribosomes and then transferred as proteins to the matrix. →most proteins are synthsied in the cytosol and then transfer to the mitochondria.(example: cytochrome proteins).

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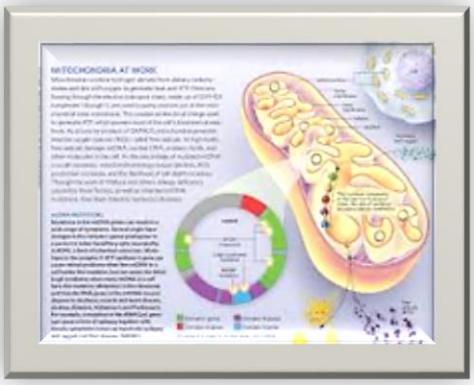
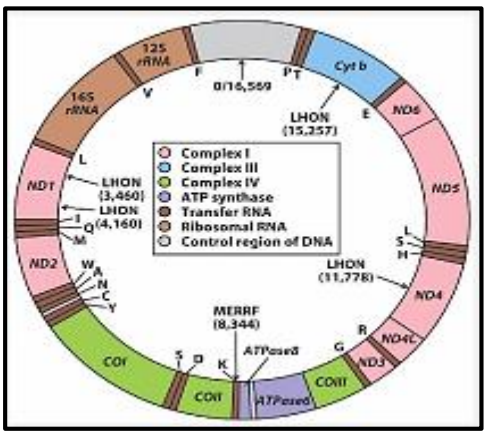
The genetic system of mitochondria

Different than nuclear DNA since it is circular and very similar to the bacterial DNA, that is why they thought that the origin of the mitochondrial DNA came from bacteria, "Bacterial cell is lived in a large animal cell in a symbiosis relationship علاقة تكافلية , not infection, each of them benefit from the other, so it became part of the mitochondrial Endosymbiosis".

- mitochondrial DNA (~16 kb in human).





- circular, multiple copies per organelle.

→encodes 13 prroteins involved in electron transport chainand oxidative phosphorylation, 2 rRNA (16S+12S of mitochondrial ribosomes), and 22 tRNA.

This tRNA is different from the cytosolic tRNA, we have 22 tRNA and 20 amino acids, 2 of the tRNA have anticodons for two stop codons and 20 tRNA for 20 amino acids, so each amino acid has only one tRNA, while in the cytosol we have 64 codons (61 for amino acids and 3 for stop codons).

<u>About mitochondrial codons, some of them may encode another amino acid .</u>

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.

- ❖ most mitochondrial proteins are synthzied on the free ribosomes in the cytosol – not on the RER ribosomes- , only small amount of mitochondrial proteins are encoded by mitochondrial DNA.

→so, I expect to need transport system for cytosolic proteins to be transferred either to: IMM, OMM, or the matrix.

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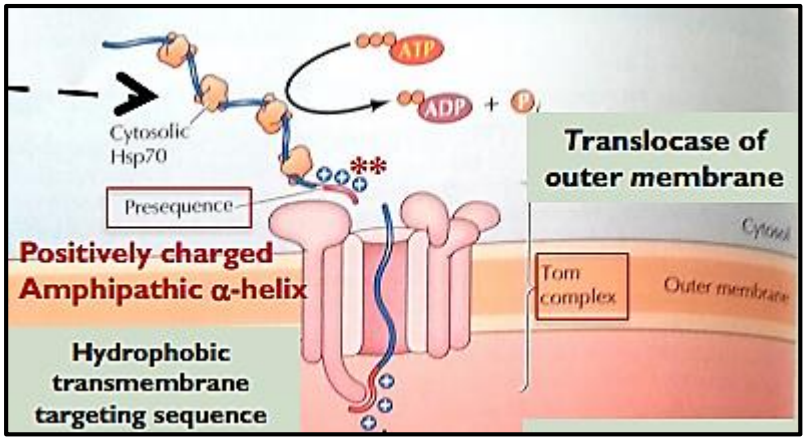
Protein import and mitochondrial assembly

IMM proteins (soluble\membrane proteins) they are going to be synthsied on the free ribosomes " translation has to be completed" , the whole polypeptide chain is hold completely unfolded by chaperon HSP70.

