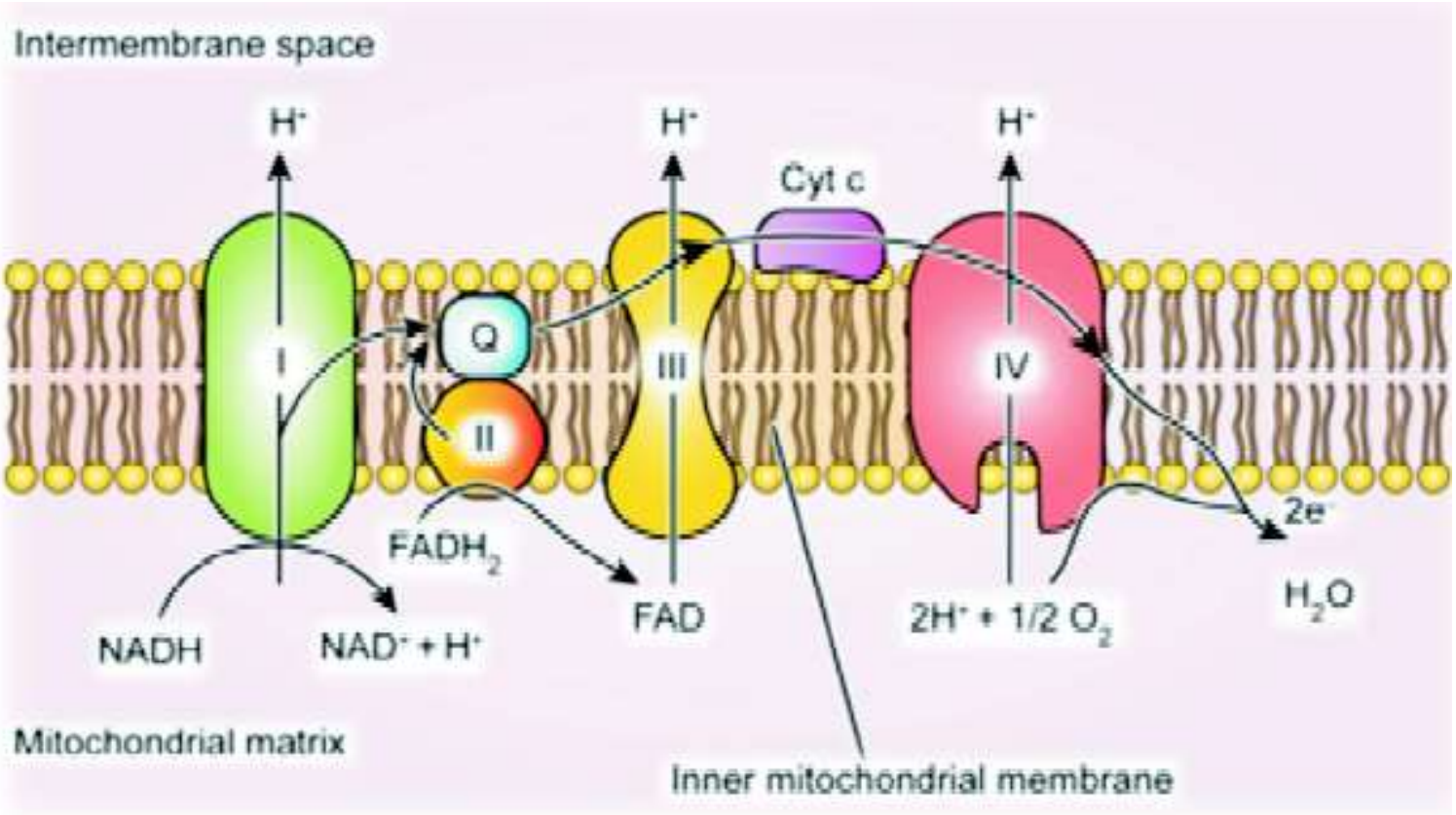
MITOCHONDRIAL RESPIRATORY CHAIN



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Since mitochondria are the site of oxidative phosphorylation, a good understanding of the structural organization of mitochondria is required for appreciation of oxidative phosphorylation. You have already been provided some details of mitochondria while studying TCA cycle in Unit 3 of BBCCT-109. Mitochondria are present in all aerobic eukaryotic cells, and they are the site of the tricarboxylic acid cycle reactions, electron transport and oxidative phosphorylation. They are often localized near structures that require ATP, which is the major energy curency in biological sysems. For example, in the flight muscles of some insects, mitochondria are regularly arranged along the myofibrils. The ATP molecules formed by these mitochondria thus need to diffuse only to a short distance to the ATP-requiring contractile elements. Mitochondria are also frequently located adjacent to cytoplasmic fat droplets, which serve as a fuel source for synthesis of biochemically relevant form of energy, ATP. Let us now discuss the structural organization of mitochondria.

Structure and Function of Mitochondria

The most intensively studied mitochondria are those present in the liver cells. Under electron microscope, they appear as structures which are about 2 mm in length and less than 1 mm in width. Their size remains about the same in bacterial cells as well.

