Mitochondria: structure

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Lesson: Mitochondria: structure
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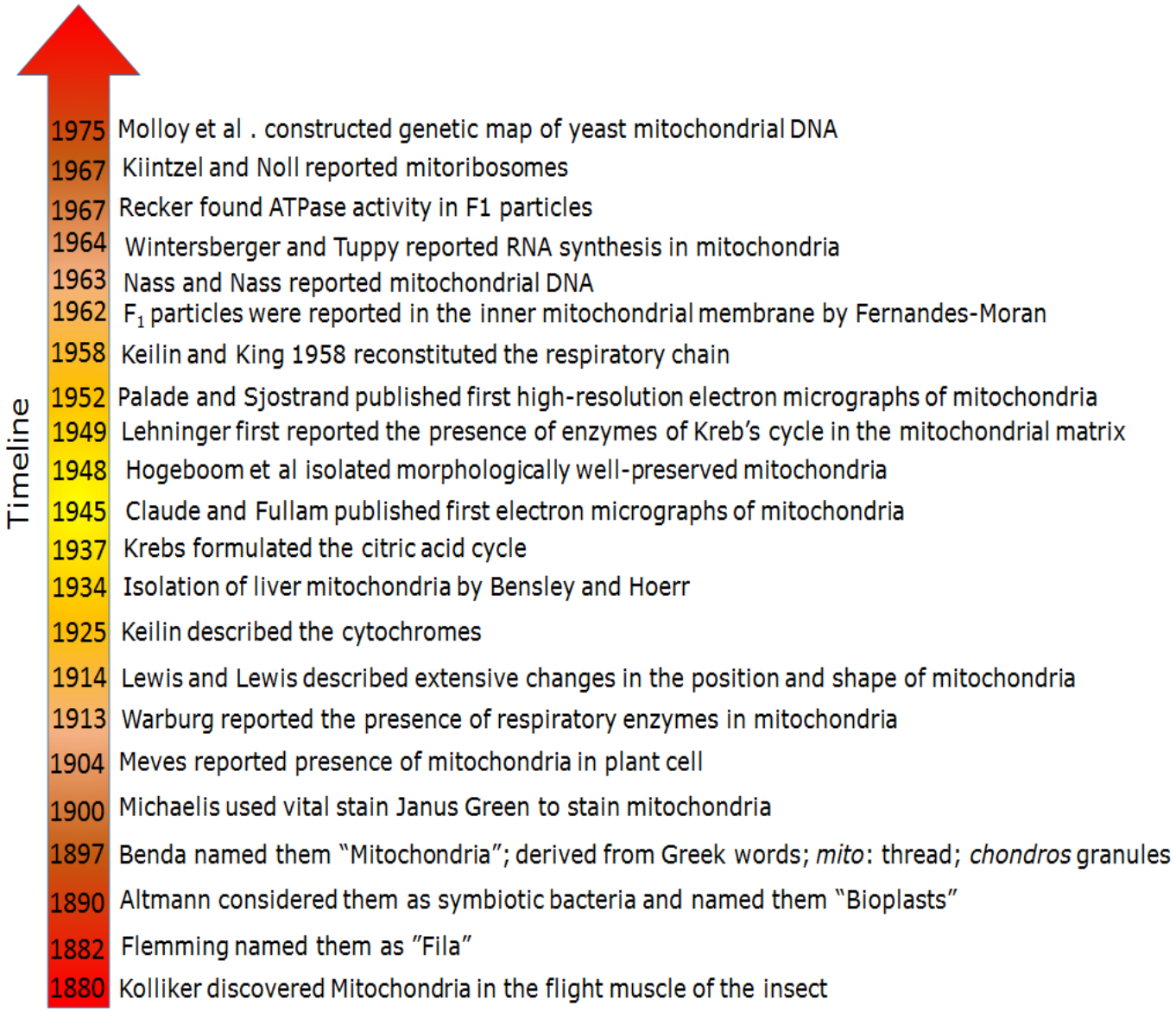
Mitochondria: Introduction

Mitochondria are important cell organelle associated with energy metabolism. They were first reported by Kolliker (1880) from the flight muscles of insects. The name mitochondria was given by Benda (1897). Mitochondrion is derived from Greek words; *mito*: thread; *chondros*: granules.



Presence

Mitochondria are found in all eukaryotic cells except mature mammalian RBCs. They are completely absent from bacteria and blue-green algae.

Mitochondria: structure

Size

Mitochondria are among one of the largest cell organelles with size comparable to that of an *E. coli*. The size of mitochondria varies from 0.5 to 10 µm.

Shape

Mitochondria have a variety of shapes like rod, filamentous or granular. The shape of mitochondria changes with cell types and also within a cell at various time intervals. They are dynamic organelle capable of changing shapes.

Number

The number of mitochondria present in a cell depends on the metabolic state of a cell. They are more abundant in actively growing and dividing cells. Unicellular eukaryotes usually have single mitochondrion. Large number of mitochondria are reported from flight muscles of insects and *Chaos chaos*, an amoeba. Sperms have fewer than 100 mitochondria. The distribution of mitochondria can also vary over time. They are usually concentrated near those regions of cell which require constant ATP supply (like at the base of flagellum). Animal cells usually contain more mitochondria as compared to plant cells. They are more in germinating seeds as compared to dormant seeds. Cyclosis (streaming movement of cytoplasm) tends to distribute mitochondria uniformly in some plant cells. All mitochondria of a cell are collectively known as **chondriome**. Mitochondria of muscle cells are known as **sarcosomes**.

Ultrastructure of mitochondria

A mitochondrion has a double membrane structure (Fig. 1) which is the phospholipid bilayer like plasma membrane. The outer and inner membrane are quite different from each other and divide mitochondrion into two distinct compartments: **intermembrane space** and **mitochondrial matrix**. Palade and Sjostrand independently obtained high resolution electron micrographs of mitochondria. Their micrographs revealed a double membrane surrounding the mitochondrion giving the first hint of the presence of two membranes.

Do you know???

Both Palade and Sjostrand were working to resolve the structure of mitochondria. They independently published high resolution electron micrographs of mitochondria. What Palade observed in the micrographs has been defined by him in one of his publications published in 1956. According to him "*Two spaces or chambers are outlined by the mitochondrial membranes, an outer chamber contained between the two membranes, and an inner chamber bounded by the inner membrane. The inner chamber is penetrated and, in most cases, incompletely partitioned by laminated structures which are anchored with their bases in the inner membrane and terminated in a free margin after projecting more or less deeply inside the mitochondrion*"

From: Palade,G. E. 1956. In Enzymes: Units of Biological Structure and Function. O. H. Gaebler, editor. Academic Press, Inc., New York. 185-215.

