Genetics
& Cell biology

◉ Sheet ○ Slides

Number

6

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Doctor

Diala

➢ Mitochondria:

The doctor mentioned some random notes about the mitochondria from the previous lecture:

- It has a double membrane:

Outer → permeable.

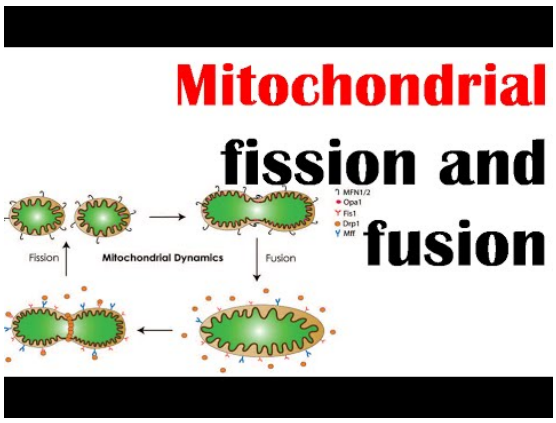
Inner → impermeable.

*The inner has high protein content (>70%), and it forms folds (cristae) to increase the surface area.

-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.

➢ 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.**



1- Fusion:

Fusion processes happen in order to:

A- Increase in mitochondrial oxidative capacity:

- Two mitochondria are fused → Large mitochondrion → The surface area increases → 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:

- Aging causes acquired changes in the genetic material, depending on the lifestyle the person lives, for instance, increasing the exposure to some chemical, physical hazards → more mutations → more diseases.

- Fusion between a mitochondrion which has acquired genetic mutations during aging and a healthy mitochondrion corrects mtDNA, since the healthy DNA will be selected and we will get rid of the damaged one.

2- Fission: (mitochondrial division)
Fission processes happen in order to:

A- Increase in resistance to oxidative stress:
Note that this point is very similar to the first point we mentioned in fusion.

Let's think about the two points logically:

- Fusion → Large mitochondrion → Large surface area→ Increased oxidative capacity.
- Fission → Dividing the large mitochondrion into small mitochondria → The <u>total</u> surface area will increase → Increase the capacity of mitochondria to resist the oxidative stress. (10 small watermelons have a larger surface area than one big watermelon).

B- Segregate the damaged mitochondria:

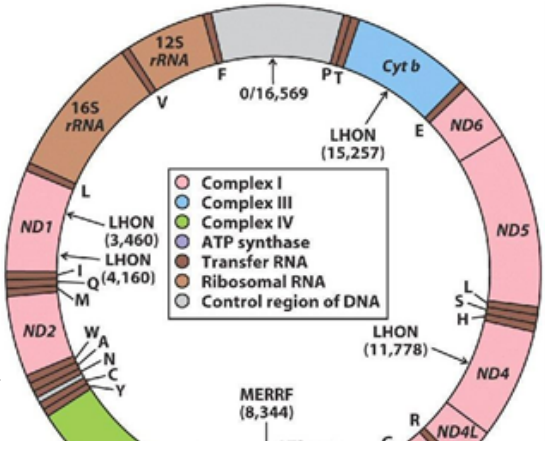
- If a mitochondrion has some sort of a problem in part of it (it's NOT totally damaged), there will be segregation between the good part and the bad part, and the cell will get rid of the bad part by Autophagy, and it will keep the good part.
- An advantage of this process to the cell is that it saves some energy, because by keeping the good part of the mitochondrion with its already existing proteins, the cell reduces the production of new proteins, so it will not have to start to produce a mitochondrion from zero with all its content.

C- Mitophagy: (autophagy of mitochondria)

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

➢ <u>The genetic system of Mitochondria:</u>

- Thought to have evolved from bacteria in a symbiotic relationship living in larger cells (endosymbiosis).
- Mitochondrial DNA (~16 kb in human),



while nuclear DNA is composed of 3 Billion base pairs.

- Circular
- Multiple copies per organelle.
- Encodes 13 proteins involved in electron transport and oxidative phosphorylation, two rRNAs (16S+ 12S of mitochondrial ribosomes), and 22 tRNAs.

➢ Mitochondrial genetic code and mutations:

- Different genetic code by tRNA.
- Only 22 tRNA.
- Germ-line mutations in mitoDNA
- Mutations in mito. tRNA genes result in:

✓ Metabolic syndrome (diabetes and obesity).

✓ Mutations in mito. genes of electron transport chain result in Leber's hereditary optic neuropathy.

Notice the differences between the universal and mitochondrial genetic codes:

- The mitochindrial tRNA is different from the cytosolic tRNA, we have 22 tRNAs and 20 amino acids, 2 of the tRNAs 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).
- Mitochondrial codons, may encode for another amino acid (as seen in the table).

