Lecture 5: Mitochondria & Peroxisomes

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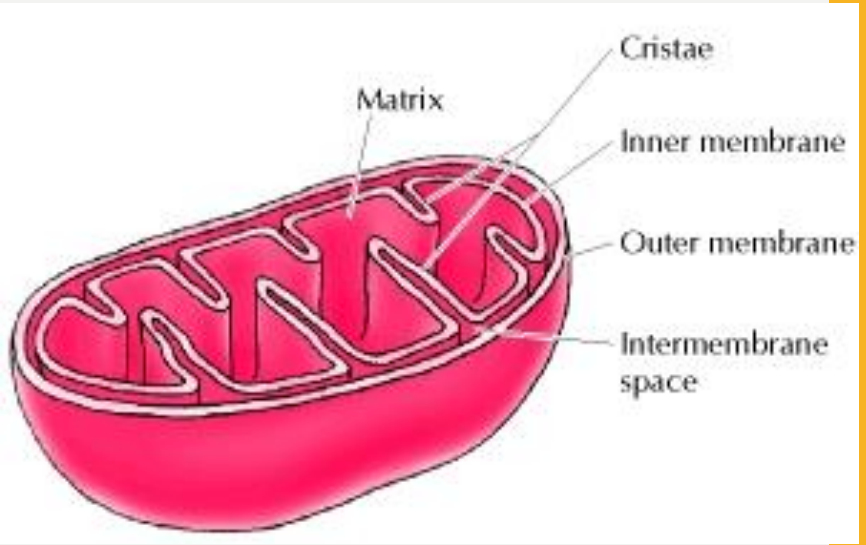
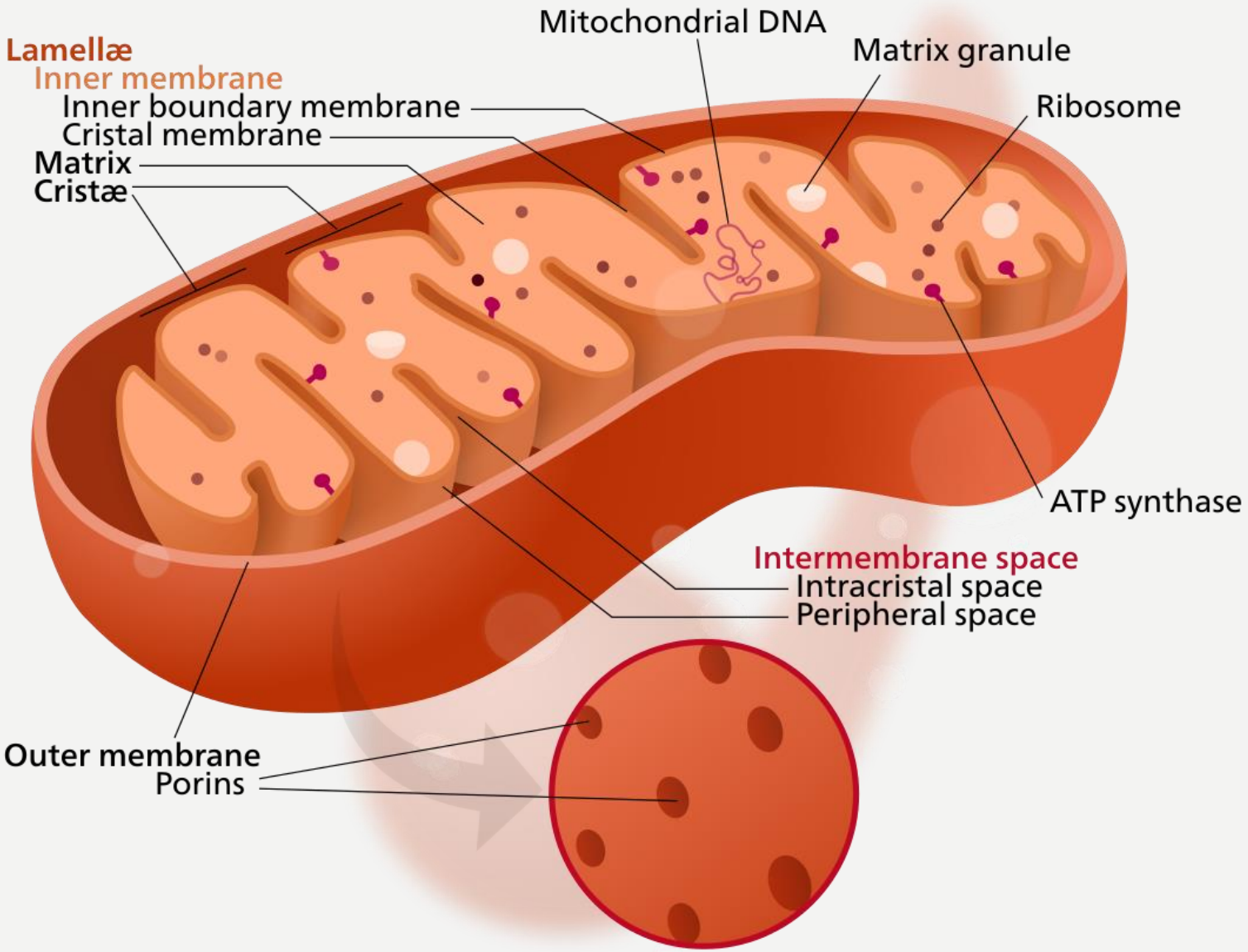
Principles of Genetics and Molecular Biology

Mitochondria

- Function: generation of metabolic energy in eukaryotic cells
  - Generation of ATP from the breakdown of carbohydrates and fatty acids
- Most mitochondrial proteins are translated on free cytosolic ribosomes and imported into the organelle.
- They contain their own DNA
- Mitochondrial DNA encodes tRNAs, rRNAs, and some mitochondrial proteins.
- Mitochondrial proteins are encoded by their own genomes and nuclear genome.