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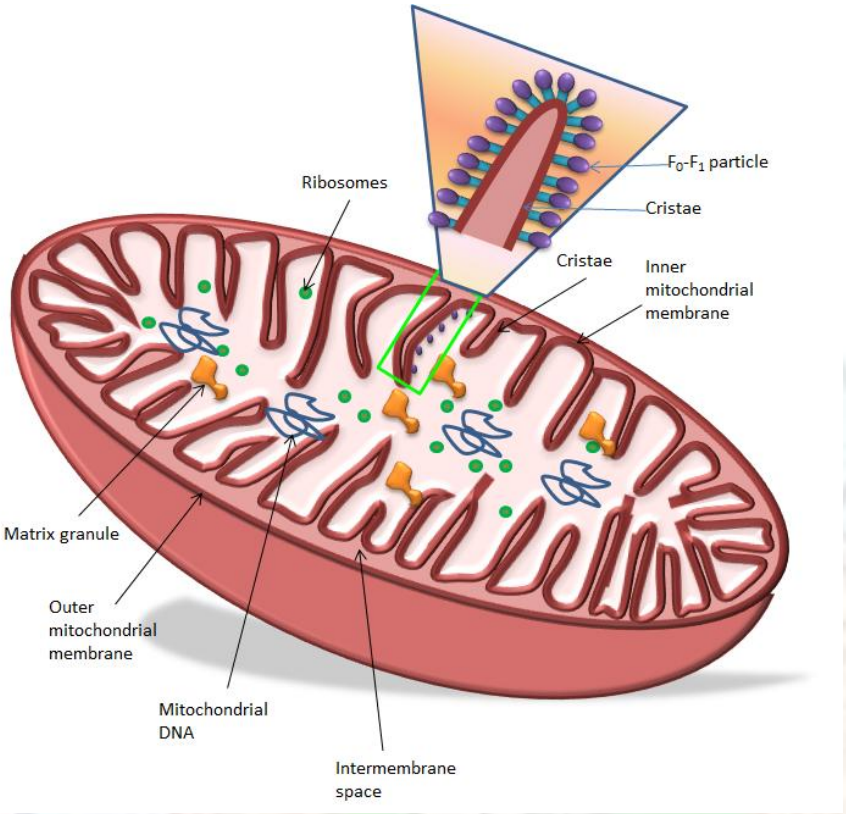

Fig. 1. Ultrastructure of mitochondria showing two membranes. The outer membrane is freely permeable due to porins and appear smooth while inner membrane is impermeable even to small ions like H+, K+, Cl- and OH-. **Source: Author**

Outer membrane:

Outer membrane appears smooth, freely permeable, contains 50% lipids and has cholesterol. The outer membrane has porin proteins (similar to porins found in bacteria) which form channels that allow molecules of upto 5000 Da to pass freely. Due to these porin proteins the concentration of intermembrane space is same as cytosol with respect to small ions.

Inner membrane:

In contrast to the outer membrane, the inner membrane is highly impermeable; restricts the passage of molecules (which require special membrane transporters to enter into the matrix) and therefore important in creating H^+ gradient. The inner membrane is also impermeable to small ions like H^+, K^+, Cl^- and OH^- but is freely permeable only to O_2, CO_2 and H_2O. The inner membrane is rich in proteins (nearly 76%), cardiolipin (like diphosphatidyl glycerol which are found in bacterial plasma membrane); and lacks cholesterol. Majority of inner membrane proteins are involved in electron transport and

oxidative phosphorylation. The inner membrane also has many folds which forms ridges inside. Palade (1952) called these ridges *cristae mitochondriales*, still these folds are known as **cristae.** In plants they appear tubular and are known as **tubuli**.

These infoldings of inner membrane increase the surface area for cellular respiration. Each crista has intracristal space. The cristae bear a number of knob like structures known as F_1 particles or oxysomes. As per some estimates, there are 10^4 to 10^5 F_1 particles per mitochondrion. However, the number of F_1 particles varies with the metabolic state of a cell. Mitochondrial cristae can be disrupted by sonication to obtain submitochondrial particles which have F_1 particles attached to the outer surface and can carry out oxidative phosphorylation (Fig. 2). If the F_1 particles are removed the ability to carry put oxidative phosphorylation is lost suggesting them to be the site of ATP synthesis.

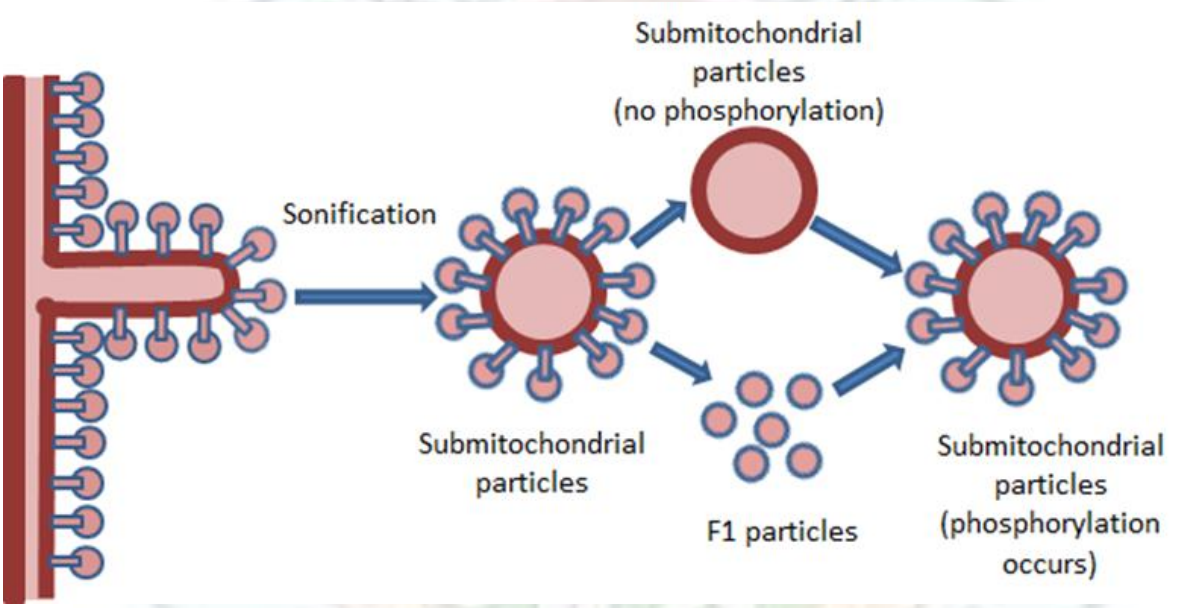

Fig. 2: Submitochondrial fractionation to separate the inner membrane to form submitochondrial particle. These submitochondrial particles have F_1 subunits attached to the outer surface and are required for oxidative phosphorylation to generate ATP.

Matrix

Mitochondrial matrix is gel-like and has high concentration of water-soluble proteins and contains enzymes for Krebs cycle, lipid oxidation and synthesis. Matrix also contains ribosomes known as **mitoribosomes** with Svedberg coefficient 55-80S, and double stranded, circular, naked DNA. The mitochondria are called **semi-autonomous** organelle as they have complete machinery for protein synthesis (mitoribosomes, tRNA, double stranded, circular, naked DNA and enzymes involved in transcription and translation). The mitochondrial DNA codes for only 1% of the total protein required by mitochondria and for rest of the proteins it is dependent on the nuclear DNA.

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Do you know??

Mitochondria can be stained using a vital dye- Janus Green used by Michaelis in 1900. The dye imparts mitochondria greenish blue color due to oxidation of dye by cytochrome oxidase.

Structure of Mitochondrial ATP synthase

Mitochondrial ATP synthase also known as F_1F_0-ATPase or complex V or F-type H^+-ATPase is made up of main components: **a globular head or F_1** and a **basal section or F_0**. F_1 can be easily dissociated from F_0 by treatment with urea. Similar types of ATP synthases are also found in bacteria and chloroplast. The F_1 is composed of five different polypeptides *α, β, γ, δ* and *ε* and is found to be conserved between bacteria, chloroplast and mitochondria. The five polypeptide chains form nonamer with a composition of $\alpha_3\beta_3\gamma\delta\varepsilon$, where *α* and *β* subunits arranged alternately (Fig. 3). The γ subunit forms a central stalk which connects F_1 with F_0 basepiece. The ε subunit helps in the attachment of γ subunit to F_0.