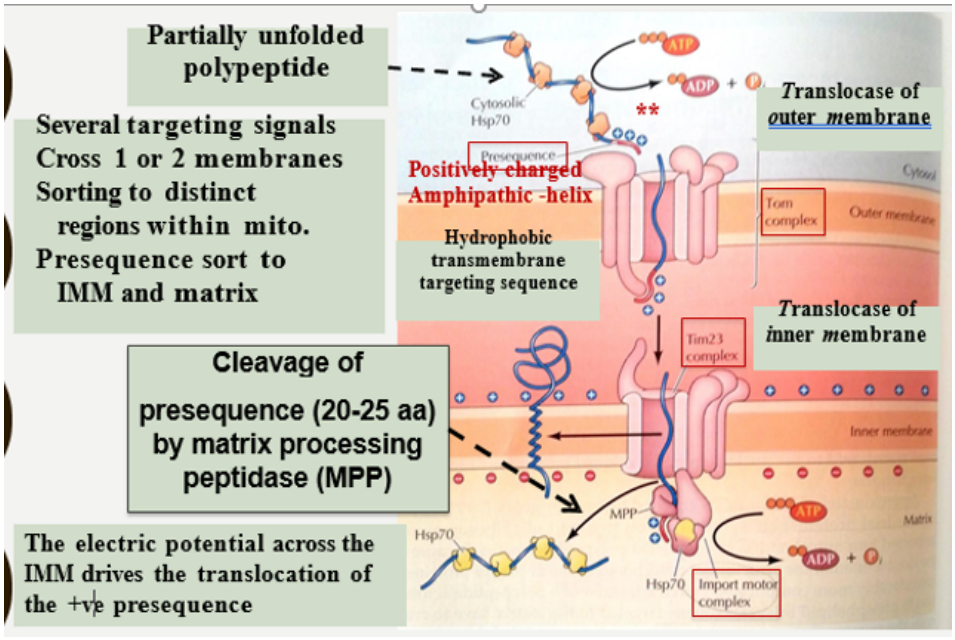
TABLE 11.1 Differences between the Universal and Mitochondrial Genetic Codes

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:

- Proteins required for DNA replication, transcription, translation, ribosomal proteins, oxidative phosphorylation, and enzymes for mitochondrial metabolism (TCA cycle).
- 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.
- Some proteins are present in all mitochondria, regardless of the cell type(<50% of proteins are common to all tissues). While others differ according to the tissue.
  For instance: Some reactions of the urea cycle happen in the mitochondria of the hepatocytes specifically, so no need to synthesize carbamoyl phosphate synthetase 1 in the mitochondria of the intestinal cells for example.
- Different types of proteins are distributed in the IM, OM, intermemranous space and matrix.



➢ Protein import and mitochondrial assembly:

1-Targeting of inner mitochondrial membrane proteins:

A- Proteins that are made from a single helix:

- They have a signal sequence (presequence) which contains positively charged amino acids.

- The presequence will be cleaved by matrix processing peptidase (MPP).
- This presequence (20-25) amino acids, will be recognized by a protein complex called TOM (Translocase Of Outer membrane), this complex will pass the protein through it, and target it to the TIM(Translocase Of Inner membrane) complex.

If it is a **membrane protein**, there will be a sequence such as the stop transfer sequence or internal signal sequence, that induces the formation of a helical structure and then insertion of the protein into the membrane will happen.

If it is a **soluble protein** (matrix protein), it'll be pulled through the channel, by a protein complex called import motor complex which requires ATP in order to push the protein inside the matrix, and (hsp70) chaperones are going to hold the protein and start its folding.

B- <u>Proteins that are made from more than one helix:</u>

- Many mitochondrial proteins are multi-pass transmembrane proteins that do not contain presequences, but have multiple internal mitochondrial import signals.
- A complex in the outer membrane will catch these proteins, then, they're going to be held by two chaperons **Tim9 and Tim10**, which are found in the intermembranous space, and direct this protein to a Tim complex (Tim22) in order to be inserted into the inner membrane.
- Again, they contain stop transfer sequences, which aid in the helical formation, and then insertion of the protein into the membrane will happen.