Mitochondria are a double membrane structure. As shown in Fig. 9.1, the outer membrane is smooth, while the inner membrane is invaginated to form folds referred to as the **cristae.** This increases the surface area of the inner membrane without a proportionate increase in the overall size of mitochondria. The internal mitochondrial matrix is proteinaceous and rich in enzymes.

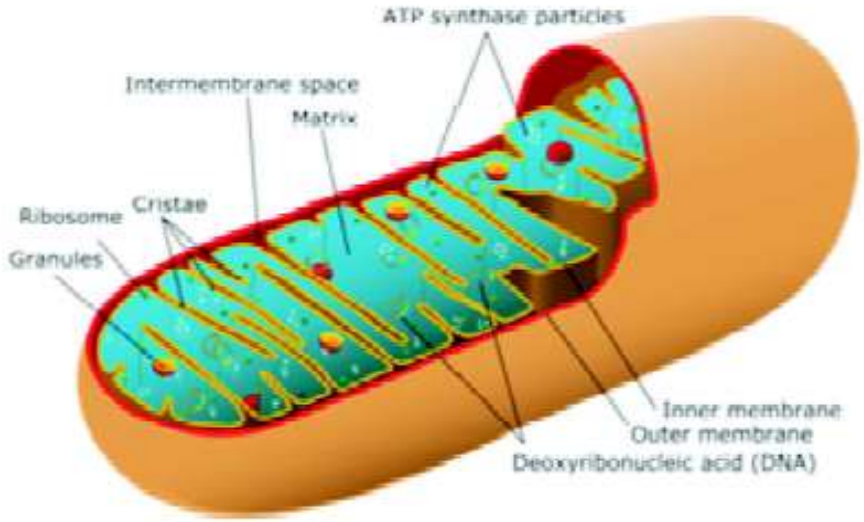

Fig. 9.1: Schematic representation of the structure of mitochondria.

The two membranes of mitochondria differ in composition and biochemical function. The outer mitochondrial membrane is permeable to many solutes but the inner mitochondrial membrane is not. The inner mitochondrial membrane contains components of the electron transport chain and oxidative phosphorylation. It also contains components of shuttle systems that can transfer electrons from cytosolic NADH to the mitochondrial electron transport chain, or from mitochondrial NADH to the cytosol. Hence, the rate of glycolysis in the cytoplasm and respiration in the mitochondria are integrated by the concentration of ATP, ADP and phosphate in the cytosol and the mitochondria. Enzymes of TCA cycle and fatty acid oxidation are present in the mitochondrial matrix. Table 9.1 lists some of the important mitochondrial enzymes and their locations.

Table 9.1: Location of certain enzymes in mitochondria.