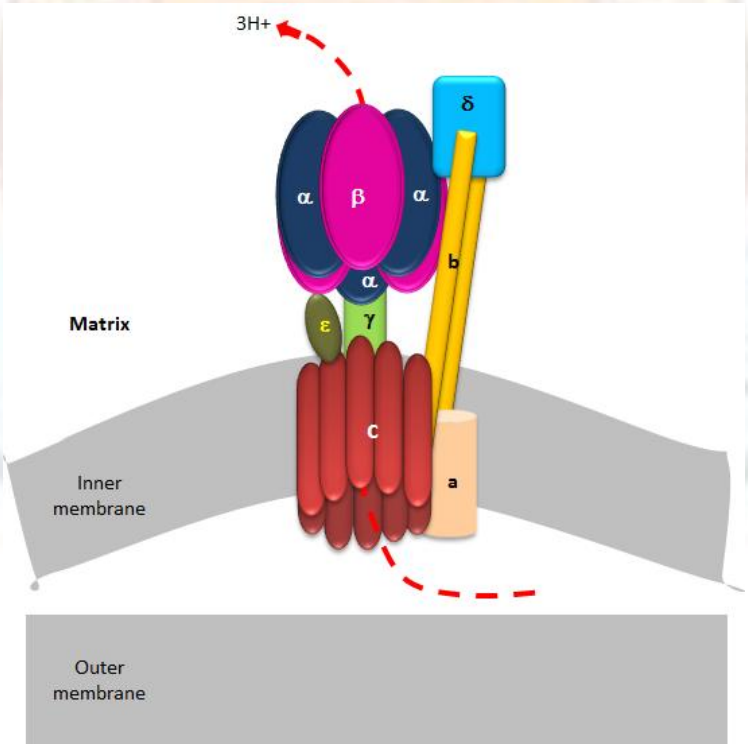

Fig. 3: The structure of ATP synthase. The F_1 is composed of five different polypeptides *α, β, γ, δ* and *ε* while the F_0 basepiece is composed of three different polypeptides *a, b* and *c.* **Source: Author**

The F_0 basepiece is composed of three different polypeptides *a, b* and *c* with composition of ab_2c_{10-14} and is found inside the inner membrane. The number of *c* subunits vary e.g. ATP synthase in bacteria and yeast mitochondria have 10 subunits, while chloroplast has 14 subunits. The *c* subunit forms a rotating motor. The *a* subunit contains the H^+ channel through which H^+ flow back into the matrix from the intermembrane space. The b subunit along with δ subunit of F_1 particle forms peripheral stalk which help positioning the $\alpha_3\beta_3$ subunit.

Mitochondrial marker enzymes

The mitochondria are site for many metabolic activities of the cell. The metabolic processes like fatty acid oxidation, Kreb's cycle take place in the matrix while electron transport and oxidative phosphorylation takes place at the inner membrane. Therefore, different metabolic processes take place at different compartments of the mitochondria and therefore the enzymes are distributed accordingly (Table 1). These enzymes serve as marker enzymes to study the integrity of mitochondria during subcellular and submitochondrial fractionation.

Table 1: Distribution of key enzymes in various mitochondrial compartment.

Mitochondrial compartment	Enzymes
Outer membrane	Fatty acid CoA ligase
	Monoamine oxidase
	NADH-cytochrome c reductase
	Kynurenine hydroxylase
	enzymes involved in phospholipid metabolism
Inner membrane	Respiratory chain enzymes like NADH dehydrogenase complex, cytochrome oxidase
	ATP synthase
	Succinate dehydrogenase
	Carnitine fatty acid acyl transferase
	Hydroxybutyrate dehydrogenase
	enzymes involved in heme synthesis
	aminolevulinic acid synthetase
Matrix	Pyruvate dehydrogenase complex
	Carnitine palmitoyl transferase II
	Enzymes of TCA cycle: • citrate synthase • aconitase • isocitrate dehydrogenase • α-ketoglutarate dehydrogenase • succinyl CoA synthetase • fumarase • malate dehydrogenase

	Enzymes for fatty acid oxidation: • Acyl-CoA dehydrogenase • Enol-CoA hydratase • Hydroxyacyl CoA dehydrogenase • Thiolase
Intermembrane space	Nucleoside diphosphokinase
	nucleoside monophosphokinase
	Adenylate kinase
	Sulfite oxidase
	yeast cytochrome c peroxidase

Mitochondrial fractionation

Mitochondria can be isolated from tissues using either differential centrifugation or by density gradient centrifugation. In differential centrifugation, the tissue is homogenized and the homogenate obtained is first subjected to centrifugation at low speeds to separate larger particles, after this the supernatant is collected and is further subjected to centrifugation with continuously increasing speeds (Fig. 4). Using this process, nuclei are the first to sediment, followed by mitochondria, lysosomes and peroxisomes which form a single pellet. Ribosomes are last to pellet.

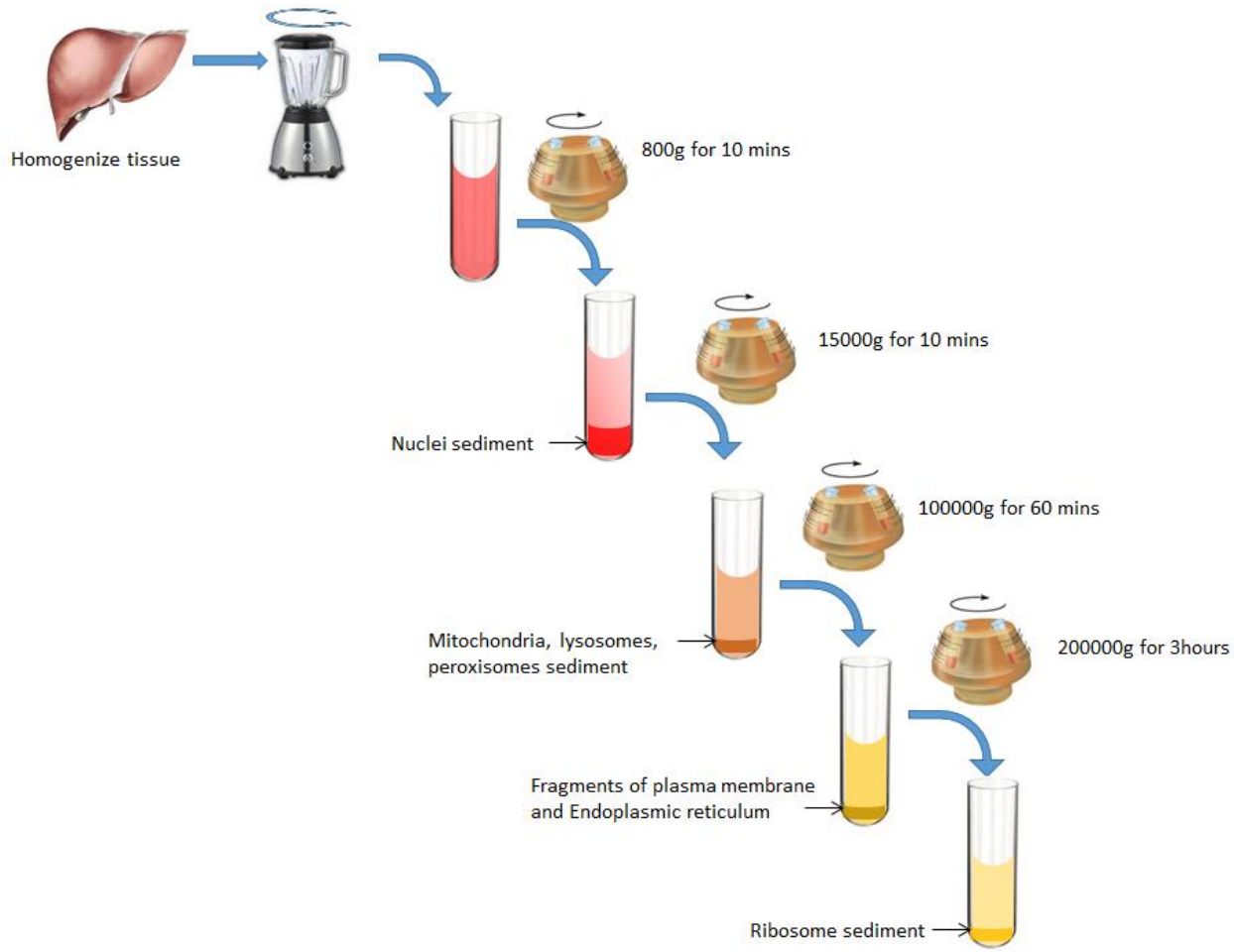

Fig. 4: Differential centrifugation. It involves centrifugation first at low speeds and collection of the supernatant which is subjected to centrifugation with continuously increasing speeds.

