Mitochondria

All cells and organisms can broadly be grouped into two categories based on the mechanism of extracting energy for their own metabolism into:

1) Autotrophs:
   In which carbon-di-oxide and water are transformed by the process of photosynthesis into elementary organic molecule, glucose from which more complex molecules are made.

2) Heterotrophs:
   Those organisms who obtain their energy from the different nutrients (Carbohydrates, Fats and Proteins) synthesized by autotrophic organisms.
In cells the transformation of energy takes place with the help of two transducing systems (systems which convert energy from one form to another) represented by two organelles viz; Mitochondria and Chloroplast.

Chloroplast are adapted to capture light energy and to transduce it to chemical energy. On the other hand, Mitochondria are the **power plants of the cell which by oxidation** release the energy contained in the fuel molecules and make other forms of chemical energy. Thus, the main function of mitochondria is Oxidative phosphorylation.
• Mitochondria (Gr Mitos = thread; chondrion = granule) is a granular or filamentous organelle present in the cytoplasm of protozoa, animal and plant cells.

• They were first observed at the end of the 19th century and described as bioblasts by Altmann (1894), these structures were called mitochondria by Benda.

• Mitochondria were isolated by fractional centrifugation of ruptured liver cells by Bensley and Hoerr in 1934.
• Hageboom and his coworkers in 1948 finally demonstrated that mitochondria is the site of cellular respiration.

• It is difficult to observe mitochondria in living cells because of their low refractive index but they can be observed in cells cultured invitro under darkfield illumination.

• The stain Janus green is specific for mitochondria.
• Mitochondria are usually 0.5 - 1.0 microns in cross – sectional diameter and vary in length up to 7 microns. They may be filamentous or granular.

• Mitochondria has a definite orientation. In cylindrical cells they are oriented in basal – apical direction parallel to main axis. In leucocytes they are arranged radially with respect to centrioles.
Structural Organization

- Mitochondria consists of two membranes and two compartments. An outer limiting membrane about 6 nm thick surrounds the mitochondria. Within this membrane and separated from it by a space of 6-8 nm is the inner membrane that projects into the mitochondrial cavity as infoldings called mitochondrial crests.
• The inner mitochondrial membrane divides the mitochondria into two chambers or compartments:

1) The outer chamber contained between the two membranes and the core of the crests and
2) The inner chamber filled with a relatively dense proteinaceous material called mitochondrial matrix.

• The mitochondrial matrix is generally homogeneous but it may contain fine filamentous material or small dense granules which contains phospholipids.

• The mitochondrial crests are incomplete septa or ridges that do not disrupt the continuity of the inner chamber.
• Protruding from the inner membrane are knob like spheres called F₁ complex or F₁ particles or elementary particles.

• Each F₁ particle is a complex of proteins and is attached by a short protein neck to a F₀ complex ( an assembly of hydrophobic proteins embedded within the inner membrane ).
• The combination of $F_0$ and $F_1$ is called $F_0F_1$ complex. Functionally it is called ATP synthetase because it is the structure responsible for ATP generation.

• Within the mitochondrial matrix are small ribosomes and a circular DNA.

• The use of negative staining has enabled recognition of details of the mitochondrial structure.
• When the mitochondria is allowed to swell and break in a hypotonic solution and then immersed in phosphotungstate the inner membrane in the crest appears to be covered by particles ( of 8.5 nm size ) called $F_1$ or elementary particles.

• These are regularly spaced at an interval of 10 nm on the inner surface of the inner membrane. According to estimates there are between 10,000 -100,000 elementary particles per mitochondria.

• The presence of $F_1$ particles on the matrix side (M side) confers a characteristic asymmetry to the inner mitochondrial membrane.
• There **are structural and chemical** differences between the outer and inner membranes.

• The **outer membrane** has 40% lipid content, contains more cholesterol, is high in phosphatidyl inositol and low in cardiolipin. Its lipid:Protein content is 0.8.

• The inner membrane has 20% lipid content and the lipid/ protein content is 0.3. The low lipid : protein ratio indicates that there is greater intercalation of proteins in the lipid bilayer.
• The mitochondrial enzymes show a distinct compartmentalization:

**Outer Membrane:**
1) Mono amine oxidase
2) Rotenone- insensitive NADH Cytochrome c- reductase
3) Kynurenine hydroxylase
4) Fatty acid COA ligase
Space between outer and inner membranes:
1. Adenylate kinase
2. Nucleoside diphospho kinase

Inner membrane:
1. Respiratory chain enzymes
2. ATP synthase
3. Succinate dehydrogenase
4. B–hydroxybutyrate dehydrogenase
5. Carnitine fatty acid acyl transferase

Matrix
1. Malate
2. Isocitrate dehydrogenase
3. Fumarase
4. Aconitase
5. Citrate synthetase
6. Alpha keto acid dehydrogenase
7. Enzymes of beta-oxidation
• The molecular organization of mitochondria is very complex. Within the realm of this organelle are more than 70 enzymes and coenzymes as well as numerous cofactors which operate in an orderly and coordinated fashion.