Outer membrane	Inner membrane
Monoamine oxidase	NADH dehydrogenase (antimycin sensitive)
Kynurenine 3-monooxygenase	Iron sulfur proteins
NADH dehydrogenase (antimycin-insensitive)	Cytochromes b, c, c_1 and aa_3
Acetyl-CoA synthetase	F1 ATPase
Phospholipase A_2	Succinate dehydrogenase
Nucleoside diphosphate kinase	Carnitine acyltransferase
Matrix	
Citrate synthase	
Isocitrate dehydrogenase	
Malate dehydrogenase	
Fumarase	
Aspartate transaminase	
Glutamate dehydrogenase	
Fatty acyl-CoA oxidation enzymes	
Intermembrane space: Adenylate kinase	

Electron Transport Chain: Organization and Function

Mitochondrial electron transport chain is also referred to as the **respiratory chain**. It consists of four complexes namely, **NADH-coenzyme Q reductase (Complex I), Succinate-Coenzyme Q reductase (Complex II), Coenzyme Q-Cytochrome *c* reductase (Complex III), and Cytochrome *c* oxidase (Complex IV)**. The sequential organization of these complexes of respiratory chain is depicted in Fig. 9.2. *Transfer of electrons from NADH or $FADH_2$ to O_2 by a series of electron carriers* generates 26 of the 30 molecules of ATP that are formed when a glucose molecule is completely oxidized to CO_2 and H_2O. In this section we will discuss the organization and function of mitochondrial electron transport chain.

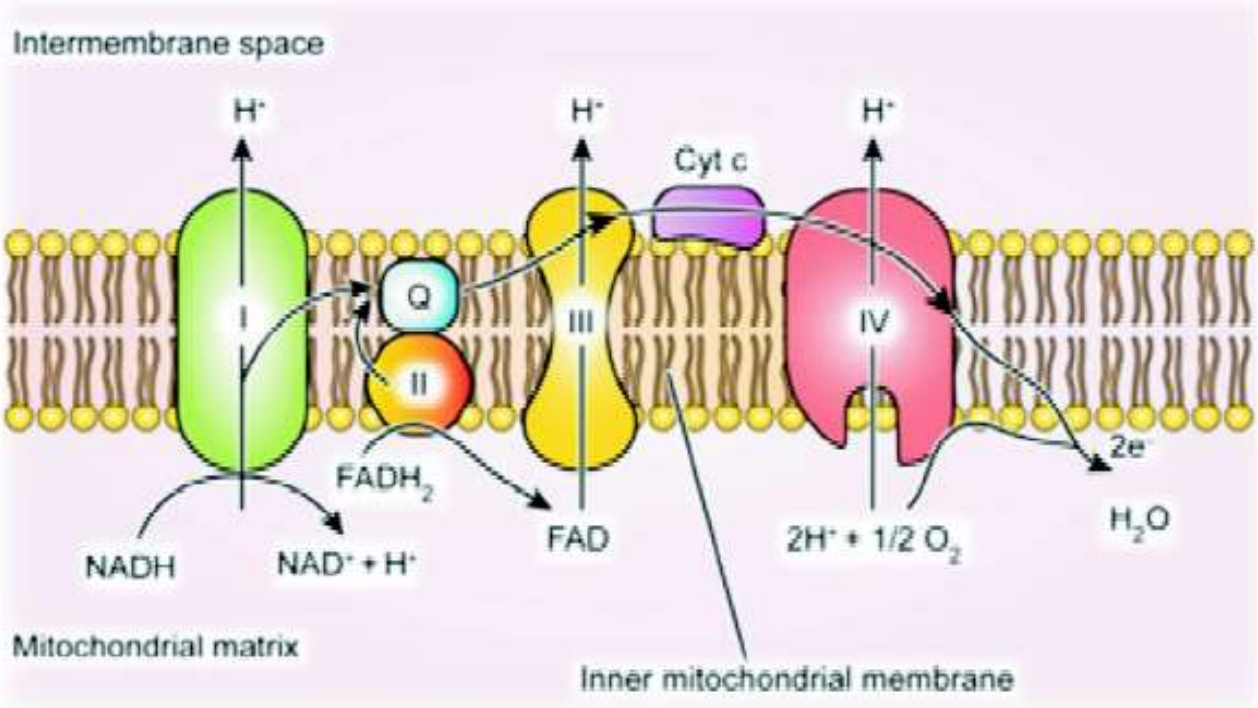

Fig. 9.2: Electron transport complexes of the inner mitochondrial membrane.

