VIVEKANANDA COLLEGE
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NAAC ACCREDITED ‘A’ GRADE

Topic:	RESPIRATION
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Chemiosmosis

The chemiosmotic hypothesis proposed by Peter Mitchell states: "The differential of electrochemical activity of the hydrogen and hydroxyl ions across the membrane generated by electron transport causes the specific translocation of hydroxyl and hydrogen ions from the active centre of the so called ATPase system thus effectively dehydrating ADP+P". This pioneered the research on the coupling of the ETC and ATP synthesis.

In chemiosmosis, the free energy from the series of redox reactions of ETC, is used to pump hydrogen ions (protons) across the membrane.

The uneven distribution of H^+ ions across the membrane establishes both concentration and electrical gradients (thus, an **electrochemical gradient**), owing to the hydrogen ions' positive charge and their aggregation on one side of the membrane.

If the membrane were open to diffusion by the hydrogen ions, the ions would tend to **diffuse back across into the matrix, driven by their electrochemical gradient**. Recall that many ions cannot diffuse through the nonpolar regions of phospholipid membranes without the aid of ion channels. Similarly, hydrogen ions in the matrix space can only pass through the inner mitochondrial membrane through an **integral membrane** protein called **ATP synthase** (Figure2).

This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient. The turning of parts of this molecular machine facilitates the addition of a phosphate to ADP, forming ATP, using the potential energy of the hydrogen ion gradient.

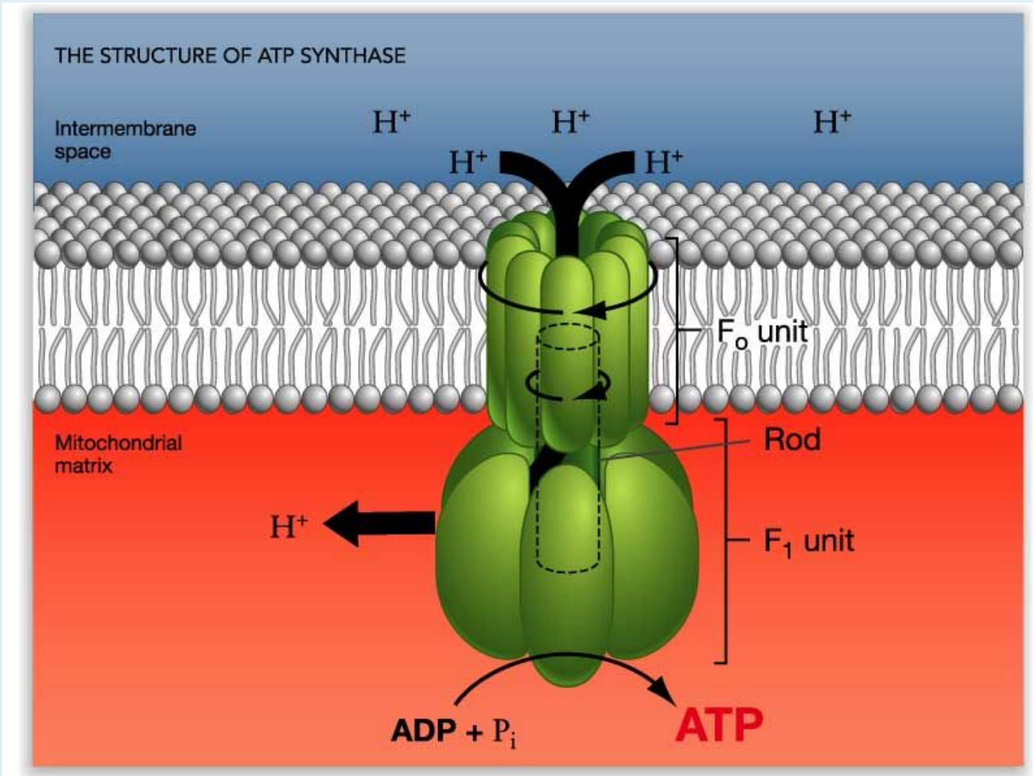


Figure 2. ATP synthase is a complex, molecular machine that uses a proton (H+) gradient to form ATP from ADP and inorganic phosphate (Pi). Dinitrophenol (DNP) is an uncoupler that makes the inner mitochondrial membrane leaky to protons.