• Most of the metabolic processes in the mitochondria occur in the inner compartment. Active flow of metabolites occurs across the two membranes.

• The outer membrane is freely permeable but the inner membrane is not and therefore it makes use of specific carriers or translocators for the movement of ions and other metabolites to achieve it.
Mitochondrial DNA

• In 1963, Margit and Sylvan Nass observed filaments within mitochondria that they interpreted as DNA molecules. This finding was confirmed in cell sections as well as DNA extracted and studied by surface spread technique.

• The mitochondrial DNA (mt-DNA) is located in the matrix and is probably attached to the inner mitochondrial membrane at a point where DNA duplication begins.

• A single mitochondria may contain one or more DNA molecules depending on its size i.e; larger the size more the no. of DNA molecules.

• Mitochondrial DNA duplication is under nuclear control and the enzymes used are imported from the cytosol.

• In most animals and plants the mt-DNA is circular although it may be highly twisted.
Mitochondrial Ribosomes

• Mitochondrial matrix has been found to contain small ribosomes. These ribosomes have a sedimentation coefficient of 55s with subunits of 35s and 25s.
• Ribosomes in mitochondria are tightly associated with the inner membrane.
• These mitochondrial ribosomes synthesize 12 hydrophobic proteins called proteolipids.
Semi autonomous organelle

By virtue of the fact that mitochondria have their own DNA as well as Ribosomes and are able to synthesize their own proteins, the term semi–autonomous organelle, is applied to them. However, their biogenesis is highly dependent on nuclear genome and the biosynthetic activity of the ground cytoplasm.

There are however, some differences between mitochondrial protein synthesis and protein synthesis in a cell.

1) Only 22 different t-RNA’s are present in mitochondria as against more than 40 t-RNA’s available for ordinary translation.

2) For most part the genetic code used by mitochondria is identical to that used by nuclear DNA with two differences a) UGA (stop codon for nuclear DNA is read as Tryptophan in mitochondria and b) AUA is read as methionine in mitochondria but isoleucine in nuclear DNA.
BIÓGENESIS OF MITOCHONDRIA

Two main mechanisms have been postulated for the biogenesis of mitochondria:
1) Origination division from parent mitochondria
2) Origination de novo by simpler building blocks.

1) Origin by division:
• Mitochondria are distributed between the daughter cells during mitosis and their number increases during interphase. It has been observed by Time Lapse Cinematography that mitochondria gradually elongate and then fragment into smaller mitochondria.
• This has been verified in choline deficient mutant of Neurospora. When it was labelled with radioactive choline and radioactivity followed in the mitochondria of the 2nd and 3rd generation by autoradiography, it was found that all the mitochondria of the original progeny were labelled but the mitochondria of next generation contained about half the radioactivity. This indicated that the mitochondria had divided.
2) **Origin de novo:**

- This has been seen in Yeast cells.
- Yeast cells grown anaerobically lack a complete respiratory chain (Cytochromes b and c are absent) and under the E.M show no typical mitochondria. However, when the yeast cells are placed in air, the mitochondrial membranes present fuse, unfold and form true mitochondria that contains the cytochromes.
- This observation is in agreement with the de novo synthesis theory of mitochondrial biogenesis.
The Symbiont Hypothesis: Mitochondria and Chloroplast are intracellular parasites

At the end of last century, scientists like Altmann and Schimper, on purely morphological ground, speculated that mitochondria and chloroplasts might be intracellular parasites that have established a symbiotic relationship with the eukaryotic cell. The bacteria were thought to have originated the mitochondria and the blue–green algae the chloroplast.

In modern cell biology, with the recognition that these organelles have a certain degree of autonomy, this hypothesis has been refrained. According to the revised theory, the original host cell is conceived to be an anaerobic organism deriving its energy from glycolysis (a process which occurs in the cytoplasmic matrix) and the parasite contains reactants of the Kreb’s cycle and the respiratory chain. It is therefore able to carry on respiration and oxidative phosphorylation.
The Symbiont hypothesis is based on many similarities between mitochondria, chloroplast and prokaryotes:

1) Similarity in the localization of respiratory chain or electron transport system:
   Certain bacteria have membranous projections extending from the plasma membrane forming the mesosomes. These mesosomes are comparable to the mitochondrial crests and have been shown to contain the respiratory chain. Thus, the inner mitochondrial membrane and the crests may represent the original symbiont enclosed within a membrane of cellular origin.