The mitochondrial electron transport chain utilizes NADH and $FADH_2$ which are energy-rich molecules generated during metabolic reactions including glycolysis, fatty acid oxidation, and citric acid cycle. These energy-rich molecules have electrons with high transfer potential which are carried through the electron transport complexes to O_2. This transfer of electrons is also accompanied by pumping of protons from the mitochondrial matrix into the intermembrane space. This proton gradient across the inner mitochondrial membrane generates a proton motive force, which is utilized to drive the endergonic reaction of phosphorylation of ADP to ATP.

Isolated mitochondria have also been demonstrated to carry out electron transport under *in vitro* conditions. The inner mitochondrial membrane can be resolved into major electron transport complexes. This process may be undertaken in laboratory, and it involves mechanical treatment such as sonication, selective solubilization using biological detergent such as digitonin, and separation methods such as centrifugation and chromatography. Four major electron transport complexes have been isolated from inner mitochondrial membranes which are shown in Fig. 9.2. These complexes, in a way, function as multienzyme systems and together transport electrons from NADH to O_2. Now let us study the function of each mitochondrial electron transport complex in detail.

Respiratory Complex I

It is the first complex of mitochondrial electron transport chain which is also known as the **NADH-coenzyme Q reductase** complex. It catalyzes the first step of electron transport from NADH to CoQ. Complex I is an integral part of the inner mitochondrial membrane. It contains a molecule of flavin mononucleotide (FMN) and several (six to seven) iron-sulfur clusters which participate in the electron transfer. This complex is the largest of all the mitochondrial electron transport complexes.

The electron transport reaction occurs in several steps with successive oxidation and reduction reactions. It begins with the transfer of electrons from NADH to flavoprotein. In the next step, the reduced flavoprotein transfers its electron to reduce the iron-sulfur protein while it gets itself reoxidized. In the third step, the reduced iron-sulfur protein then donates its electrons to coenzyme Q, which in turn gets protonated to $CoQH_2$. **Coenzyme Q is also called ubiquinone**. Transfer of two electrons from NADH to CoQ results in transport of four protons from mitochondrial matrix to the intermembrane space (Fig. 9.3).

$$NADH + H^+ + CoQ \rightarrow NAD^+ + CoQH_2$$

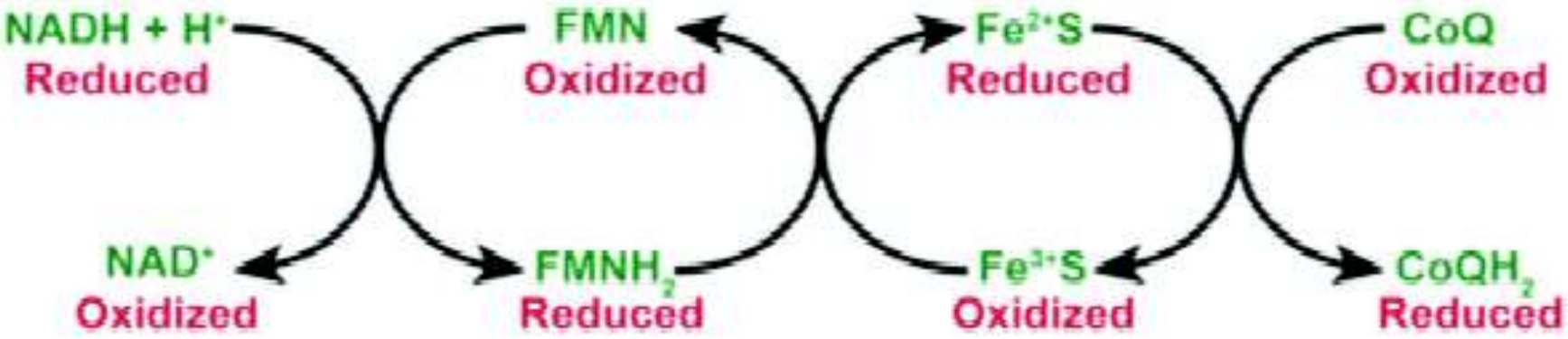

Fig. 9.3: Transfer of electrons from NADH to CoQ.