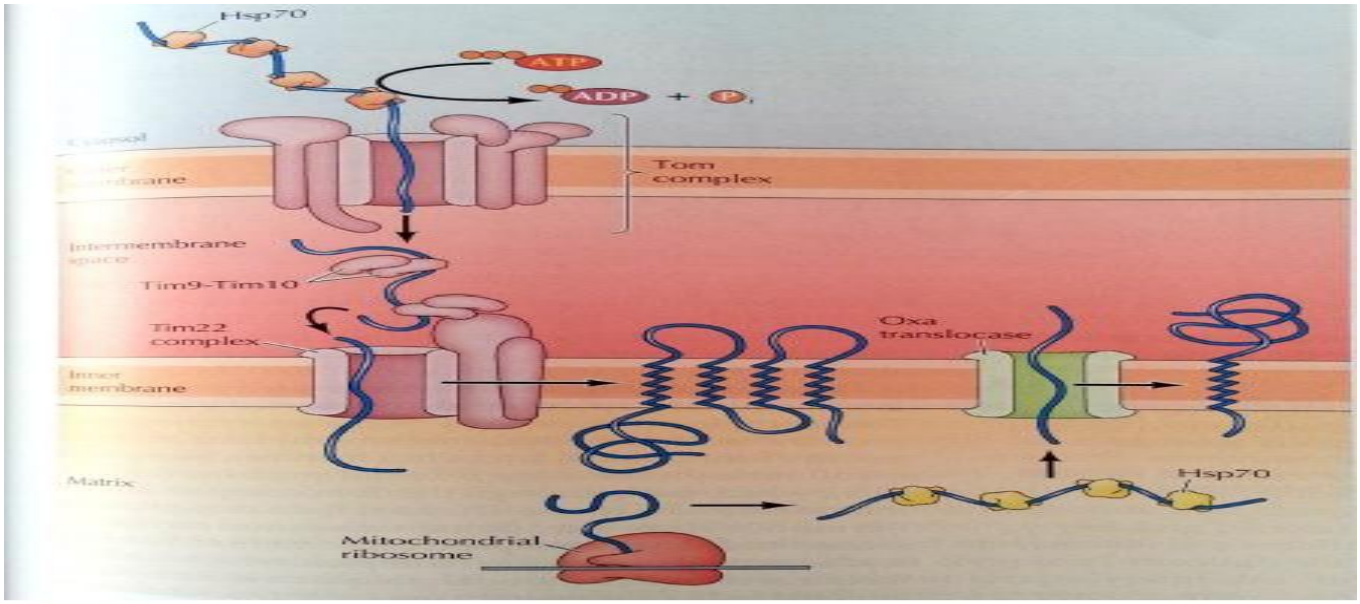
<u>Remember that: All the above-mentioned proteins are synthesized on free ribosomes</u>**<u>.</u>**

<u>So, what about the proteins synthesized on the mitochondrial ribosomes?</u>

After their synthesis in the matrix, they will be held by chaperones to prevent their folding because they need to be

inserted in the inner membrane, this insertion will happen by another protein complex which is **Oxatranslocase.**

Note: Mitochondrial ribosomes are more important for the synthesis of the inner mitochondrial membrane proteins, because most of the proteins that are encoded by mtDNA are enzymes that are important in the oxidative phosphorylation and krebs cycle.



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2-Targeting of outer membrane proteins:

- The mitochondrial outer membrane has much less protein content in comparison to the inner membrane.
- They have NO presequence to be targeted to the TIM complex.
  Again:

  If it is a **membrane protein**, the stop transfer sequence is going to induce the formation of a helical structure and then insertion into the outer membrane will happen.

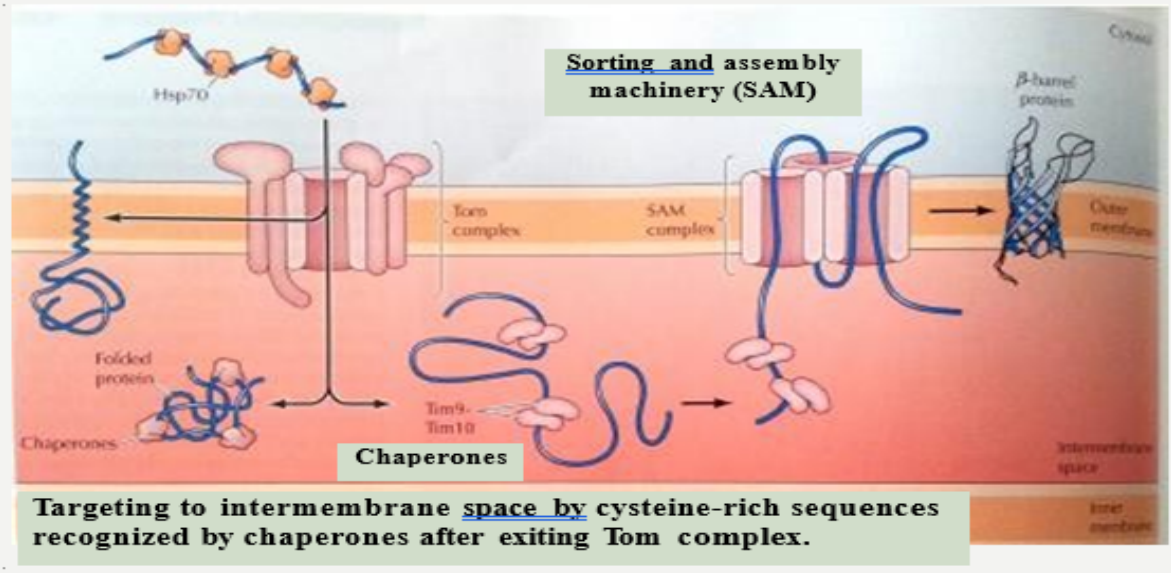
  If it is a **soluble protein** (an intermembranous space protein), it's going to enter the intermembranous space and then it'll be caught by the chaperons which complete their folding (TIM9 and TIM10).
- We have different types of proteins in the outer membrane:
  A- Helical structured proteins → inserted by TOM complex.
  B- Beta-barrel proteins such as porins (sheet structure)