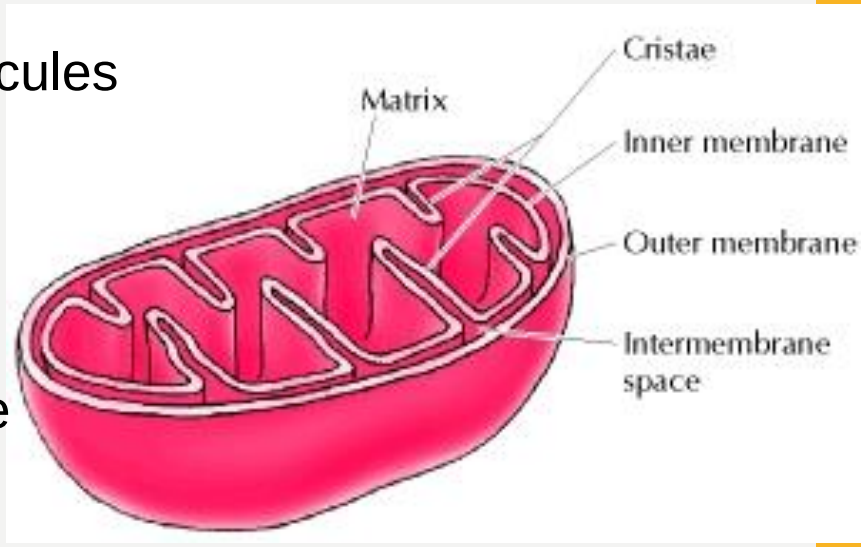


Mitochondrial organization & function



Mitochondrial structure

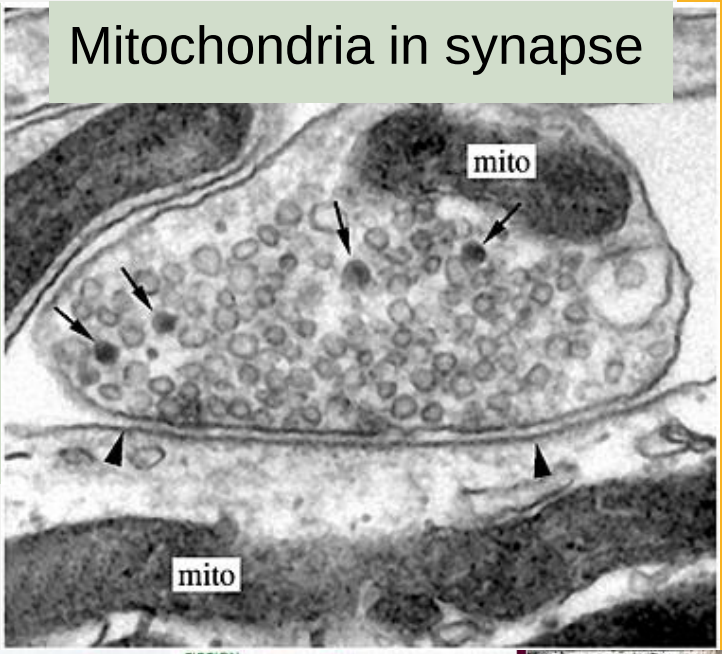
1. Outer membrane
   - ✓ Highly permeable to small molecules (~1000 Da) because of porins
2. Inner membrane
   - ✓ High protein content (>70%)
   - ✓ Forms folds (cristae) to increase surface area
   - ✓ Function; oxidative phosphorylation, ATP generation, transport of metabolites (pyruvate and fatty acids)
   - ✓ Impermeable to most ions and small molecules, thus maintains H+ gradient that drives oxidative phosphorylation.
3. Intermembrane space: similar in composition to the cytosol
4. Matrix
   - ✓ Contains the mitochondrial genetic system and the enzymes responsible for Krebs cycle