It may be noted that NADH can only participate in two-electron transfer, while both FMN and CoQ can accept and donate either one or two electrons. On the other hand, the cytochromes present in the Complex III, which receive electrons from reduced CoQ, can only undergo one-electron reductions. Therefore, FMN and CoQ mediate electron transfer between the two-electron donor NADH and the one-electron acceptors, the cytochromes. CoQ is a mobile electron carrier as it has a hydrophobic tail which allows it to be present and be mobile within the lipid bilayer of inner mitochondrial membrane.

Respiratory Complex II

It is also known as **Succinate-CoQ reductase**. It is the second of four membrane-bound complexes. It contains the dimeric citric acid cycle enzyme succinate dehydrogenase and three other small hydrophobic subunits. This complex transfers electrons from succinate to coenzyme Q. The electron transfer in this complex is facilitated by a covalently bound FAD, three Fe-S clusters and one cytochrome b_{560}. The substrate succinate (from the citric acid cycle) is oxidized to fumarate by a flavin enzyme succinate dehydrogenase, and electrons are transferred to Fe-S clusters, and then to CoQ. **No proton pumping across membrane is associated with this complex ie no protons are pumped during this reaction.**

The overall reaction is

Succinate + CoQ → Fumerate + $CoQH_2$

Respiratory Complex III

This complex is also called the **CoQ-Cytochrome c reductase** complex. It is the third complex which is an integral part of the inner mitochondrial membrane. Here the electrons received from reduced coenzyme Q are passed on to cytochrome *c* in a multistep process. This complex contains two *b* type cytochromes, one cytochrome c_1, and one Fe-S cluster.

Cytochromes are redox active proteins found in almost all organisms. They contain heme groups that reversibly alternate between Fe (II) and Fe (III) oxidation states during electron transport. The reduced cytochrome, which has Fe (II) oxidation state, exhibits prominent absorption spectra having three distinct peaks, α, β and γ (Soret bands). The wavelength of the α-peak varies and it is used for differentiating cytochromes. The α-peak is absent in oxidized cytochromes which have Fe (III) oxidation state. **Mitochondrial membranes are known to have three types of cytochromes, cytochromes *a*, cytochrome *b* and cytochrome *c*.** Slight differences may exist around the heme groups within each type of cytochrome. Therefore, each type of cytochrome may further be divided into several other subtypes.

The complex III is located asymmetrically within the inner mitochondrial membrane. Both cytochrome c_1 and the non-heme iron-sulfur (Riske) protein are located towards the outer surface, whereas cytochrome *b* spans through the membrane.

Cytochrome *c* is a peripheral membrane protein which is loosely bound to the outer surface of the inner mitochondrial membrane. It functions to shuttle electrons from cytochrome c_1 of Complex III and cytochrome *c* oxidase (Complex IV) by alternately binding them.

The overall reaction that takes place within this complex is

$CoQH_2$ + 2 Cyt *c* [Fe (III)] → CoQ + 2Cyt *c* [Fe (II)] + $2H^+$

As we have seen, coenzyme Q carried two electrons, whereas the reduction of Fe (III) to Fe (II) requires only one electron. Consequently, two molecules of cytochrome c are required for every molecule of coenzyme Q.

Respiratory Complex IV

This complex is also called the **cytochrome *c* oxidase** complex. It catalyzes the one-electron oxidation of four consecutive reduced cytochrome *c* molecules, and the concomitant four-electron reduction of one molecule of O_2. This is the final steps of mitochondrial electron transport.