Beta-barrel proteins will not be inserted directly into the membrane, they'll enter just like the soluble proteins, then the chaperones are going to hold them in this format, until they get inserted into the outer membrane by another protein complex called SAM (sorting and assembly machinery).

So, B-barrel proteins are inserted by → SAM complex.

Targeting of outer membrane proteins

- Tom complex inserts proteins with -helical transmembrane domains.
- SAM complex inserts -barrel proteins such as porins.

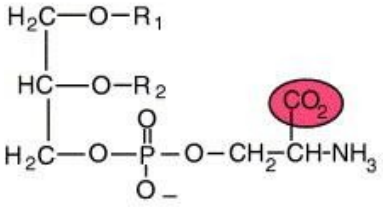


Targeting to intermembrane space by cysteine-rich sequences recognized by chaperones after exiting Tom complex.

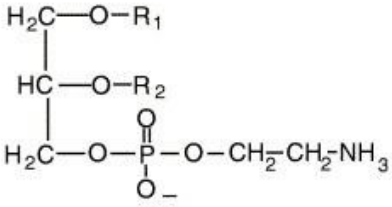
➢ Mitochondrial phospholipids:

- Phosphatidylcholine and phosphatidylethanolamine are synthesized in the ER and carried to mitochondria by phospholipid transfer proteins.
- Phosphatidylserine is synthesized from phosphatidylethanolamine by the mitochondria.

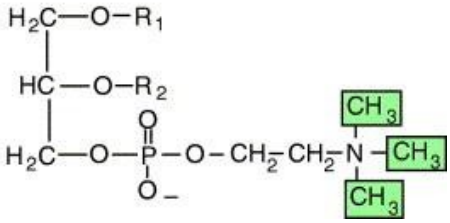
Phosphatidylserine



Phosphatidylethanolamine



Phosphatidylcholine



- There is a special type of phospholipids that is exclusively present in the inner mitochondrial membrane which is **cardiolipin.**

Cardiolipin structure:

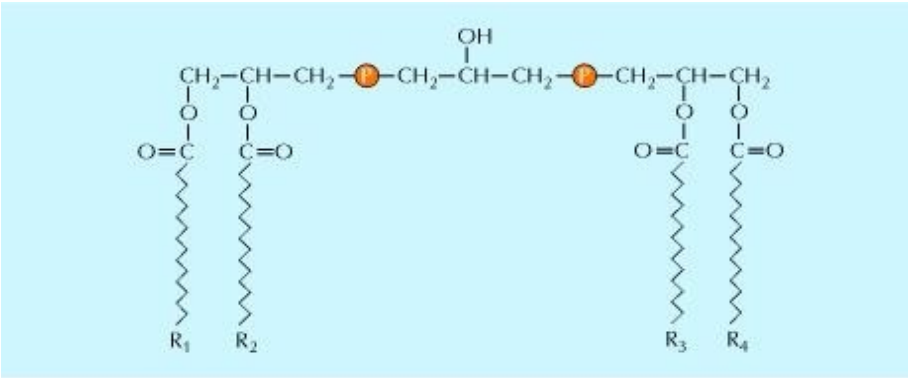
-2 glycerol molecules each connected to 2 fatty acids (so we have 4 fatty acids in total).

-2 phosphate groups.

-One more glycerol molecule (the head group) that is connected via carbon no. 1 to one phosphate group and carbon no.3 to the other phosphate group.