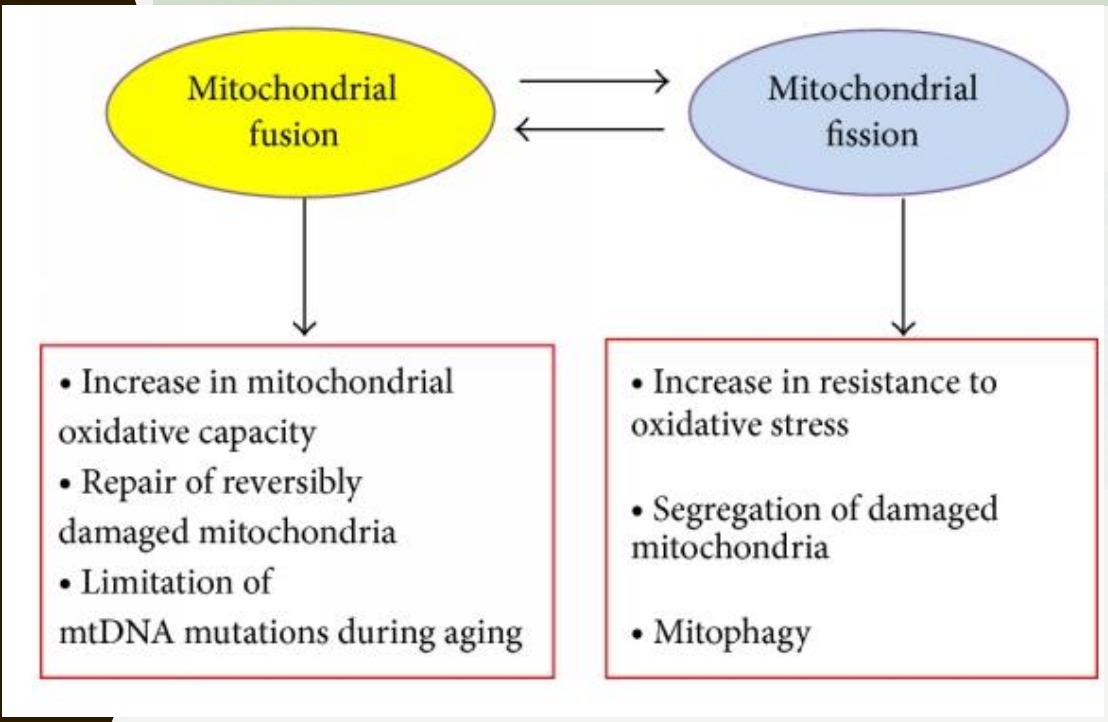


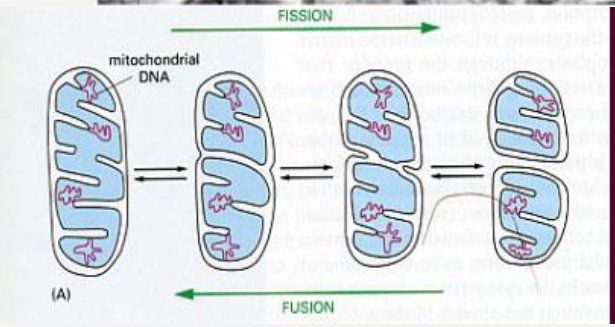
Mitochondrial fission versus fusion

- Located in cells requiring high-energy
- Dynamic organelles (fusion and division)
  - Exchange genetic material
  - Regulate autophagy
  - Cell survival

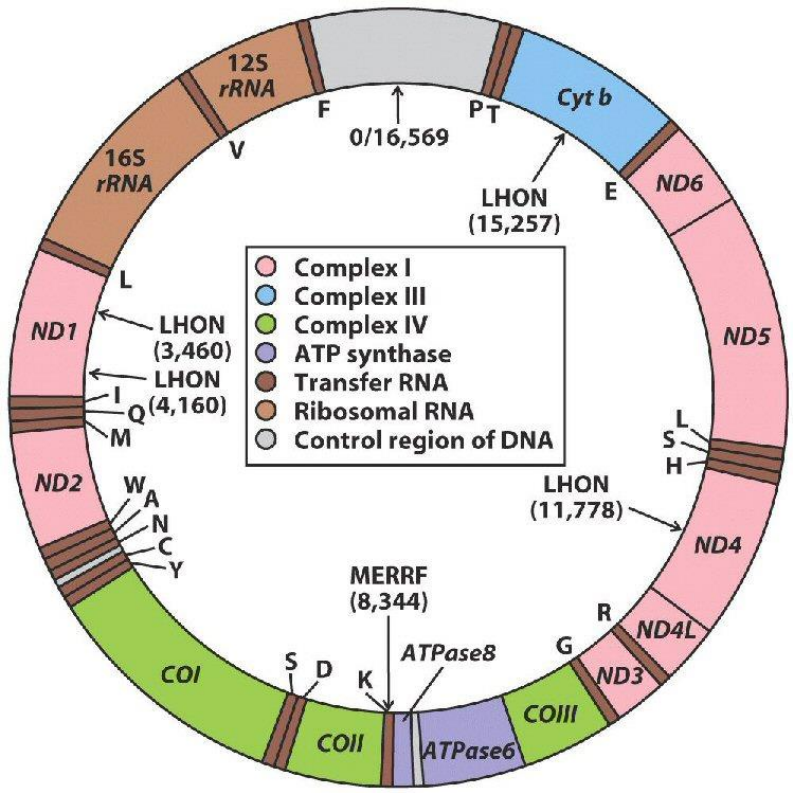
Mitochondria in synapse







The Genetic System of Mitochondria



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

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 encoded by nuclear genes:

- ✓ 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.

- ✓ Mitochondria from different tissues contain different proteins (<50% of proteins are common to all tissues)