In density gradient centrifugation, a continuous sucrose-density gradient is created with density being high at the bottom of the tube (Fig. 5). In the first step the tissue is broken by using a homogenizer in an isotonic buffered solution. The lysate is then subjected to density gradient centrifugation where different cellular fractions are separated based on their densities. For isolating mitochondria, the usual protocol that is followed is first separating the cellular components using differential gradient centrifugation to obtain pellet containing mitochondria, lysosomes and peroxisomes. This pellet is collected, re-suspended in a continuous sucrose density gradient and is centrifuged at 65,000g for 2 hours. Mitochondria form an intermediate band between lysosomes and peroxisomes.

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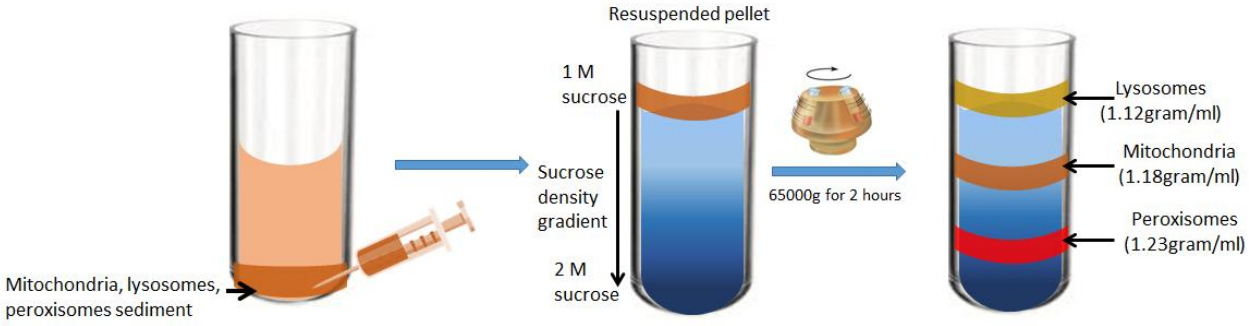

Fig. 5: Density gradient centrifugation. In this process a continuous sucrose-density gradient is created which separates the components based on their density. Using sucrose-density gradient mitochondria form an intermediate band between lysosomes and peroxisomes.

The outer and inner mitochondrial membranes as well as the contact sites between the two membranes can be separated by the process commonly called submitochondrial fractionation. It was known since 1950s that mitochondria have two membrane but the attempts to separate them were made successfully by Levy et al. (1966) and Schnaitman et al. (1967). They used low concentrations of a detergent digitonin which binds cholesterol present on the outer membrane to remove the outer membrane of liver mitochondria to obtain **mitoplasts**. This was followed by differential centrifugation to separate the fractions of outer and inner membrane.

Many modifications of the existing methods have been done by many workers. One method involves disrupting mitochondria using sonication in hypotonic solution containing EDTA. The homogenate is subjected to centrifugation to separate small vesicles from intact mitochondria and **mitoplasts**. These vesicles are then separated using a continuous sucrose-density gradient in density gradient centrifugation. The outer membrane is separated as low-density band, inner membrane as low-density band while the contact sites as intermediate density band (Fig. 6).

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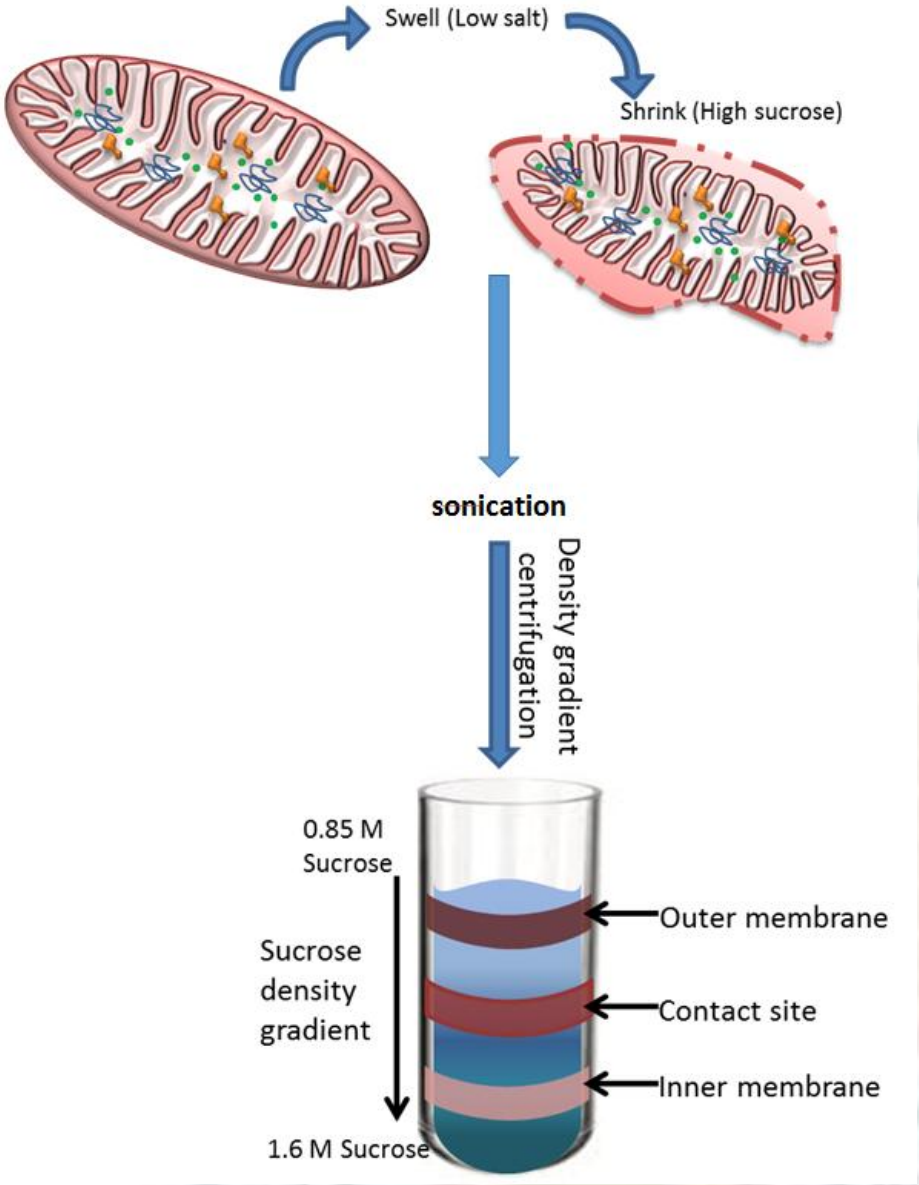

Fig. 6: Submitochondrial fractionation to separate inner and outer membranes and the contact sites. The components are separated by using a continuous sucrose gradient from 0.85-1.6 molar. The outer membrane is separated as low-density band (at 1 M), inner membrane as low-density band (1.36 M) while the contact sites as intermediate density band (1.2 M).