The overall reaction is

$$4\text{Cyt } c \text{ [Fe (II)]} + 4H^+ + O_2 \rightarrow 4\text{Cyt } c \text{ [Fe (III)]} + 2H_2O$$

Like the other respiratory complexes, cytochrome oxidase is also an integral part of the inner mitochondrial membrane and contains cytochromes *a* and a_3, as well as two Cu ions (Cu_A and Cu_B) that are involved in the electron transport process. The reduction of O_2 to 2 H_2O by cytochrome *c* oxidase takes place on the cytochrome a_3-Cu_B binuclear complex which involves four consecutive one-electron transfers from Cu_A and cytochrome *a* sites. Proton pumping across the inner mitochondrial membrane also takes place as a result of this reaction.

The overall flow of electrons through the four complexes of mitochondrial electron transport chain is summarized in (Fig. 9.4).

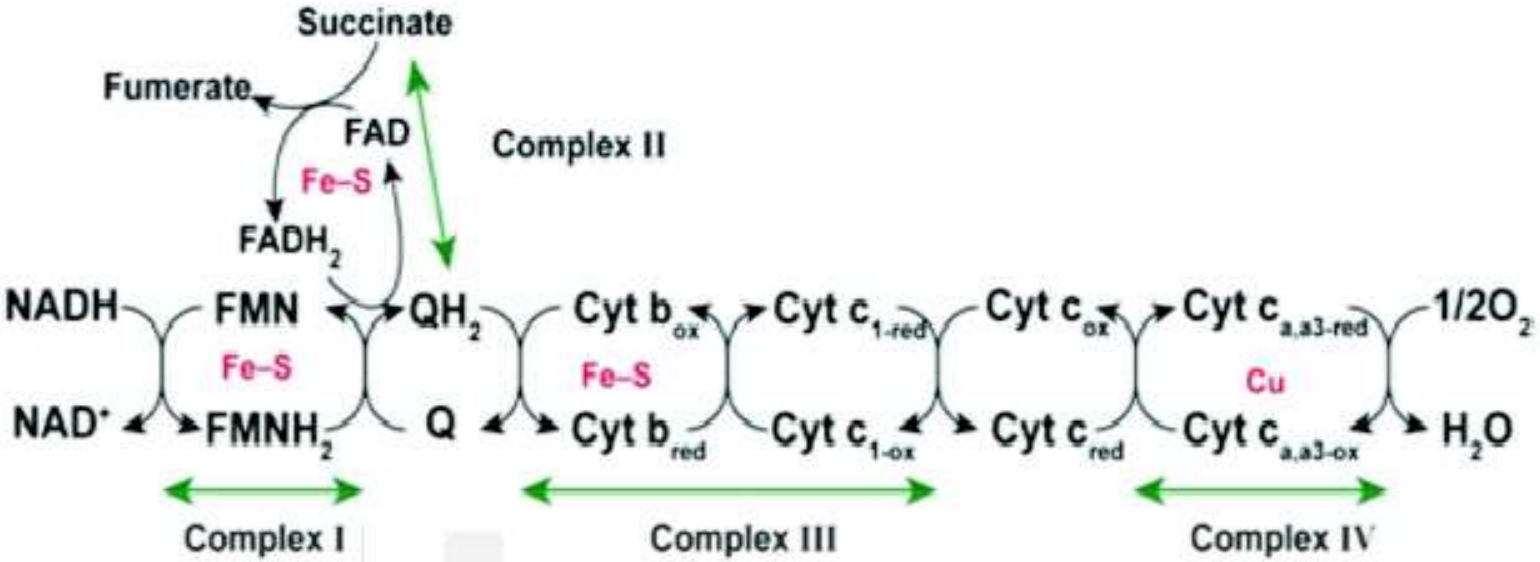

Fig 9.4: Flow of reducing equivalents through respiratory complexes (electron transport chain) of mitochondria.

Here you must take a note that the mitochondrial electron transport chain is associated with vectorial proton pumping across the inner mitochondrial membrane which generates a proton gradient. The energy stored in the form of proton gradient is dissipated in an orderly manner to drive the phosphorylation of ADP to produce ATP. This is known as oxidative phosphorylation. The two processes electron transport and oxidative phosphorylation are coupled together which will be discussed in the coming sections.