Why we need to keep the polypeptide unfolded?

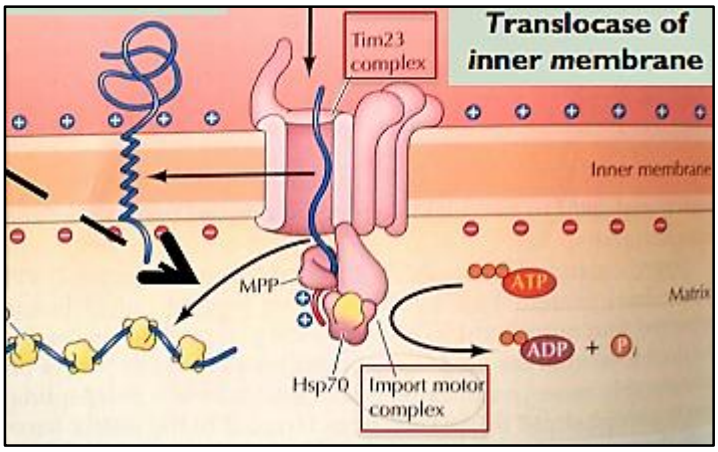
- ✓ to be completely synthezied.
- ✓ folding process change the overal 3D shape of the proteins making them difficult to translcated throug the channel (may be wider than).



The polypeptide is going to be identified at the outer membrane by multiprotein sununit, **TOM** complex →**T**ranslocase of the **O**uter **M**embrane.

TOM binds to a signal sequence (pre-sequence), " N-terminal stretch of positively charged amino acids" , the channel will open to allow the entery of the polypeptide chain to the intermembrane space.

To translocate it to the IMM →**TIM** – Translocase of the Inner Membrane-, will recognize the same sequence and allow the entery of the polypeptide chain to the matrix.



- During its entery it is goind to be received by another complex : ***Import motor complex***, this complex is made up of several proteins (subunits), one of them is a chaperon HSP70 , holded the protein unfolded , another subunit is "MPP", Matrix Processing Peptidase, will cut the signal sequence , since the signal sequence will not be a part of the protein.

Once the trnaslocation is done, the protein will be bounded by another chaperon HSP70 for final folding (in this case we talked about soluble proteins).

→in the case of membrane protein→it is going to have a stretch of(20-25) hydrophobic amino acids to span the membrane, it is going to form a helix within the lumen of TIM complex and then move laterally and get inserted into the IMM.

Notice the figure above:- why is at the inner leaflet of the IMM we have negative charges while at the outer leaflet we have positive charges?

→because of the action of the proton pump, that pump H+ to the intermembranous space to facilitate the oxidative phosphrylation process.

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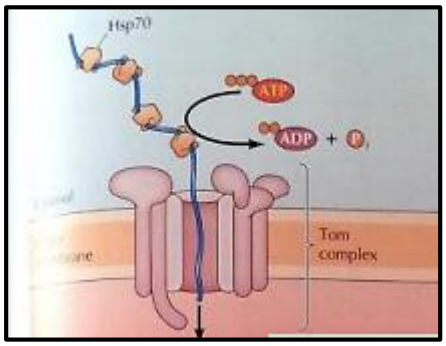
Targeting of the inner mitochondrial proteins

Another way to transfer the IMM proteins from the cytosol and insert them in the IMM :

Again, we have a polypeptide chain that is synthsied on the free ribosomes, it is going to be identified by TOM complex.



note :

There is no pre-sequence, there is another sequence that is going to be identified by TOM complex.

- Then the polypeptide will be transferred thtough the outer membrane, in the intermembrane space, it is recognized by soluble proteins (mobile chaperon) " **TIM 9 +10**".
- TIM 9 +10 bind to the polypeptide and move it and bind to TIM complex and allow the entery of this protein.

Since it is a membrane protein, it should contain hydrophobic stretchs called:" Internal stop sequence", it is going to form a helix, move laterally through the membrane and inseted in it.