Two states of mitochondria

Based on the metabolic state of the cell, mitochondrion exists in two forms known as **inactive or orthodox state** and **active or condensed state**. Inactive or orthodox state of mitochondria is characterized by large amount of matrix and fewer cristae and is seen when ATP synthesis is low. Active or condensed state is characterized by less matrix and abundant cristae and is seen during high metabolic needs of the cell.

Mitochondria: structure

Origin of mitochondria

The evolutionary origin of mitochondria was described in 1970s by Lynn Margulis in a theory known as **endosymbiotic theory**. This theory suggests the origin of mitochondria from an obligate aerobic prokaryote that was engulfed by an anaerobic eukaryote (Fig. 7). During the course of evolution, many of the genes of the symbiont were either lost or transferred to the nucleus of the host cell.

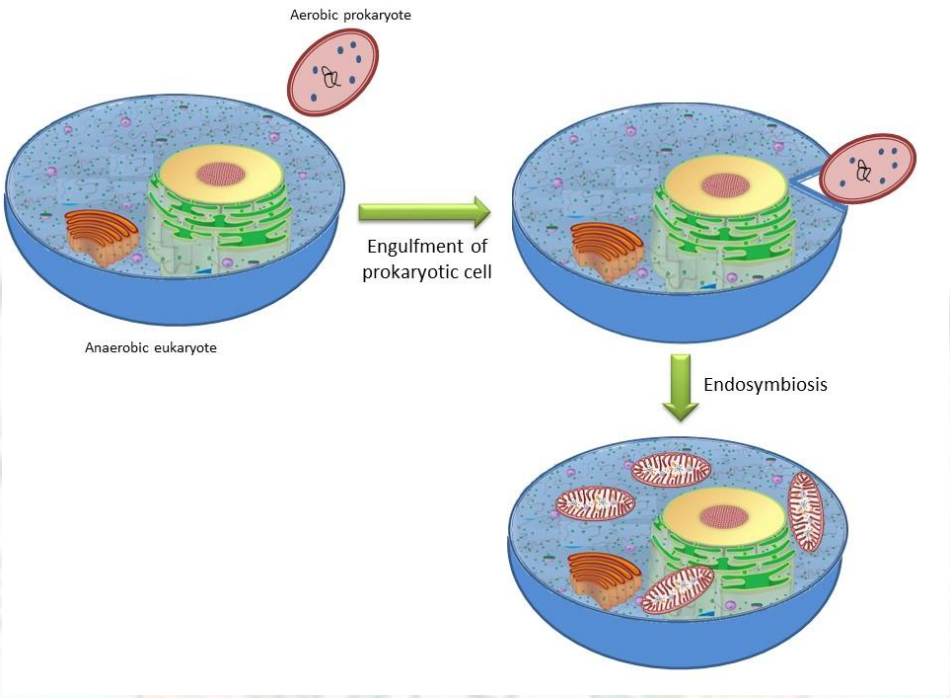

Fig. 7: The endosymbiotic theory suggesting the proposed evolution of mitochondria from an obligate aerobe. **Source: Author**

The DNA sequence analysis has revealed that the prokaryote was probably belonging to *alpha proteobacteria*, more precisely *Rickettsia prowazekii* which is an obligate intracellular parasite. The genome sequencing of *Rickettsia prowazekii* suggested the presence of genes same like mitochondria. ATP production in *Rickettsia* is also found to be same as in mitochondria. The genome of *Rickettsia prowazekii* lacks a number of genes involved in the biosynthesis of amino acids. These are also found to be absent from mitochondria suggesting the evolution of mitochondria from this *Rickettisa*-like proteobacteria.

Dynamic Nature of Mitochondria

Mitochondria are not static organelle. They are rather dynamic, constantly fusing with one another (**Mitochondrial fusion**) or splitting in two (**Mitochondrial fission**). All mitochondria arise from pre-existing mitochondria by fission. The number and length of mitochondria is determined by the balance between fusion and fission. When there are more

fusions as compared to fission the mitochondria appear more elongated while they become more in number and distinct when there are more fissions than fusions.

Mitochondrial Fusion: Two large GTPases, **Mitofusins** and **OPA1** are required for mitochondrial fusion. Two mitofusins have been found to play an important role: Mitofusins 1 and 2 (Mfn1, Mfn2) and share more than 80% sequence similarity and also similar topology. Mfn1 and Mfn2 are found on the outer mitochondrial membrane and have a cytosolic GTPase domain and two C-terminal coiled-coil regions. (Fig. 8) These coiled coil regions are important as they help the Mfn molecules present on the adjacent mitochondria to undergo oligomerization. OPA1 is dynamin-related protein found in the intermembrane space. The co-ordinated actions of both Mitofusins and OPA1 are required for mitochondrial fusion. While mitofusins are crucial for fusion of the outer membranes, OPA1 is essential for inner membrane fusion (Fig. 8).

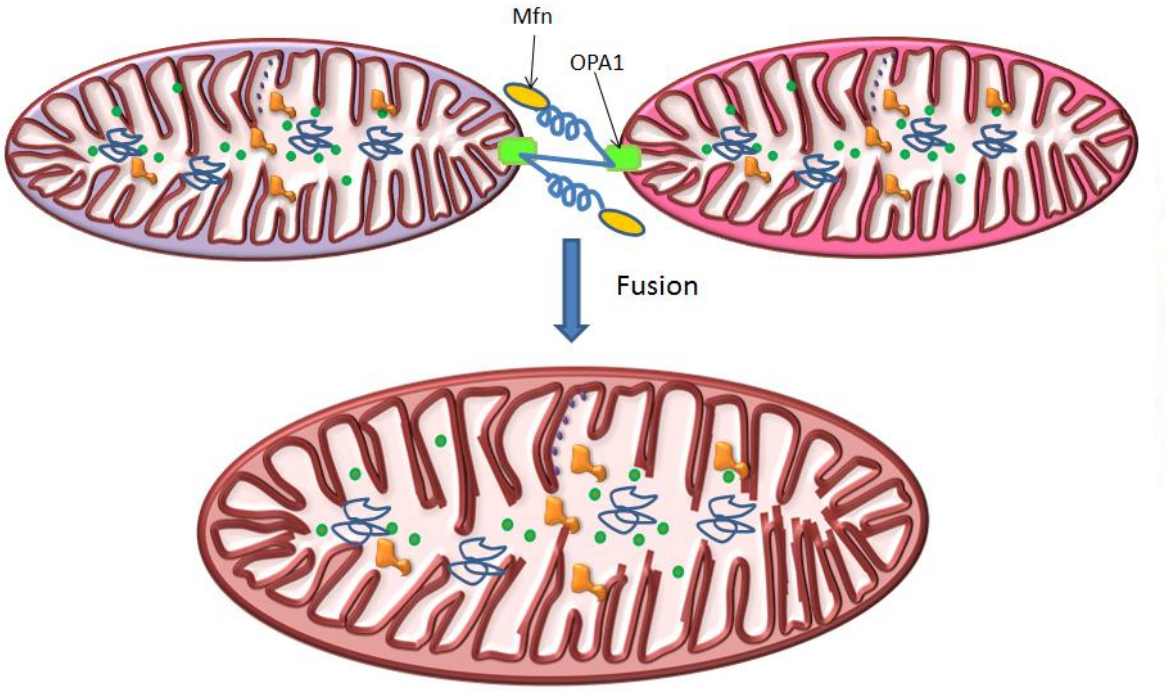

Fig. 8: Mitochondrial Fusion: As shown in the figure two large GTPases, **Mitofusins** and **OPA1** are required for mitochondrial fusion. Mitofusins are required for fusion of the outer membranes, OPA1 is important for inner membrane fusion. **Source: Author**

Mitochondrial Fission: Mitochondrial fission requires **Fission protein 1 (Fis1)** and **dynamin-related protein 1 (Drp1).** Fission protein 1 (Fis1) is found on the mitochondrial outer membrane. Dynamin-related protein (Drp1) is largely localized to the cytosol and contains N-terminal GTPase and C-terminal **GTPase effector domain (GED).**

It has been proposed that interactions between the GTPase and GED regions are required for GTPase function. Drp1 hydrolyses GTP to bring mitochondrial membrane constriction and fission (Fig. 9).