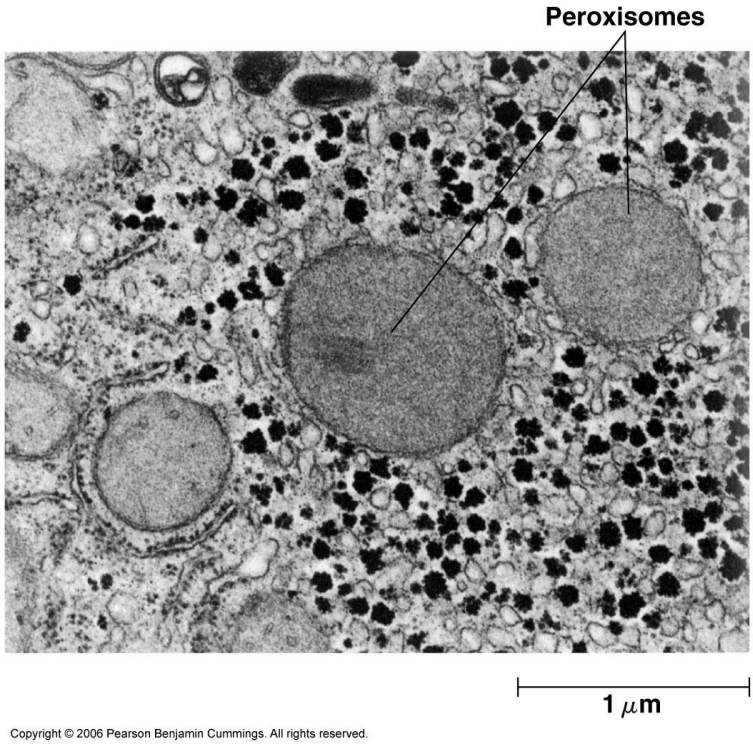
Cardiolipin function:

It improves the efficiency of oxidative phosphorylation by restricting proton flow across the membrane.



➢ Peroxisomes:

- Small, spherical-structured, single membrane-enclosed organelles that vary a lot in size.
- Contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism.
- They replicate by division.
- Can rapidly regenerate even if entirely lost.
- Most human cells contain 500 peroxisomes.



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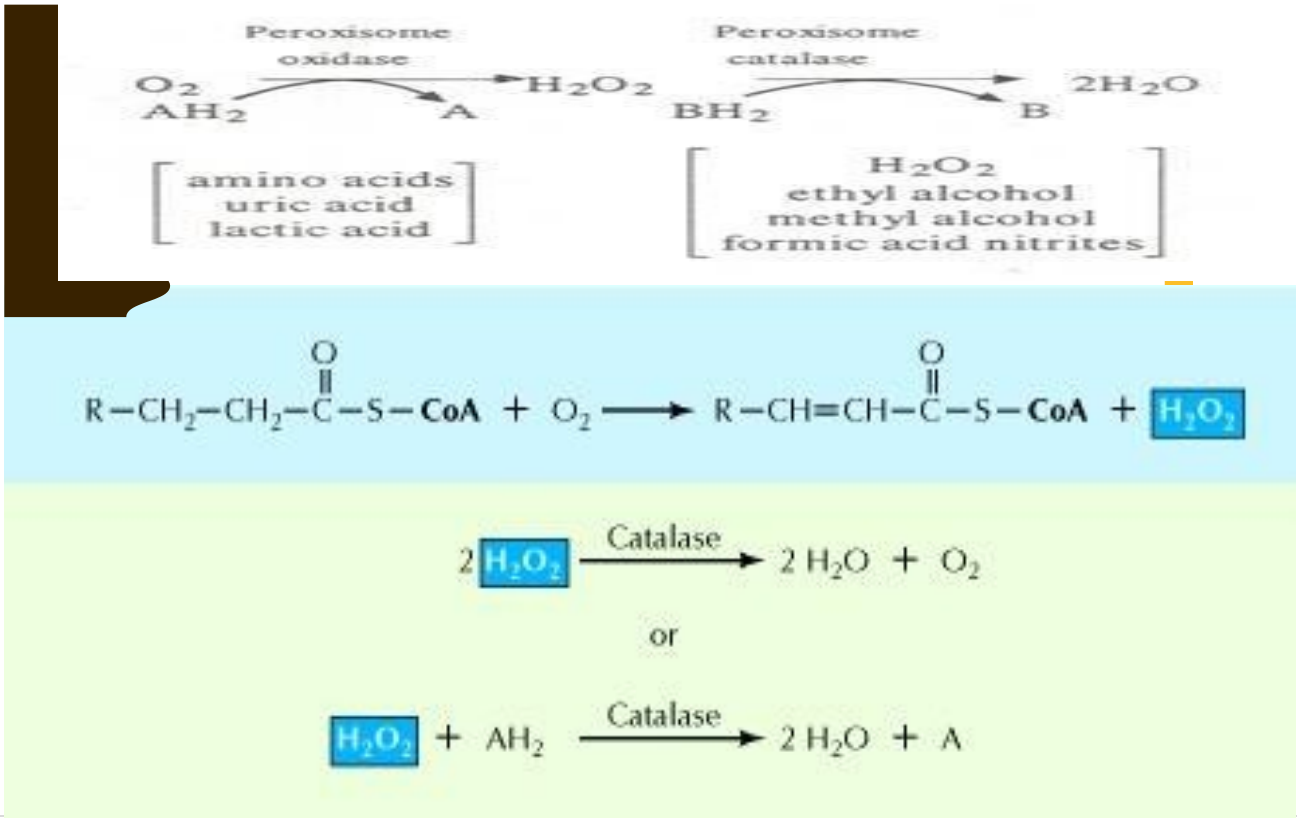
- Their proteins are called peroxins (Pex1, Pex2, etc).