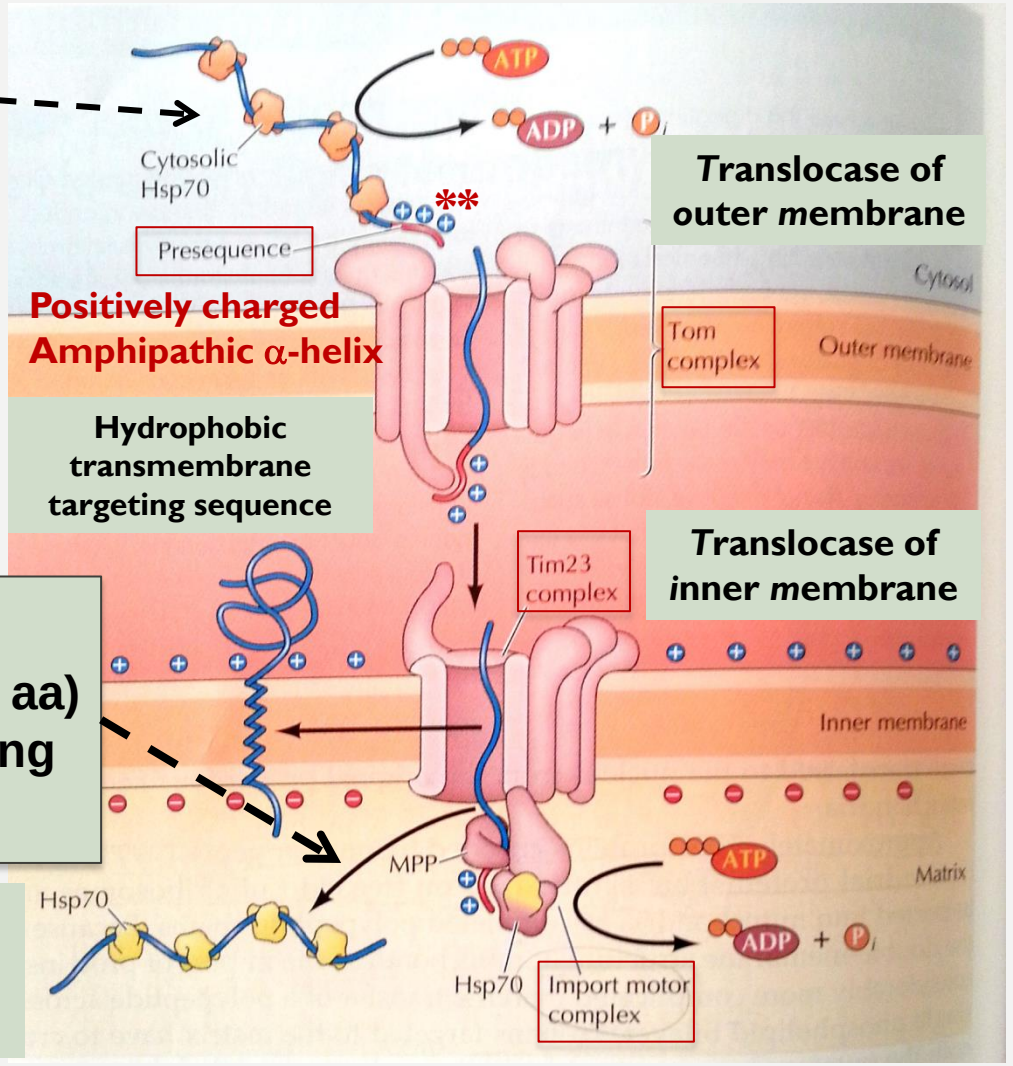
Protein Import and Mitochondrial Assembly

Partially unfolded polypeptide

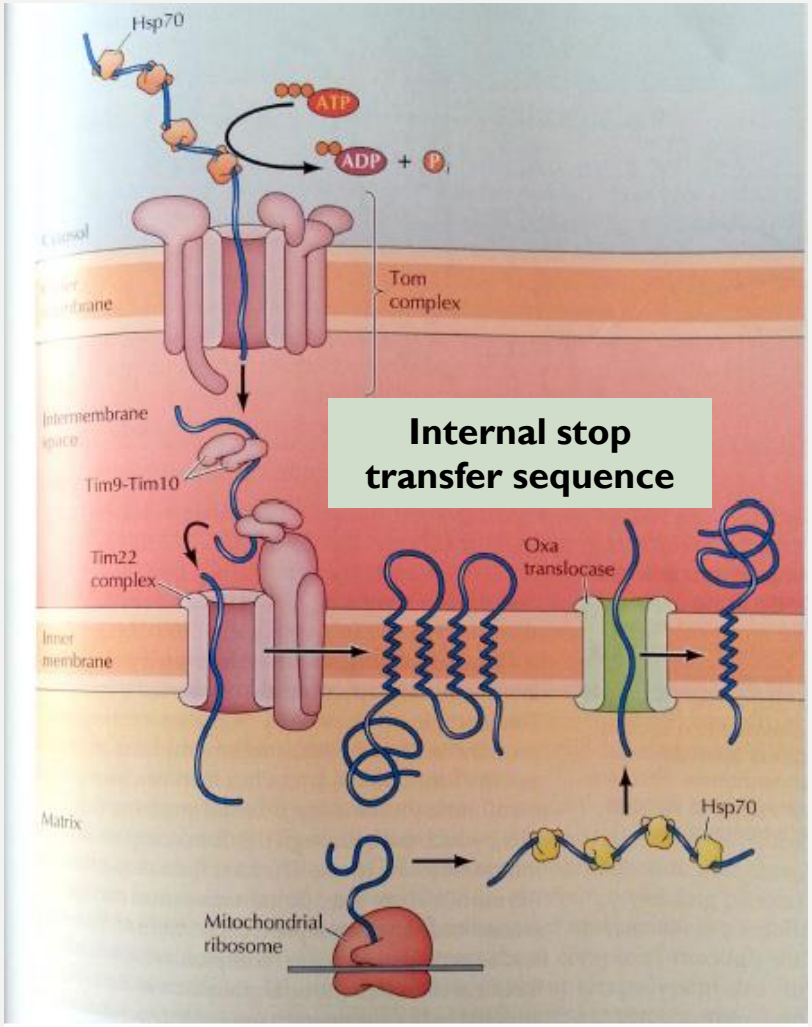
- ✓ **Several targeting signals**
- ✓ **Cross 1 or 2 membranes**
- ✓ **Sorting to distinct regions within mito.**
- ✓ **Presequence sort to IMM and matrix**

Cleavage of presequence (20-25 aa) by matrix processing peptidase (MPP)

The electric potential across the IMM drives the translocation of the +ve presequence