2) The mitochondrial DNA is circular as is the case in the chromosomes of prokaryotes.

3) Protein synthesis in both mitochondria and bacteria is inhibited by Chloramphenicol whereas the extra mitochondrial protein synthesis of higher cells is not affected.

4) In mitochondria, there is evidence of DNA dependent – RNA synthesis which indicates partial autonomy of this organelle.
5) Another support of the possible support of the prokaryotic origin of mitochondria and chloroplast comes from the fact that intracellular synbiosis is found in nature too eg, Paramecium have been found to contain bacteria.

Endosymbiosis of a photosynthetic prokaryote confers on one hand to the host, the ability to capture light energy to synthesize various products and on the other hand provides for the prokaryote, a constant environment in which to grow and reproduce.

In evolutionary terms it is possible to believe that a symbiotic relationship could have evolved into the present situation, in which, the organelles have a certain degree of autonomicity but depend on the nucleus and cytosol of the cell for the synthesis of most of their specific components.
Molecular Organization and Function

• The main function of mitochondria is of an energy transducing organelle (i.e.; it converts one form of energy into another). In mitochondria, therefore, major degradation products of cell metabolism penetrate and are converted into ATP to be used in various activities of the cell. The entire process requires the entrance of ADP, Oxygen and inorganic phosphate and brings about the exit of ATP, water and carbon-di oxide.

• The chemical energy of nutrients is stored in the covalent bonds between the atoms of each molecule. This energy is not released suddenly but is made available in a stepwise and controlled manner.
• The process of energy transformation in mitochondria occurs in the following three coordinated steps:

• 1) **Kreb’s cycle**: Carried out by a series of soluble enzymes present in the mitochondrial matrix, which produces CO2 by decarboxylation and removes electrons from the metabolites.

• 2) **Respiratory chain or Electron Transport system**: Which captures the pairs of electrons and transfers them through a series of electron carriers, this finally leads to the formation of water by combination with activated oxygen.

• 3) **Phosphorylating System**: Is tightly coupled with the respiratory chain, which at 3 points gives rise to ATP molecules.
Kreb’s Cycle/TCA cycle/Citric Acid Cycle

- Takes place in the mitochondrial matrix
- It is the first step in a common pathway for the degradation of fuel molecules
- These molecules are acted upon metabolically (in the cytoplasm) to produce acetyl groups which are taken into the cycle by acetyl coenzyme-A
- The primary function of TCA cycle is to oxidize acetyl groups that enter the cycle
- The reaction forms a cycle because the acetyl group is not oxidized directly but only after it has been covalently added to a larger molecule, oxaloacetate which is regenerated at the end of one turn of the cycle
- The cycle begins with a reaction between acetyl CoA and oxaloacetate to form tricarboxylic acid called citric acid
- Each turn of the cycle generates two carbon dioxide molecules and four pairs of hydrogen atoms which enter the respiratory chain. Out of these three pairs are accepted by NAD and one pair accepted by FAD
- It takes two turns of the cycle to metabolize the two acetate molecules that are produced by glycolysis from one molecule of glucose
- A total of 30 molecules of ATP are generated from each glucose molecule
ELECTRON TRANSPORT SYSTEM

- Starts with NAD$^+$ which is reduced to NADH. This is followed by a flavo protein, coenzyme-Q and a series of cytochromes ending with oxygen
- The respiratory chain and the phosphorylating system are in the inner mitochondrial membrane
- The components of the respiratory chain may be separated into multi-molecular complexes in which lipids are essential for the activity
- **Complex I or NADH-Q reductase** is the largest and has 15 subunits. It contains FMN and 6 iron-sulphur centers. The NADH reaction site is at the M side (matrix)
- **Complex II or succinate-Q-reductase** contains FAD and 3 Iron-sulphur centers. This complex transfers electrons from succinate to Co-Q
- **Complex III or QH$_2$–Cytochrome-reductase** contains cytochrome b1, c1, and 1 iron-sulphur protein. Cytochrome C is on the cytosol side
- **Complex IV or cytochrome C oxidase** is a large complex with cytochrome a and a3 and 2 copper atoms
# Metabolic functions of different components of mitochondria

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