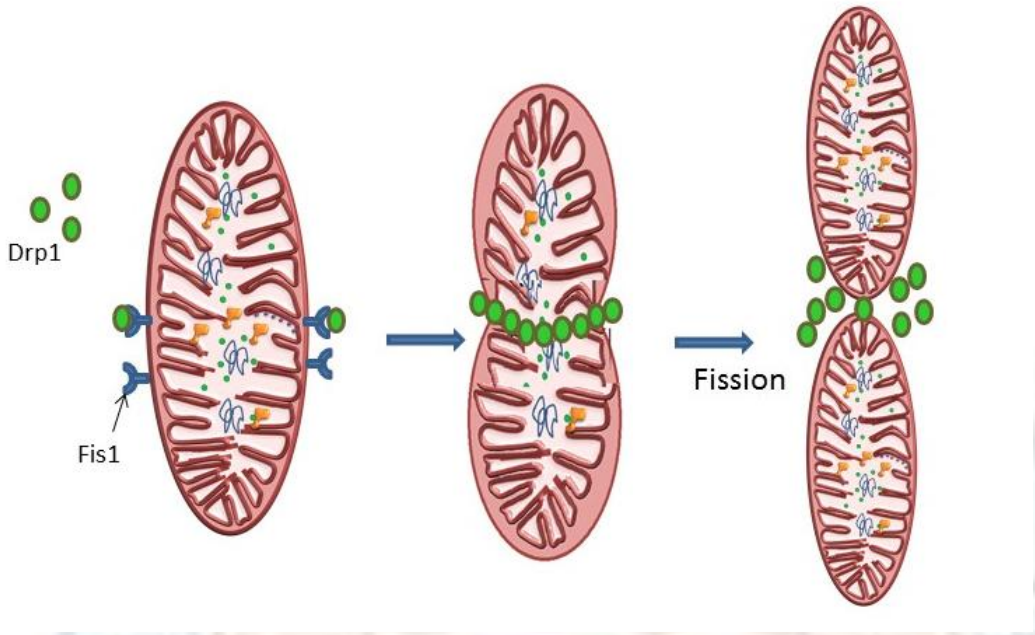

Fig. 9: Mitochondrial Fission: Mitochondrial fission requires Fission protein 1 (Fis1) and dynamin-related protein 1 (Drp1). As shown in the figure Drp1 brings mitochondrial membrane constriction and fission. **Source: Author**

Mitochondrial DNA (mtDNA)

Mitochondria contain double stranded, circular, naked DNA which resembles with the DNA found in prokaryotes. However some organism like ciliated protozoans have linear mitochondrial DNA. A mitochondrion usually has multiple copies of DNA. Vertebrates have 5-10 copies while plants have 20-40 copies per mitochondrion. In humans the size of mtDNA is 16,500 base pair and contains 37 genes (Fig. 10).

Mitochondria: structure

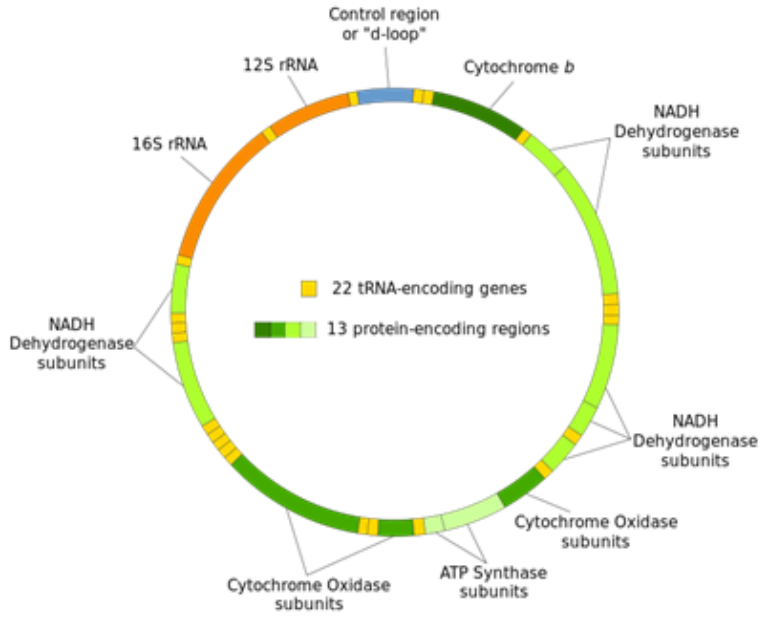

Fig. 10: Human mitochondrial DNA map. **(Adopted from Wikipedia).**

Out of these, the protein encoded by 13 genes are involved in electron transport and oxidative phosphorylation. The remaining genes code for 2 rRNA (12S rRNA and 16S rRNA) and 22 tRNA. The smallest known mtDNA is from *Plasmodium falciparum* (6 Kb) which codes for only 3 proteins. Plants have large mtDNA which is more than 200Kb. *Arabidopsis thaliana* has mtDNA of 367 Kb. However, the large size of plants mtDNA do not correlate with the number of genes. For example human mtDNA with size of 16.5 Kb code for 37 genes while *Arabidopsis thaliana* mtDNA with size of 367 Kb codes for only 58 genes. The size of mitochondrial DNA varies in different organisms (Table 2). On an average, the animals have small mtDNA about 15-16 Kb in size, protists, fungi and algae have intermediate size mtDNA about 20–100 kb in size while plants have larger mtDNA about 200–400 kb in size. Plants mtDNA codes for additional genes not found in animal or fungal mtDNA like genes for ribosomal proteins, subunits of respiratory chain complexes and cytochrome-c-biogenesis.

The organisms with smaller mtDNA like human usually lack introns and have less intergenic spacer while organisms with larger mt DNA have more introns and intergenic spacer region. In vertebrates, the two strands differ in density, designated as Heavy (H) and Light (L) and therefore easily isolated. Most of the genes are encoded by H strand. Mitochondria despite of having their own DNA require proteins synthesized on the cytosolic ribosomes.

Table 2: The size of mitochondrial genome in different organisms.

Organism	**Size (in Kb)**
Plasmodium falciparum	6
Tetrahymena pyriformis	47
Paramecium caudatum	40-44

Yeast: *Hanseniaspora uvarum* *S. cerevisiae*	 11 85
C. elegans	13.7
Drosophila	16-19.5
Xenopus laevis	17.5
Mus musculus	16.3
Canis familiaris (domestic dog)	16.7
Domestic cat	17
Human	16.5
Prototheca wickerhamii (a green algae)	58
Arabidopsis thaliana	367
Pisum sativum	110
Marchantia polymorpha	184

mtDNA is more prone to undergoes mutations because they are not complexed with histone proteins and they have high concentration of reactive oxygen species (ROS) generated because of the metabolic activities. Mutations in mtDNA are responsible of a number of genetic disorders (see section on mitochondrial disorders).