- ✓ Then the cycle will continue →another internal stop tansfer sequence →form a helix →move laterally ,and so on .

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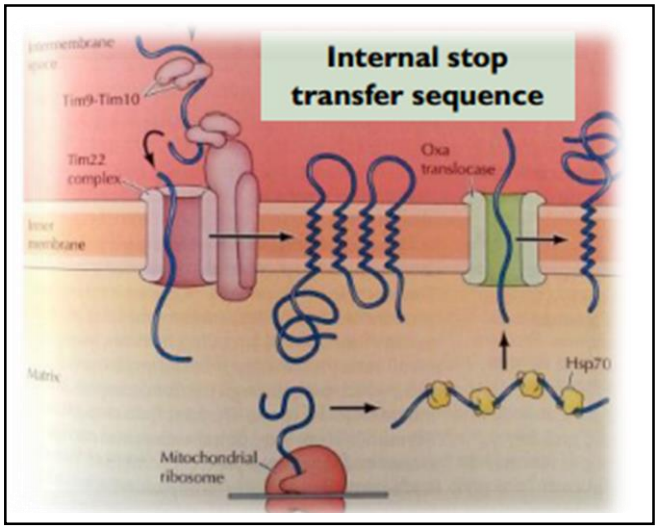
Inner membrane proteins encoded by mitochondrial genome are inserted via Oxa translocase.

HOW?



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These proteins are synthsied completely by the mitochondrial ribosomes, chaperons are going to hold these proteins in an unfolded form, then it is going to be directed by the action of Oxa translocase (specific proteins synthsied by mitochondrial ribosomes).

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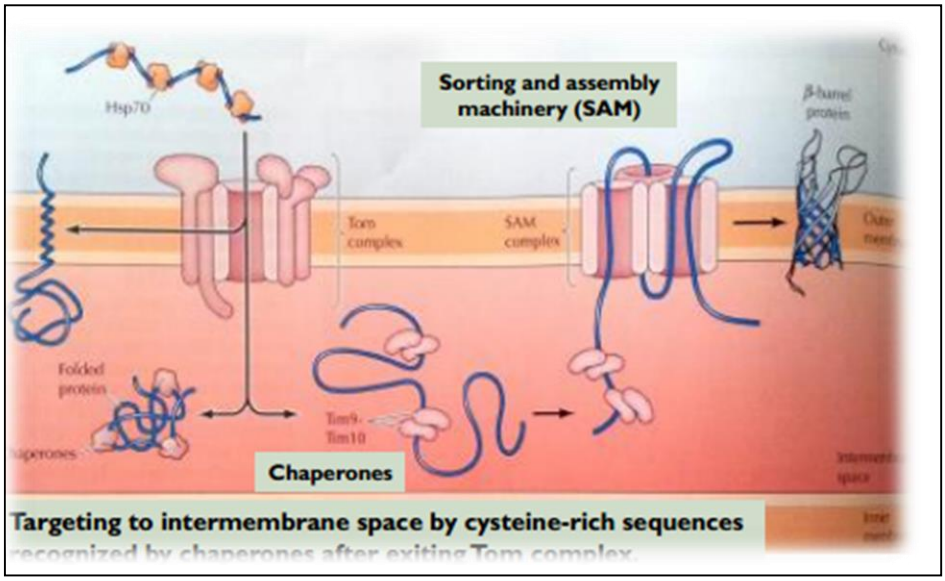
Targeting of outer membrane proteins



The outer membrane proteins are:

- Alpha helical transmembrane proteins(single helix or multiple).
- Beta barrel proteins such as porins (increase the permeability).

Again, synthsied on the free ribosomes, holded unfolded, identified by TOM complex, move to the intermembrane space.

→if it is a soluble protein, it is going to fold inside.

→if it is a membrane protein made up of helices, it have hydrophobic stop transfer sequence form helix, then movement of the helix through the membrane: if it is a beta barrel sheeth, then it will be directed to "**SAM** complex", and then inserted as beta barrel protein in the outer mwmbrane. (SAM= **S**orting and **A**ssembly **M**achinery).

End ot text ☺

Sorry for any mistake.