Chemiosmosis (Figure 3) is used to generate 90 percent of the ATP made during aerobic glucose catabolism; it is also the method used in the light reactions of photosynthesis to harness the energy of sunlight in the process of photophosphorylation. Recall that the **production of ATP using the process of chemiosmosis in mitochondria is called oxidative phosphorylation**. The overall result of these reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These atoms were originally part of a glucose molecule. **At the end of the pathway, the electrons are used to reduce an oxygen molecule to oxygen ions.** The extra electrons on the oxygen attract hydrogen ions (protons) from the surrounding medium, **and water is formed.**

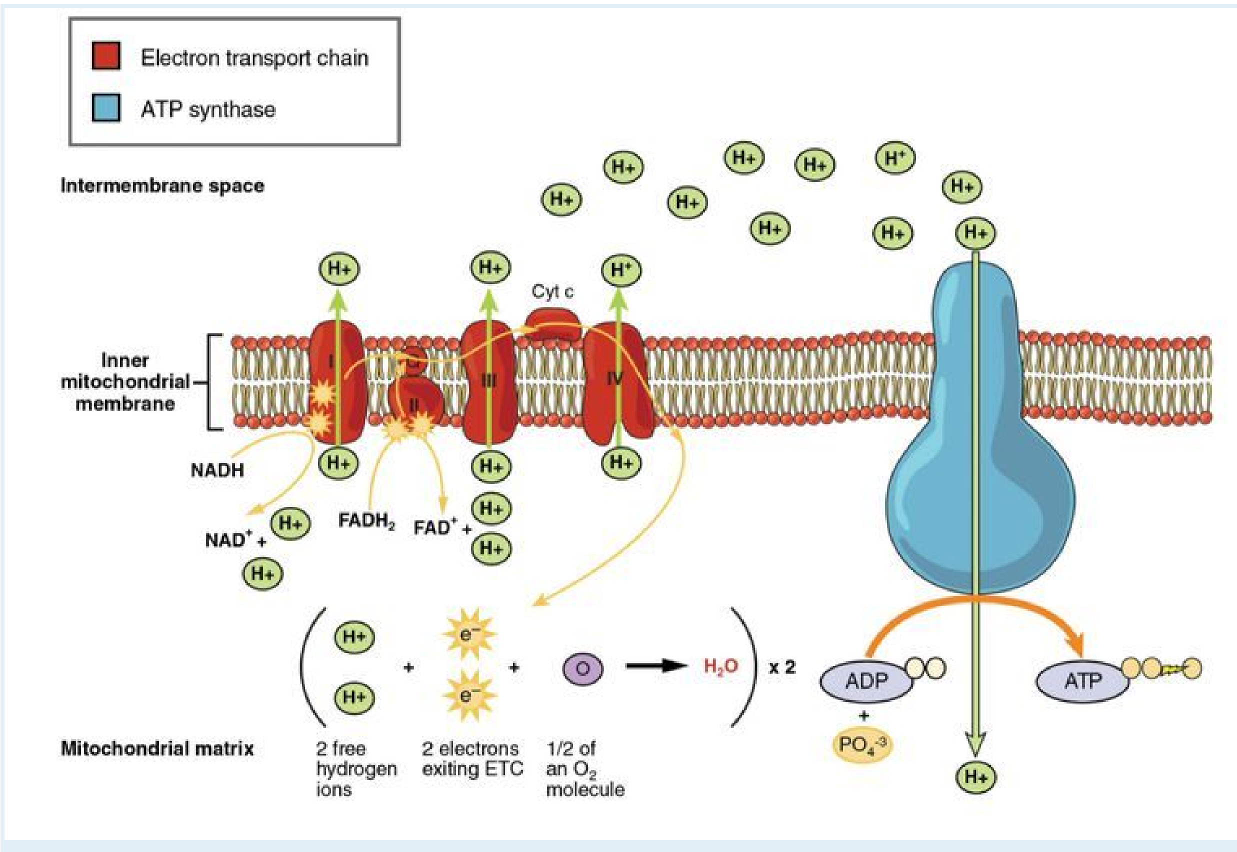
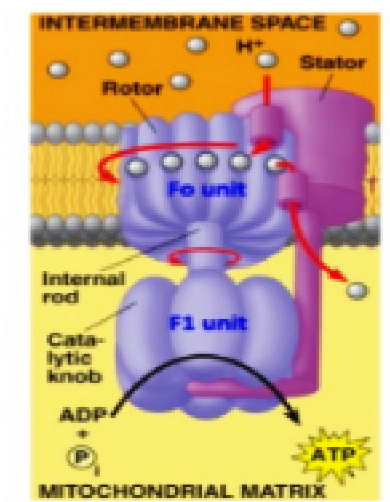