Mitochondrial inheritance

Mitochondria have their own DNA and therefore can pass the genetic information to the next generation. All the mitochondria are inherited from the egg and therefore mitochondrial genes show uniparental inheritance, more precisely maternal inheritance as most of the cytoplasm of zygote is derived from egg. Well studied cases of mitochondrial inheritance include male sterility in higher plants, petite mutants (mutants fail to grow on carbon sources like sucrose) in yeast and poky character (having absormal cytochromes) in yeast. mtDNA analysis has become a powerful tool for analyzing the maternal lineages in humans.

Mitochondrial genetic code

The mitochondria uses slightly different genetic code which is different from the universal genetic code (Table 3). These differences are found in the mtDNA of not only vertebrates but also in invertebrates, yeast and protozoans. For example, the codons AGA and AGG which specify arginine serve as stop codons in vertebrate mitochondria. Human mt DNA codes for only 22 tRNA which are the only tRNA required for translation in the mitochondria. In human U present in the anticodon tRNA can pair with all the four bases present at the third codon position (this is in accordance to wobble hypothesis, but present an extreme case).

Table 3. Table showing differences in the mitochondrial genetic code from standard codons.

	Mitochondrial	Standard
Vertebrates		
AGA	Ter*	Arg
AGG	Ter*	Arg
AUA	Met	Ile
UGA	Trp	Ter
Invertebrates		
AGA	Ser	Arg
AGG	Ser	Arg
AUA	Met	Ile
UGA	Trp	Ter*
Yeast		
AUA	Met	Ile
CUU	Thr	Leu
CUC	Thr	Leu
CUA	Thr	Leu
CUG	Thr	Leu
UGA	Trp	Ter*
CGA	Absent	Arg
CGC	Absent	Arg
Protozoan		
UGA	Trp	Ter*

Mitochondrial transport system

The mitochondria synthesize ATP from ADP and P_i. The ATP produced should move out of the mitochondria to cytosol to drive important biological processes requiring energy. Also, ADP and P_i should move into mitochondria to continue ATP synthesis. The inner mitochondrial membrane contains **ATP-ADP translocator** which exchanges ADP for ATP. The antiport is electrogenic because of differences in the charges of ATP and ADP. This exchange is energetically favorable and is drive by proton motif force (pmf).

P_i is moved into the mitochondria by **phosphate carrier** which is a P_i-H^+ symporter. Mitochondria plays an important role in regulating the Ca^{2+} concentration and therefore it has specific mechanism for influx and efflux of Ca^{2+}. The Ca^{2+} influx is determined by the membrane potential ($\Delta\Psi$). The efflux is driven by antiport which exchange Ca^{2+} for Na^+.

Mitochondrial Diseases

Mitochondria have their own genetic system which resembles prokaryotes. The mtDNA can undergo mutations like nuclear DNA. The offspring inherits mitochondria from egg and therefore follows maternal inheritance. A number of genetic disorders are known caused due to mutations in mtDNA. Some of these diseases are explained below.

1. **Leber's Heredity Optic Neuropathy (LHON):** This disease was first described by German ophthalmologist Theodor Leber. The disease results in blindness due to degeneration of optic nerve. In 1988 the team of Douglas Wallace identified the mutation resulting in this rare genetic disorder. The mutation takes place at 11,778 position causing the substitution of histidine with arginine in NADH dehydrogenase. However, three other mutations are also responsible for this condition: two affecting complex I subunit and one affecting complex III.

2. **Leigh disease (juvenile subacute necrotizing encephalomyelopathy or subacute necrotizing encephalomyelopathy (SNEM):** Named after British neuropsychiatrist Denis Leigh the disease affects central nervous system. This genetic disease has been associated with the mutations in the gene coding for subunits of ATP synthase responsible for ATP production.

3. **Mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS):** A complex genetic disorder affecting brain, nervous system and muscles.

Treatment of Mitochondrial disorders

Currently there is no effective cure for genetic disorders caused by mutations in mtDNA. Genetic counselling and pedigree analysis can help in understanding the risk of these genetic disorders. Researchers are looking for methods that can be employed to prevent the passing of mitochondrial genetic disorders. A newer method like **mitochondrial donation** has gained much popularity. In this method, the nuclear DNA is removed from the egg which contains defective mitochondria and is transferred to the donor's egg (nuclear DNA from donor's egg is removed) containing healthy mitochondria.

Mitochondria, diseases and aging

Mitochondria are important cell organelle responsible for variety of metabolic functions. Mitochondrial dysfunction has been correlated with onset of many diseases like Type II diabetes, neurodegenerative disorders, bipolar disorders and various cancers. It has now become increasingly clear that mutations in mitochondrial DNA has direct correlation with certain cancers like breast, liver, prostate, bladder and pancreatic cancer. Studies have also indicate the link between mitochondria and aging. It has been proposed the accumulation of mutations in mtDNA results in decrease in the metabolic functioning and ATP production resulting in progression of aging.

Similarities between mitochondria and prokaryotes

1. Presence of double stranded, circular and naked DNA
2. Presence of 70S ribosomes

3. Division by fission
4. Inner mitochondrial membrane contains cardiolipin which is also found in the bacterial plasma membranes
5. Folds of membrane (cristae can be considered analogous to mesosomes)

Summary

Mitochondria are important cell organelle first reported by Kolliker (1880) from the flight muscles of insects. The name mitochondria was given by Benda (1897). Mitochondria are found in all eukaryotic cells except mature mammalian RBCs. The mitochondria vary from 0.5 to 10 μm in size and have a variety of shapes like rod, filamentous or granular. The number of mitochondria present in a cell depends on the metabolic state of a cell.

A mitochondrion has a two membranes which are quite different from each other and divide mitochondrion into two distinct compartments: intermembrane space and mitochondrial matrix. Outer membrane is freely permeable and has porin proteins which allow molecules of upto 5000 Da to pass freely. The inner membrane is highly impermeable and is rich in proteins, cardiolipin and lacks cholesterol. The inner membrane has many folds which forms ridges inside known as cristae which bear a number of knob like structures known as F_1 particles or oxysomes. Mitochondrial ATP synthase (F_1F_0-ATPase) consists of a globular head or F_1 and a basal section or F_0. The F_1 has the composition $\alpha_3\beta_3\gamma\delta\varepsilon$, where α and β subunits arranged alternately. The γ subunit connects F_1 with F_0, ε subunit helps in the attachment of γ subunit to F_0. The F_0 has the composition ab_2c_{10-14} and is found inside the inner membrane. The *c* subunit forms a rotating motor and *a* subunit contains the H^+ channel through which H^+ flow back into the matrix from the intermembrane space. Matrix has high concentration of water-soluble proteins including enzymes for Krebs cycle, lipid oxidation and synthesis and mitoribosomes and double stranded, circular, naked DNA. The mitochondria are called semi-autonomous organelle as they have complete machinery for protein synthesis.

The mitochondria are site for many metabolic activities of the cell fatty acid oxidation, Kreb's cycle, electron transport and oxidative phosphorylation. These metabolic processes take place at different compartments of the mitochondria and so the enzymes are distributed accordingly.

Mitochondria can be isolated from tissues using either differential centrifugation involves centrifugation first at low speeds and collection of the supernatant which is subjected to centrifugation with continuously increasing speeds or by density gradient centrifugation where a continuous sucrose-density gradient is created with density being high at the bottom of the tube. Submitochondrial fractionation used to separate the outer and inner mitochondrial membranes involves disrupting mitochondria using sonication in hypotonic solution followed by centrifugation to separate small vesicles. These vesicles are then separated using density gradient centrifugation.