➢ Peroxins:

• 85 genes encode peroxins.
• Most peroxins are metabolic enzymes.
• Internal proteins (soluble proteins) are synthesized on free ribosomes and then imported into peroxisomes.
• Transmembrane peroxisomal proteins are synthesized in ER to be inserted directly into the membrane.
• Other membrane proteins act as receptors for the import of internal proteins.

➢ Function of peroxisome:

- Peroxisomes from a single tissue contain at least 50 enzymes.
- Peroxisomes carry out oxidation reactions leading to the production of hydrogen peroxide.
- Because hydrogen peroxide is harmful to the cell, peroxisomes contain the enzyme catalase, which reduces H2O2 levels, thereby reducing oxidative stress.
- Substrates like uric acid, purines, amino acids, and fatty acids are broken down by oxidative reactions in peroxisomes to provide energy.
- Fatty acids are oxidized in both peroxisomes and mitochondria.



➢ Molecules that can be synthesized in peroxisomes:

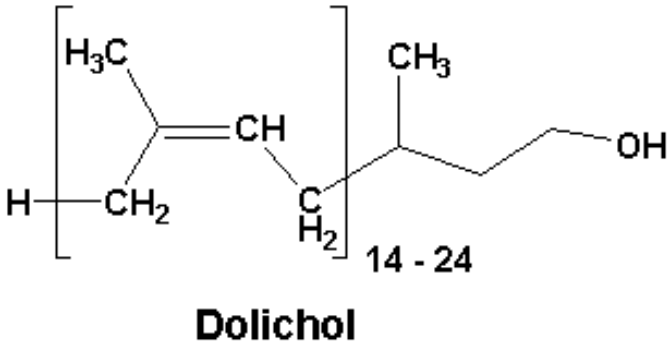
Lysine amino acid

Lipids:

• Cholesterol

• Dolichol:

– made from farnesyl



Dolichol

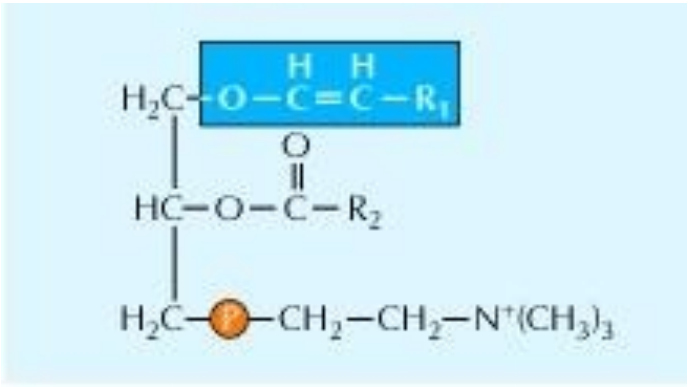
–carries the sugar group that will be added to proteins in N-linked glycosylation.

• Bile acids that are synthesized from cholesterol (liver).

• Plasmalogens:

– Phospholipids with one ether bond.

– important in membranes of heart and brain.



➢ Peroxisomal assembly:

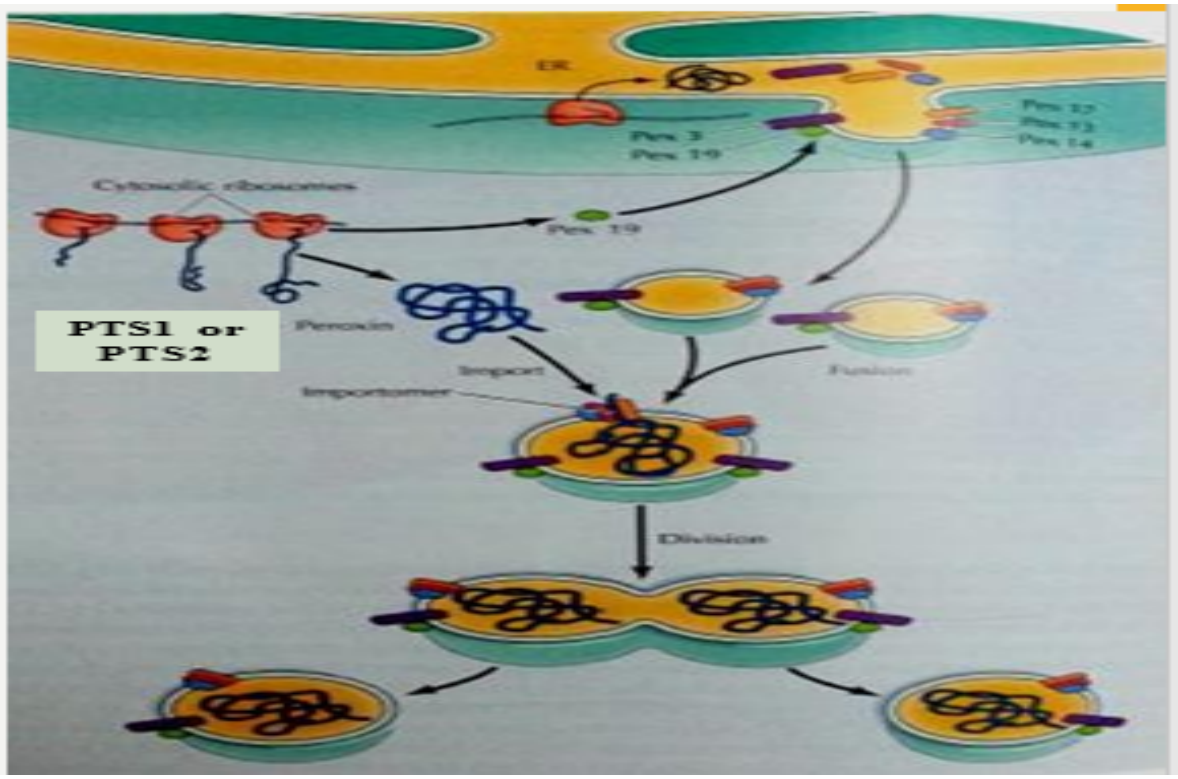
- The budding process takes place at certain locations in the ER.
- These locations contain certain proteins; transmembrane proteins such as Pex3 (synthesized on ribosomes attached to ER) and soluble proteins such as Pex19(synthesized on free ribosomes and then directed to ER to become a farnesylated protein found largely in the cytosol).
- **Pex3** protein recruits **Pex19** to initiate budding of peroxisome from ER.
- The vesicle fuses with a new or an older one.

So again, **Pex.19** → synthesized on free ribosomes → directed to the surface of the vesicle (it is located beside **Pex3** which is a membrane protein of the peroxisome).

It's like having a receptor that is present on the membrane to recruit the soluble proteins to the ER, therefore, they'll become a part of the vesicle that will be directed to the peroxisome (they don't enter inside the vesicle as soluble proteins).

Pex3, Pex19, and other peroxisomal membrane proteins then act as receptors for the import of internal matrix proteins (soluble proteins).