Figure 3. In oxidative phosphorylation, the pH gradient formed by the electron transport chain is used by ATP synthase to form ATP.

Oxidative phosphorylation (overall reaction) : $24H^{+} + 6O_2 + 34ADP + 34Pi \rightarrow 12\ H_2O_2 + 34\ ATP$

Structural Assembly of F1 - F0 ATP Synthase

- The ATP synthase (fig A) has two major subunits designated F_0 and F_1.
- The F_0 part, bound to the inner mitochondrial membrane is involved in proton translocation, whereas the F_1 part found in the mitochondrial matrix is the water soluble catalytic domain.
- F1 is involved in oxidative phosphorylation. It was named so from the term 'Fraction 1'.
- F_0 was named so as it is a factor that conferred oligomycin sensitivity to soluble F_1 .



figA:structure of ATPase

- The structure of enzyme ATP synthase mimics an assembly of **two motors** with a shared common **rotor shaft** and stabilized by a **peripheral stator stalk**.

- The F1 part of ATP synthase is made up of 8 subunits, 3α, 3β, γ, δ and ε, where the γ, δ and ε subunits add up to the central stalk (or the rotor shaft) and an alternate arrangement of 3α and 3β form a hexameric ring with a central cavity.
- The γ subunit inserted in the central cavity protrudes out to meet ε which binds on its side and together they bind the F_0.
- Fo subunit has several subunits including a,b,c.
- There are 10-12 c subunits, which have an asp residue critical for the H+ channel.
- b makes up half the stalk and acts as oligomycin sensitivity-conferring protein (OSCP) and a is the proton channel.
- Subunits of F0 form the peripheral stalk, which connect both F_1 and F_0 and keep the stators (F_1-$\alpha\beta_3$ and F_0a) from spinning along with the rotor (γδε and F_0c).
- Other additional subunits such as subunit e, f, g, and A6L extend over the membrane cohort with F_0.