Mitochondria exist in two forms known as inactive or orthodox state and active or condensed state. Inactive or orthodox state of mitochondria is seen when ATP synthesis is low while Active or condensed state is seen during high metabolic needs of the cell.

Mitochondria: structure

Mitochondria is originated by endosymbiosis mitochondria from an obligate aerobic prokaryote that was engulfed by an anaerobic eukaryote. Mitochondria are dynamic organelles constantly undergoing fusion and fission. Mitochondrial Fusion requires two large GTPases, Mitofusins and OPA1 while fission requires Fission protein 1 (Fis1) and dynamin-related protein 1 (Drp1).

In humans the size of mtDNA is 16,500 base pair and contains 37 genes in total. Animals have small mtDNA about 15-16 Kb in size, protists, fungi and algae have intermediate size mtDNA about 20–100 kb in size while plants have larger mtDNA about 200–400 kb in size. The mitochondria uses slightly different genetic code which is different from the universal genetic code. For example, the codons AGA and AGG which specify arginine serve as stop codons in vertebrate mitochondria. All the mitochondria are inherited from the egg and therefore mitochondrial genes show maternal inheritance as most of the cytoplasm of zygote is derived from egg.

Mitochondria has transporters located on the inner mitochondrial membrane for exchange of ATP and ADP (ATP-ADP translocator) and for P_i import (phosphate carrier). Mitochondria have their own genetic system still they are dependent on nuclear DNA for about 99% of their 1000 proteins. The mitochondrial proteins are synthesized at the cytosolic ribosomes and are then imported into the mitochondria.

Exercise/ Practice

A. Multiple choice questions:

1. Mitochondria was discovered by

   (a) Benda (b) Altmann (c) Flemming (d) Kolliker
2. The dye used for staining of Mitochondria is

   (a) Schiff's reagent (b) Acetocarmine (c) Janus Green (d) Fast Green
3. Which of these is not the characteristic feature of inner mitochondrial membrane?

   (a) Cristae (b) proteins rich (c) cardiolipin (d) cholesterol
4. Oxysomes are found in

   (a) Matrix (b) cristae (c) outer membrane (d) intermembrane space
5. Which of the following protein(s) are required for mitochondrial fission

   (a) Tiny tims (b) Drp1 (c) mitofusins (d) Hsp70

B. Fill in the blanks:

1. The number of mitochondria present in a cell depends on _____
2. The infoldings of inner membrane which enhance the surface area for cellular respiration are known as _____
3. The characteristic lipid found in the inner mitochondrial membrane is

Mitochondria: structure

4. The ribosomes present inside mitochondrial matrix are of _____ type and are known as _____
5. The state of mitochondria characterized by large amount of matrix and fewer cristae is known as _____
6. The state of mitochondria characterized by less matrix and abundant cristae is known as _____
7. Mitochondrial fusion requires _____
8. Mitochondrial fission requires _____
9. The size of mitochondrial DNA in humans is of size _____ and contains _____
10. Mitochondria are thought to have evolved from a proteobacteria like_____

C. True/False

1. Mitochondria are more in actively growing and dividing cells.
2. Mitochondria are absent from plant cell.
3. The outer mitochondrial membrane contains more proteins as compared to inner membrane.
4. The inner mitochondrial membrane is folded into cristae.
5. Mitochondria resemble bacteria in their organization.
6. Mitochondria are thought to evolve from *Rickettisa*-like proteobacteria due to similarity in DNA.
7. Mitochondria are static organelles.
8. Plants have larger mitochondria than other organisms.
9. Succinate dehydrogenase is a marker enzyme present in the matrix.
10. The F_1 has the composition $ab_2c_{10-14.}$

D. Expand the following

1. Mfn
2. Fis1
3. Drp
4. mtDNA
5. GED

E. Match the scientist with their contribution

Mitochondria: structure

A	B
1. Kolliker	a) citric acid cycle,
2. Michaelis	b) description of the cytochromes
3. Meves	c) discovered Mitochondria in flight muscle of the insect
4. Warburg	d) Mitochondrial DNA
5. Palade and Sjostrand	e) Reconstitution of the respiratory chain
6. Fernandes-Moran	f) Mitochondria synthesize RNA
7. Lehninger	g) The first electron micrographs of mitochondria
8. Recker	h) First isolation of mitochondria from liver of guinea pigs
9. Bensley and Hoerr	i) ATPase activity in F1 particles
10. Nass and Nass	j) presence of Kreb's cycle enzymes in mitochondrial matrix
11. Wintersberger and Tuppy	k) F_1 particles,
12. Kiintzel and Noll	l) Ultrastructure of mitochondria
13. Keilin and King	m) vital stain Janus Green to stain mitochondria,
14. Keilin	n) Mitoribosomes
15. Krebs	o) presence of mitochondria in plant cell
16. Claude and Fullam	p) the presence of respiratory enzymes in mitochondria

Glossary

ATP-ADP translocator: antiporter present on the inner mitochondrial membrane, exchanges ADP for ATP

Chondriome: collection of all mitochondria of a cell

Condensed state: metabolically active state of mitochondria, characterized by less matrix and abundant cristae

Cristae: Infolding of inner mitochondrial membrane, enhance the surface area for cellular respiration

Endosymbiotic theory: A theory suggesting the origin of mitochondria and chloroplasts

Mitochondrial donation: involves the removal of nuclear DNA from the egg containing defective mitochondria and is transferred to the donor's egg containing healthy mitochondria

Mitofusins: large GTPases required for mitochondrial fusion

Mitoplasts: mitochondria without outer membrane

Mitoribosomes: 70S ribosomes present in mitochondria

Mitochondria: structure

Orthodox state: metabolically inactive state of mitochondria, characterized by large amount of matrix and fewer cristae

Porins: proteins which form channels on the outer mitochondrial membrane, allow molecules of upto 1000 Da to pass freely

Sarcosomes: Mitochondria of muscle cells

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Answers

1. (d) Kolliker
2. (c) Janus Green
3. (d) cholesterol
4. (b) cristae
5. (b) Drp1

Mitochondria: structure

B. Fill in the blanks:

1. metabolic state of a cell
2. cristae
3. cardiolipin
4. 70S, mitoribosomes
5. orthodox state
6. condensed state
7. Mitofusins and OPA1
8. Fission protein 1 (Fis1) and dynamin-related protein 1 (Drp1)
9. 16,500 base pair, 37 genes
10. *Rickettsia prowazekii*

C. True/False

1. True
2. False; Mitochondria are also found in plant cells
3. False; the outer membrane has less proteins as compared to inner membrane
4. True
5. True
6. True
7. False; Mitochondria are dynamic organelles undergoing constant fission and fusion
8. True
9. False; it is present in the inner membrane
10. False; F_1 has the composition $\alpha_3\beta_3\gamma\delta\varepsilon$,

D. Expand the following

1. Mitofusins
2. Fission protein 1
3. dynamin-related protein
4. Mitochondrial DNA
5. GTPase effector domain

E. Match the scientist with their contribution

1. c) discovered Mitochondria in flight muscle of the insect
2. m) vital stain Janus Green to stain mitochondria
3. o) presence of mitochondria in plant cell
4. p) the presence of respiratory enzymes in mitochondria
5. l) Ultrastructure of mitochondria
6. k) F_1 particles
7. j) presence of Kreb's cycle enzymes in mitochondrial matrix
8. i) ATPase activity in F1 particles

Mitochondria: structure

9. h) isolation of mitochondria from liver of guinea pigs
10. d) Mitochondrial DNA
11. f) Mitochondria synthesize RNA
12. n) Mitoribosomes
13. e) Reconstitution of the respiratory chain
14. b) description of the cytochromes,
15. a) citric acid cycle,
16. g) The first electron micrographs of mitochondria,