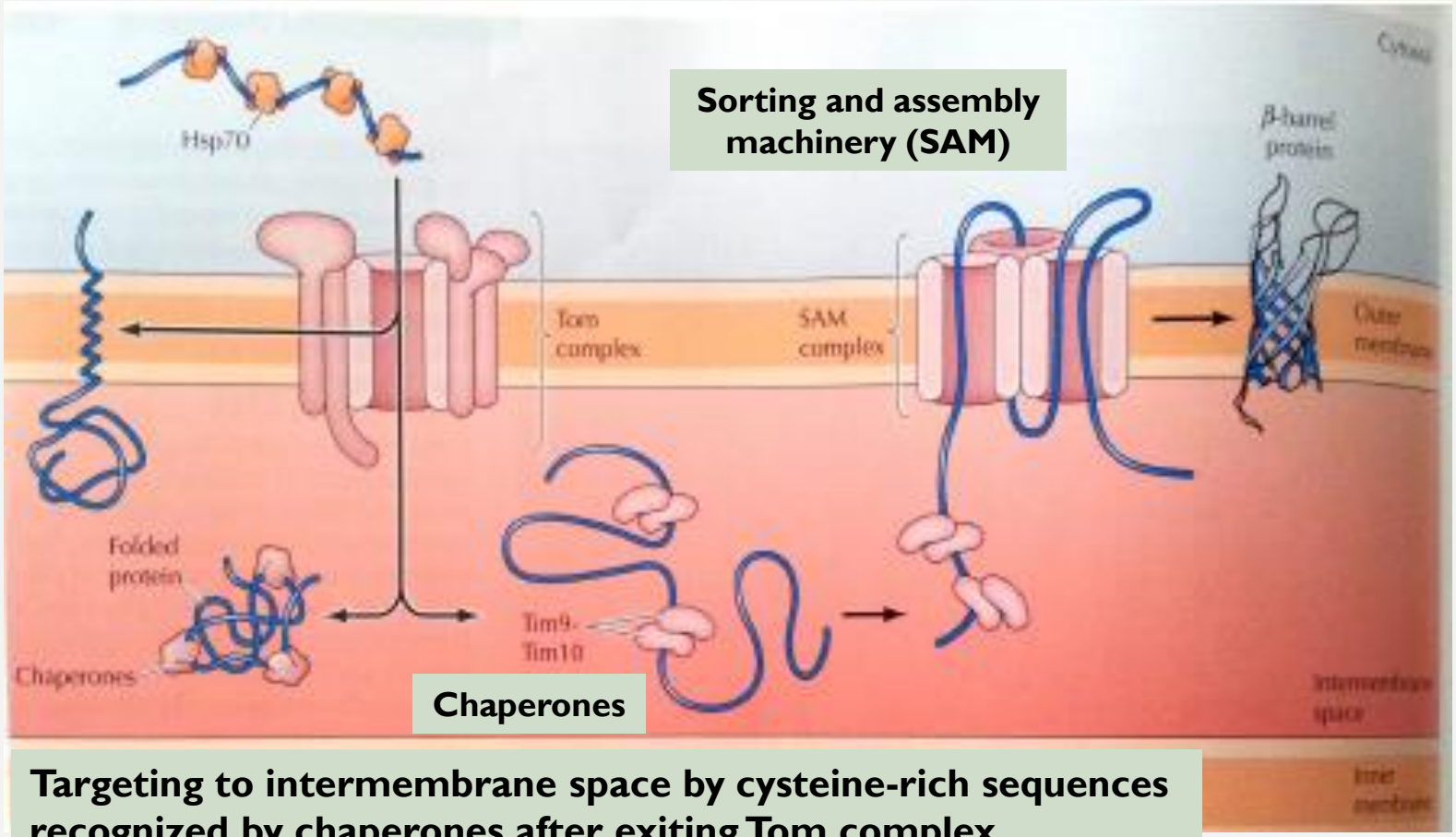
Targeting of inner membrane proteins



- Many mitochondrial proteins are multi-pass transmembrane proteins that do not contain presequences, but have multiple internal mitochondrial import signals
- They are recognized by mobile chaperones (Tim 9 & 10) in the intermemebrane space.
- These chaperones transfer the protein to a Tim complex (Tim 22).
- Inner membrane proteins encoded by mitochondrial genome are inserted via Oxa translocase.

Targeting of outer membrane proteins

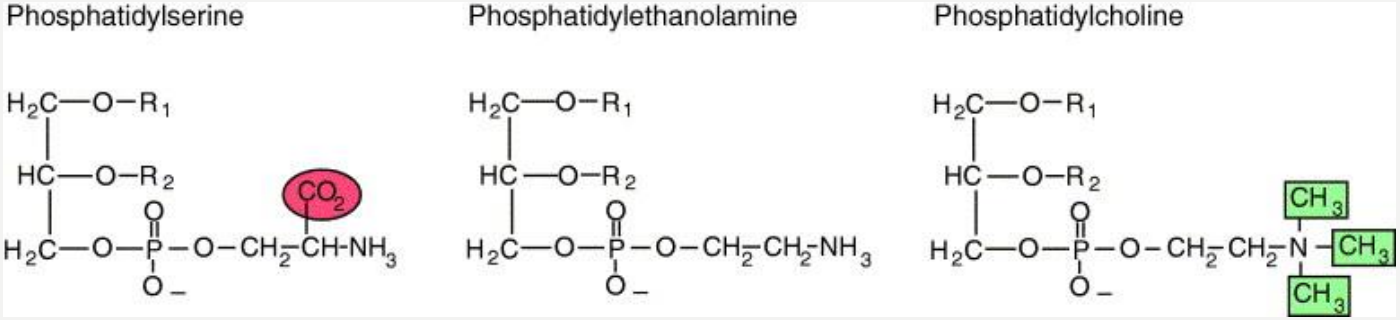
- Tom complex inserts proteins with α-helical transmembrane domains.
- SAM complex inserts β-barrel proteins such as porins.