F_0F_1–ATP SYNTHASE COMPLEX

The energy stored in the form of proton motive force is conserved by driving the endergonic reaction of ATP synthesis from ADP and Pi. Fig. 9.5 is a schematic diagram to depict the relationship between generation of H^+ gradient across the inner mitochondrial membrane, and its dissipation to drive phosphorylation of ADP to produce ATP. This complex is also called the **coupling factor**, since it couples the proton motive force to the process of ATP synthesis.

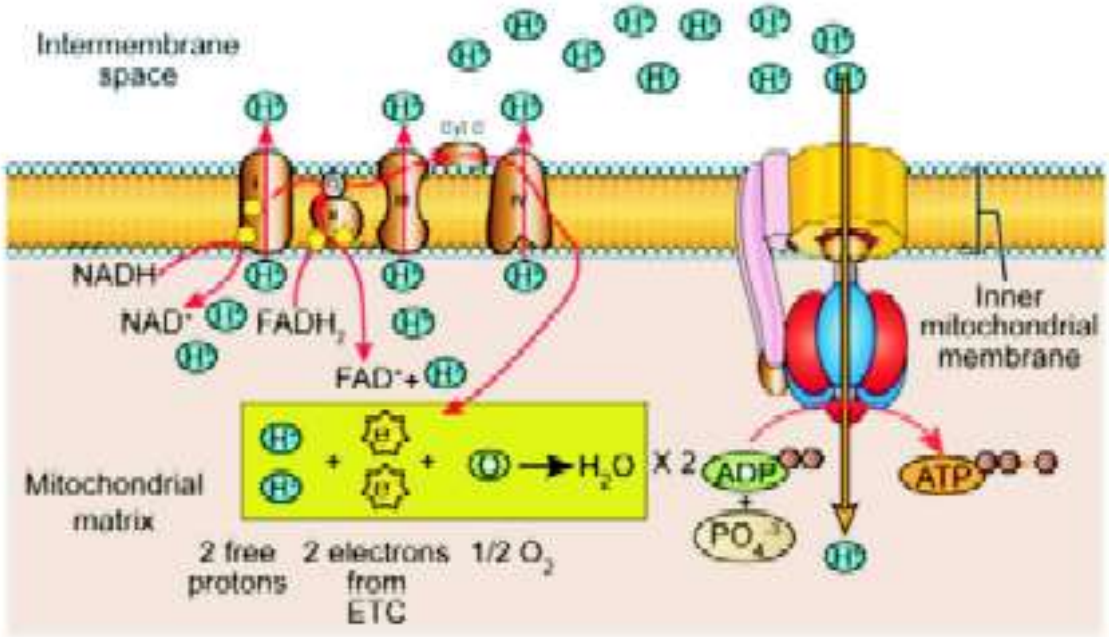

Fig. 9.5: A Schematic diagram showing connection between mitochondrial electron transport and phosphorylation of ADP to give ATP.