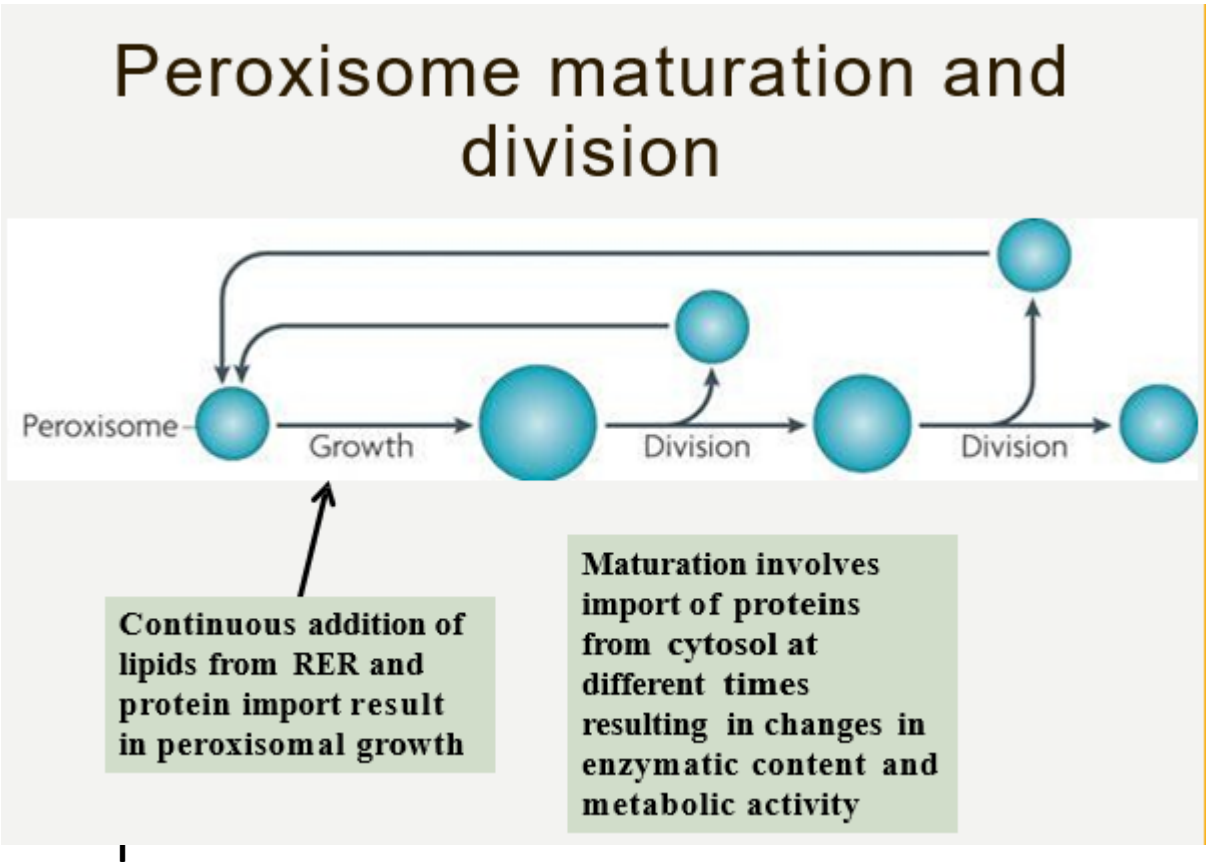
- Matrix proteins are targeted mostly by peroxisome targeting signal 1 (PTS1) or PTS2.
- These signals are recognized by cytosolic receptors and proteins are imported via a channel (importomer).
- More than one vesicle containing peroxins (transmembrane and soluble) will fuse together, forming the peroxisome as an organelle.



➢ Peroxisome maturation and division:

- Dynamic processes that can happen to peroxisomes.
  - ✓ Division processes → smaller peroxisomes.
  - ✓ Fusion processes → growth of the peroxisome.
- Division and fusion processes occur depending on the need of the cell (for example, the level of oxidative stress in the cell), affecting their size and number inside the cell.

- ✓ For example: the stroke (الجلطه الدماغيه)→ less blood supply to a certain region of the brain → death of cells → formation of a small localized area of dead tissue 'infarct' →the cells in the area surrounding the infarct will be under oxidative stress that is the result of hypoxia → the biogenesis (formation) of peroxisomes increases in these peripheral cells because of their role in reducing oxidative stress.
- ✓ That's why peripheral cells respond by increasing the biogenesis of peroxisomes with their content of enzymes to increase the capacity of anti-oxidants effect and try to repopulate the area and repair damage.
- ✓ That's why stroke patients might improve over time.



diseases:

- Single peroxisomal enzyme deficiencies:
  -Defective specific peroxisomal enzymes
- Peroxisomal biogenesis disorders (PBDs): involve multiple peroxisomal enzyme deficiencies due to failure of import.
  -Example: Zellweger syndrome → in this syndrome the proteins are not synthesized and imported to the peroxisomes efficiently, due to the presence of certain mutations (mutations in at least 10 genes such as the receptor of PTS1)

therefore, the whole structure will be disturbed. Accordingly, this disease might be lethal.

- X-linked adrenoleukodystrophy (XALD):

-Defective transport of very long chain fatty acids (VLCFA) across the peroxisomal membrane.

Note: VLCFAs presence is important in brain cells, and they are metabolized specifically in the peroxisomes. So, if we can't import them to the peroxisomes in brain cells or even other cells due to certain diseases, many problems will happen, because they are considered as one of the energy sources in our body, as well as a source of acetylcholine. Therefore, many problems occur in the adrenal gland and leukocytes.

-X-linked → more propensity for males to be affected.

➢ Mitochondrial diseases:

http://www.ncbi.nlm.nih.gov/books/NBK27914/

This article contains many mitochondrial diseases, most of them are metabolic diseases, because most of the mitochondrial genome encodes for proteins involved in metabolic processes.

The Doctor said that she might ask us a **general** question from this article in the exam.

➢ Defects of mitochondrial DNA (mtDNA):

- These disorders are associated with dysfunction of the respiratory chain because all 13 subunits encoded by mtDNA are subunits of respiratory chain complexes.
- Diseases due to point mutations are transmitted by maternal inheritance.
- 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.

- 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)

- 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.
- 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.
  The figure compares the vision in a healthy person with the vision in LHON patient, notice the difference.

Difficult roads often lead to beautiful destinations

GOOD LUCK!