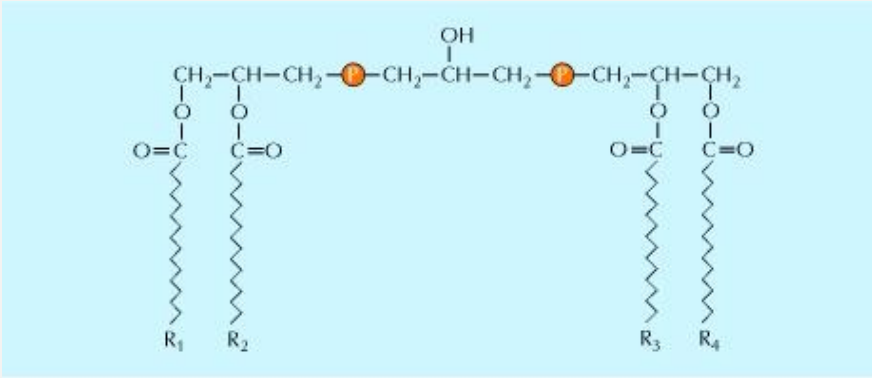
Targeting to intermembrane space by cysteine-rich sequences recognized by chaperones after exiting Tom complex.

Mitochondrial phospholipids

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

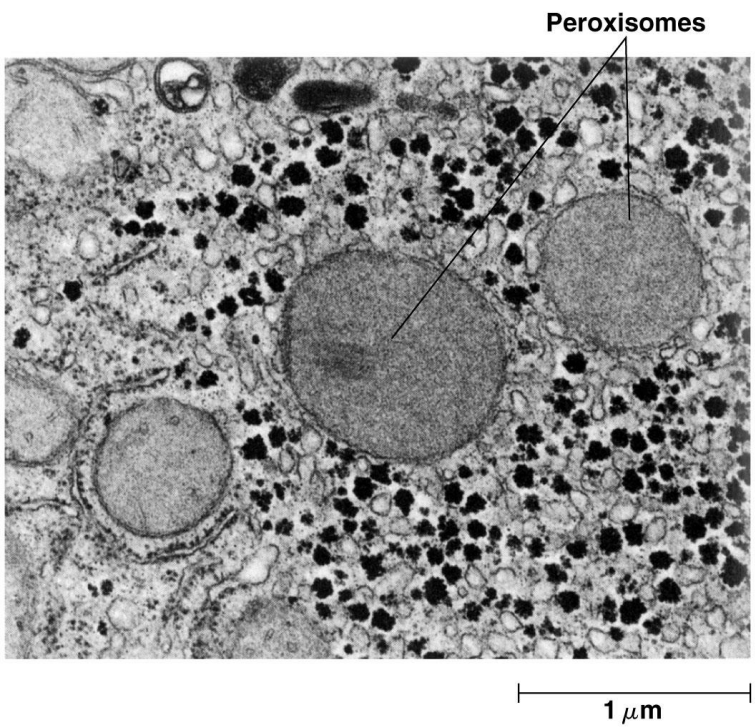


- Cardiolipin is an unusual phospholipid, that contains four fatty acid chains
- Cardiolipin is synthesized in the mitochondria.
- Cardiolipin improves the efficiency of oxidative phosphorylation by restricting proton flow across the membrane



Cardiolipin

Peroxisomes



Structural features

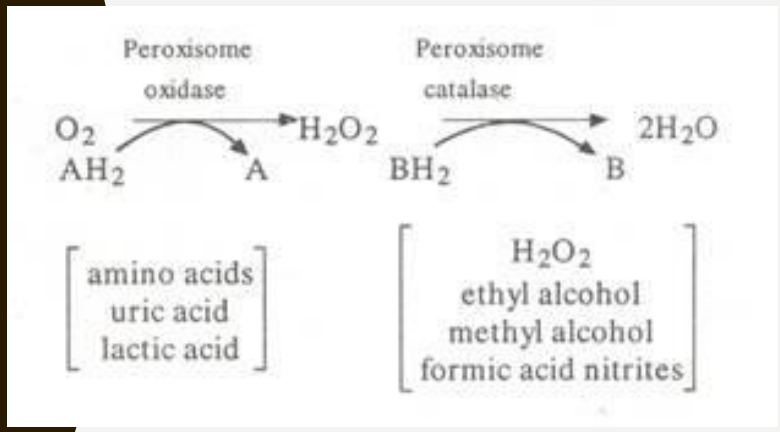
- Small, single membrane-enclosed organelles
- Contain enzymes involved in a variety of metabolic reactions, including several aspects of energy metabolism.
- They replicate by division.
- Can rapidly regenerate even if entirely lost
- Most human cells contain 500 peroxisomes.
- Their proteins are called peroxins (Pex1, Pex2, etc)