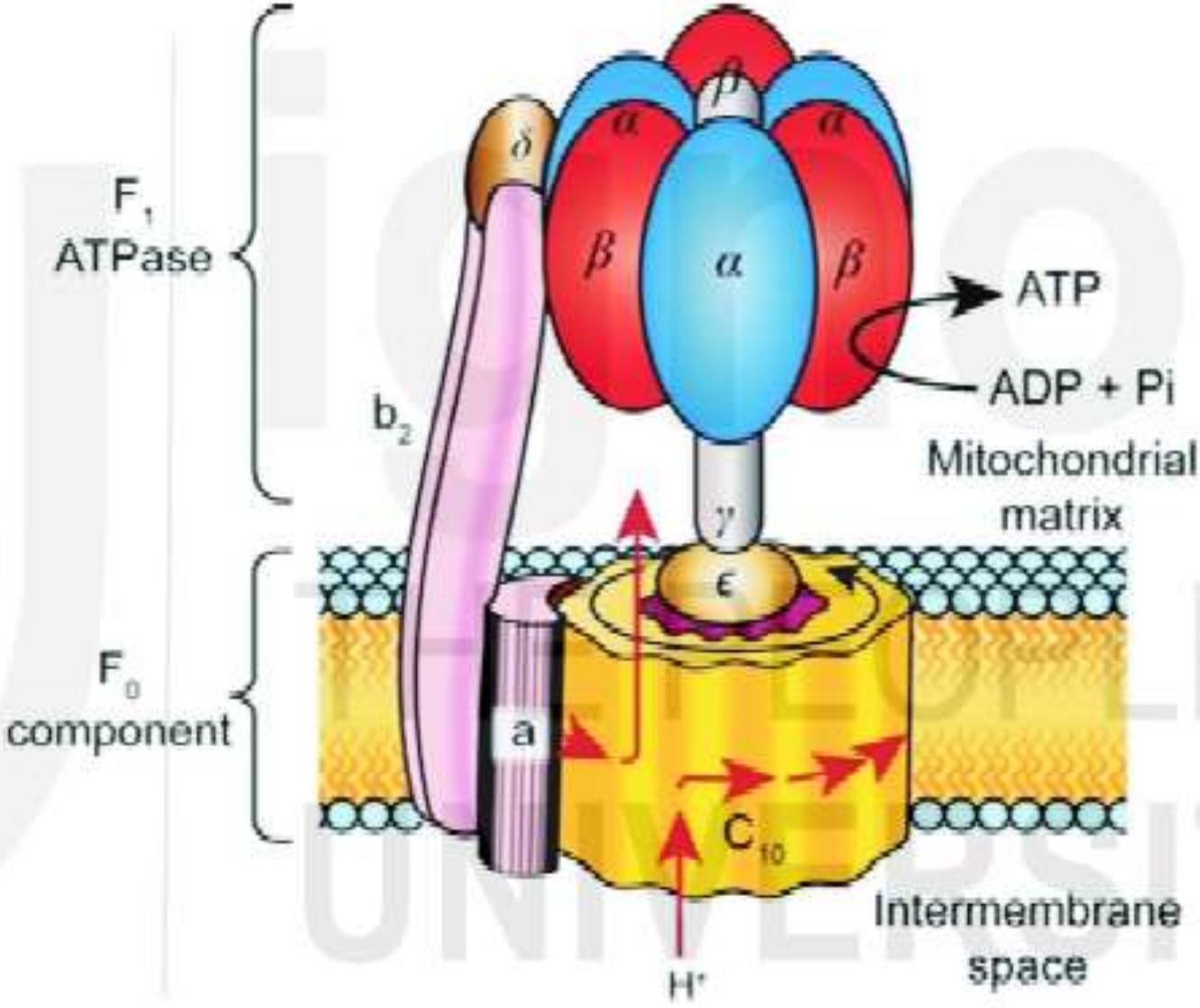

Fig. 9.6: Structure of the F_0F_1 ATP Synthase complex of inner mitochondrial membrane.

The F_0F_1-ATP synthase complex is constituted by two structurally distinct units, each of which has several subunits. The F_0 unit is located within the inner mitochondrial membrane and forms a transmembrane channel which allows movement of protons along the concentration gradient. The role of F_0 unit is in dissipation of the energy stored in the form of H^+ gradient across the inner mitochondrial membrane. This energy is utilized by the F_1 unit for ATP synthesis.

The F_0 unit consists of three hydrophobic subunits named a, b and c. The subunit stoichiometry is represented by $\mathbf{a_1b_2c_{10\text{-}15}}$, which means a single F_0 unit has 1 molecule of the **a** subunit, 2 molecules of the **b** subunits, and 10 to 15 molecules of the **c** subunit. The F_1 unit of the F_0F_1-ATP synthase complex is spherical in shape. It is linked to the F_0 unit and protrudes out from the inner mitochondrial membrane into the mitochondrial matrix. The F_1 unit consists of five different protein subunits called α, β, γ, δ, and ε. The subunit stoichiometry is represented as $\boldsymbol{\alpha_3\beta_3\gamma\delta\varepsilon}$ (Fig. 9.6).