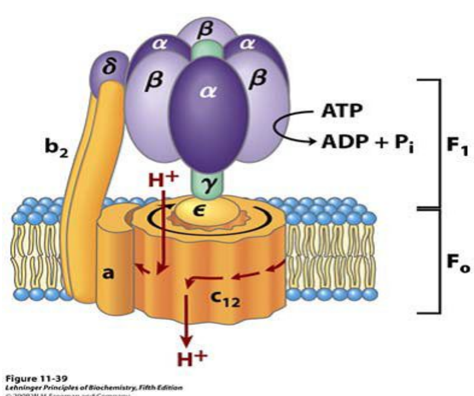


fig B: subunits of ATPase

Rotational catalysis and ATP generation

- Paul Boyer proposed a simple catalytic scheme, commonly known as the **binding change mechanism (**Figure a), which predicted that F-ATPase implements a rotational mechanism in the catalysis of ATP .
- The movement of subunits within the ATP synthase complex plays essential roles in both transport and catalytic mechanisms.
- Each catalytic site would achieve and change three conformations during a complete 360° turnover and a cycle would be completed at a different catalytic site with a rotation of 120°.
- When a nucleotide binds to ATPase, it undergoes a conformational change in order to be tightly bound to ATP.
- Another subsequent change in conformation brings about the release of ATP.
- These conformational changes are accomplished by rotating the inner core of the enzyme. The core itself is powered by the proton motive force conferred by protons crossing the mitochondrial membrane.

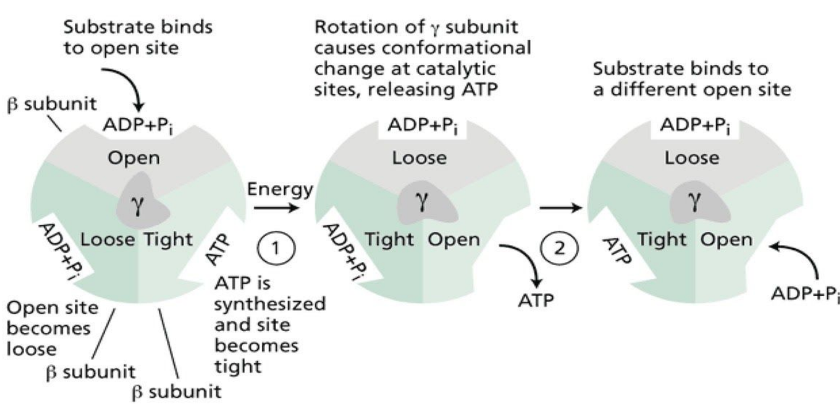


fig a

The binding-change mechanism as seen from the top of the F1 complex.

- There are three catalytic sites in three different conformations: **loose, open, and tight**. (For clarity, only the three β subunits are shown.)
- Substrate (ADP + Pi) initially binds to the open site and is converted to ATP at the tight site.
- In step 1, rotation of the γ subunit causes a conformational change, resulting in a change in the formation of the sites. As a result, ATP is released from the enzyme.
- In step 2, substrate again binds to the open site, and another ATP is synthesized at the tight site.

DIFFERENCE BETWEEN SUBSTRATE LEVEL AND OXIDATIVE PHOSPHORYLATION

Substrate Level Phosphorylation vs Oxidative Phosphorylation	
Substrate level phosphorylation directly transfers a phosphate group from the substrate (phosphorylated compound) to ADP to produce ATP.	Oxidative phosphorylation is a process by which energy released by chemical oxidation of nutrients is used for the synthesis of ATP.

Energy Used	
Energy is generated from a coupled reaction for this process.	Energy generated from the reaction of the electron transport chain is used for this process.
Redox Potential	
A small difference of redox potential is generated in substrate level phosphorylation.	A large difference in redox potential is generated to power this phosphorylation.
Conditions	
This occurs under both aerobic and anaerobic conditions.	This occurs under aerobic conditions.
Oxidation of Compounds	
Substrates are partially oxidized.	Electron donors are completely oxidized.

Locations	
Substrate level phosphorylation occurs in the cytosol and mitochondria	Oxidative phosphorylation occurs in the mitochondria.
Occurrence	
This can be seen in glycolysis and Krebs cycle.	This occurs only during the electron transport chain.
Association with Electron Transport Chain and ATP Synthase	
Substrate level phosphorylation is not associated with electron transport chain or ATP synthase	This is associated with electron transport chain and ATP synthase.
Involvement of O2 and NADH	
This does not use O_2 or NADH for the formation of ATP.	This uses O_2 and NADH to produce ATP.