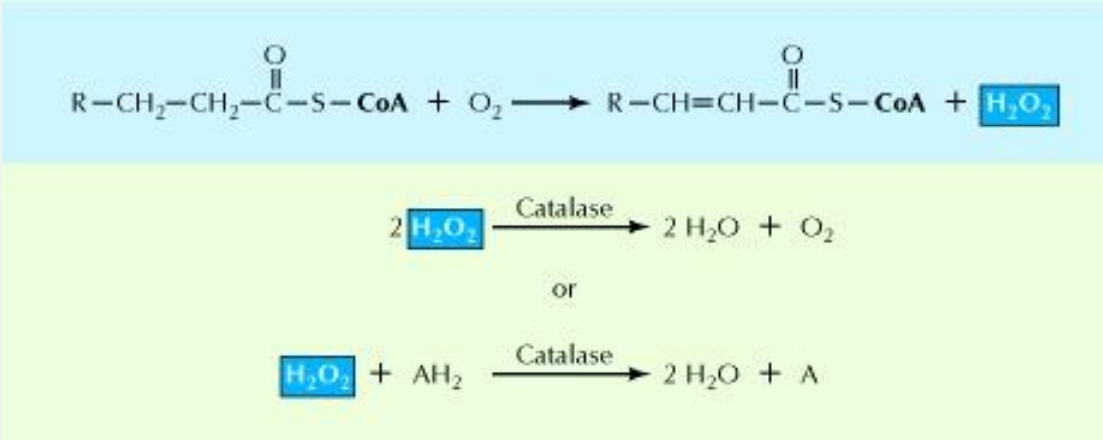
Peroxins

- 85 genes encode peroxins
- Most peroxins are metabolic enzymes.
- Internal proteins are synthesized on free ribosomes and then imported into peroxisomes.
- Transmembrane peroxisomal proteins are synthesized in ER.
- Other membrane proteins act as receptors for the import of internal proteins.

Function of peroxisomes

- 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.





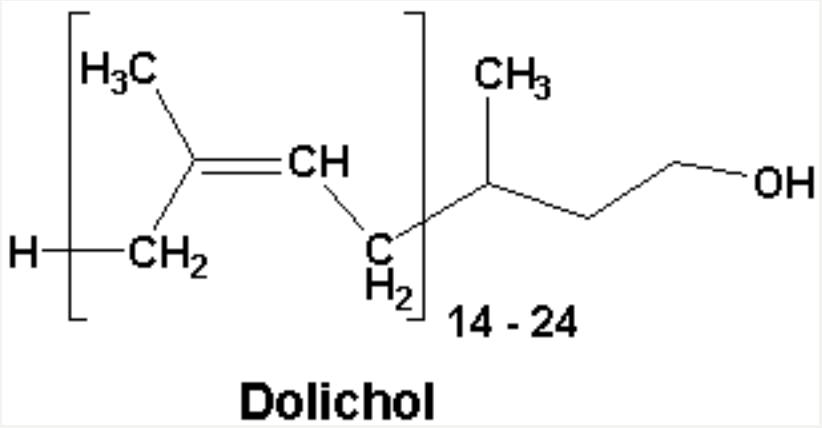
- Substrates like uric acid, purines, amino acids, and fatty acids are broken down by oxidative reactions in peroxisomes to provide energy.
- Fatty acids are oxidized in both peroxisomes and mitochondria.

Synthesis in peroxisomes

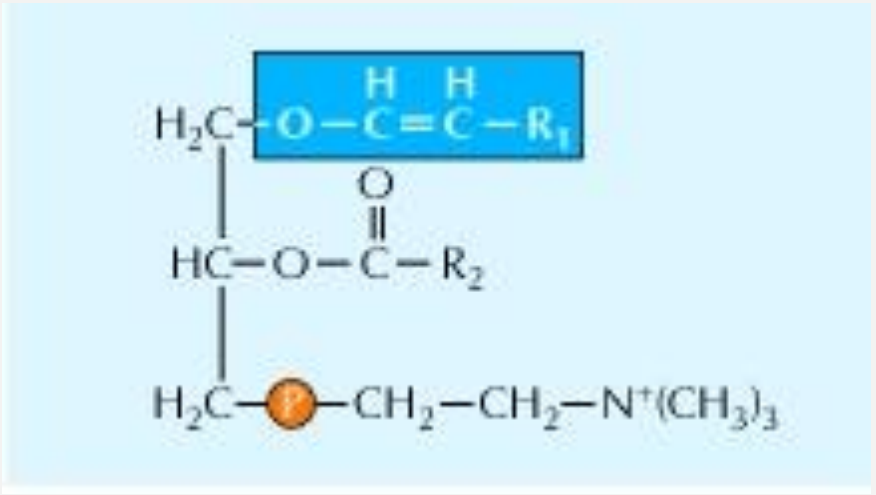
Lysine amino acid

Lipids:

- Cholesterol
- Dolichol
  - made from farnesyl
- Bile acids (liver)
- Plasmalogens
  - Phospholipids with one ether bond
  - important in membranes of heart and brain

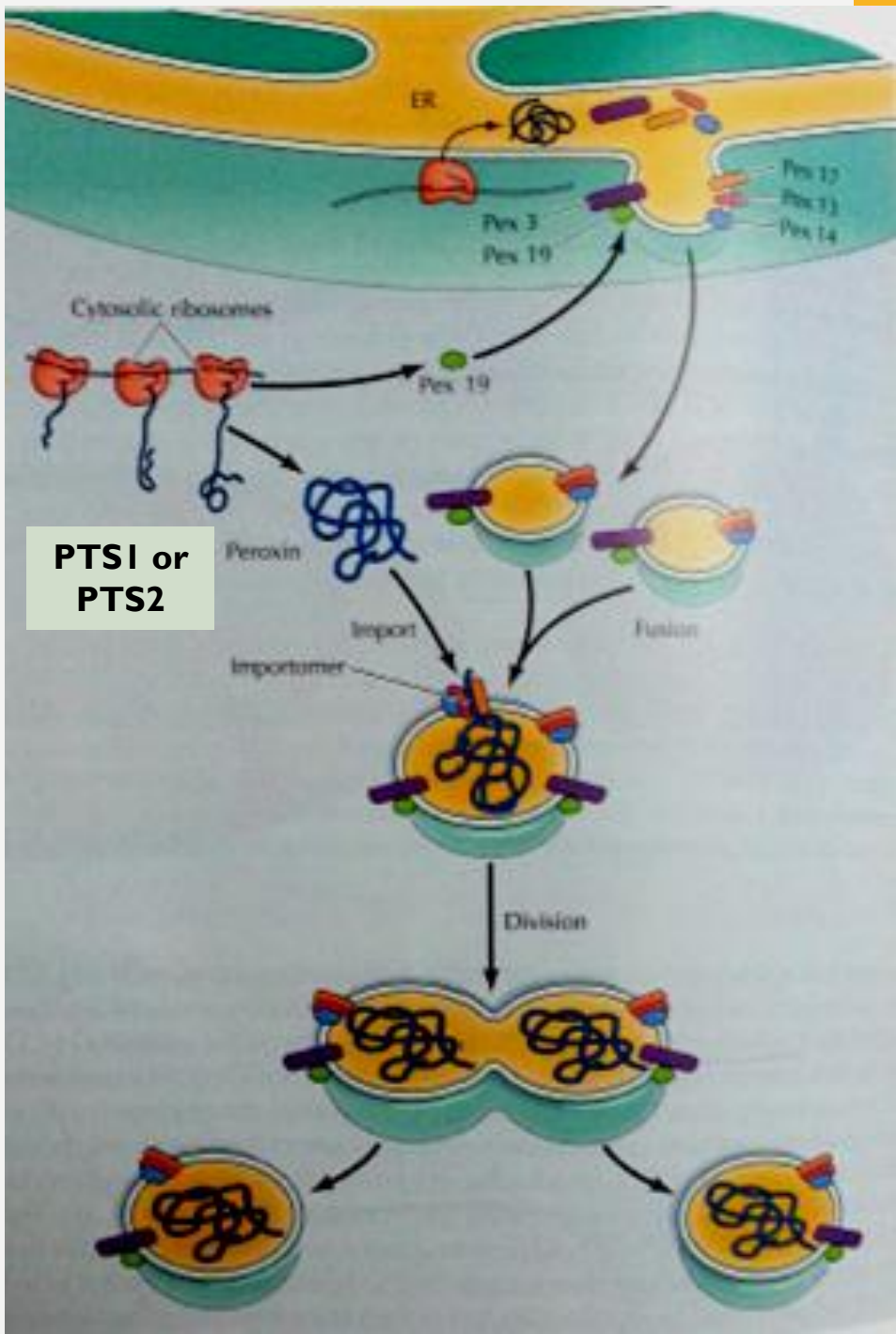


Dolichol

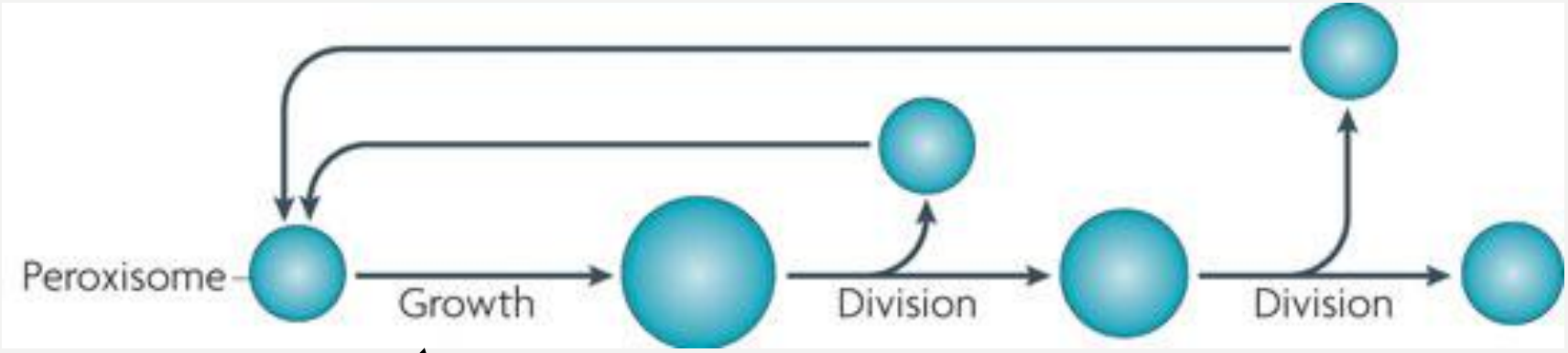


Peroxisomal Assembly

- **Pex3 protein recruits pex19 to initiate budding of peroxisome from ER.**
  - **The vesicle fuses with a new or an older one.**
- **Membrane proteins act as receptors for the import of internal matrix 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).**



Peroxisome maturation and division



Continuous addition of lipids from RER and protein import result in peroxisomal growth

Maturation involves import of proteins from cytosol at different times resulting in changes in enzymatic content and metabolic activity

Peroxisomal diseases

- Single peroxisomal enzyme deficiencies
  - Defective specific peroxisomal enzymes
- Peroxisomal biogenesis disorders (PBDs) that involve multiple peroxisomal enzyme deficiencies due to failure of import, e.g. Zellwegar syndrome
    - Lethal
    - Due to mutations in at least 10 genes such as the receptor of PTS1
- X-linked adrenoleukodystrophy (XALD).
  - Defective transport of very long chain fatty acid (VLCFA) across the peroxisomal membrane.

Mitochondrial diseases

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

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**