VIVEKANANDA COLLEGE
THAKURPUKUR
KOLKATA-700063

NAAC ACCREDITED ‘A’ GRADE

Topic : Oxidative phosphorylation
Course Title : Membrane Biology and Bioenergetics
Paper : CC8
Unit : II
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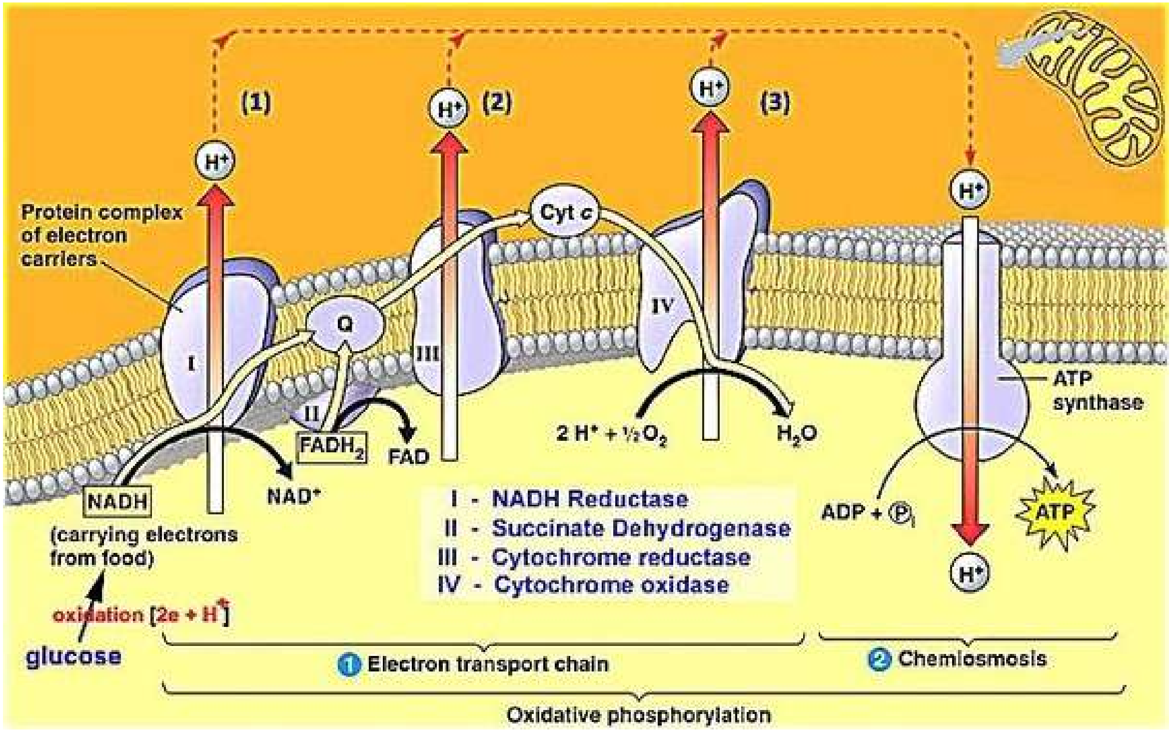
Name of the Teacher : Dr. Kakali Roy
Name of the Department : Biochemistry

Oxidative phosphorylation

Oxidative phosphorylation is the metabolic pathway in which cells use enzymes to oxidise nutrients, thereby releasing the chemical energy of molecular oxygen, which is used to produce adenosine triphosphate (ATP). In most eukaryotes, this takes place inside mitochondria.

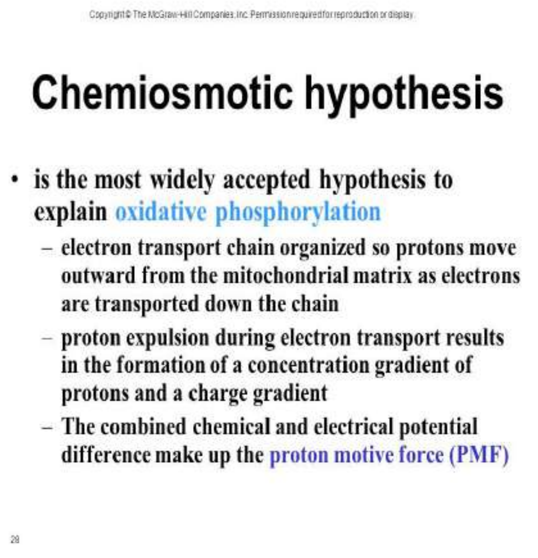
The energy transferred by electrons flowing through the electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called electron transport. This generates potential energy in the form of a pH gradient and an electrical potential across this membrane. This store of energy is tapped when protons flow back across the membrane and down the potential energy gradient, through a large enzyme called ATP synthase; this process is known as chemiosmosis. The ATP synthase uses the energy to transform adenosine diphosphate (ADP) into adenosine triphosphate, in a phosphorylation reaction. The reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.



P/O Ratio :

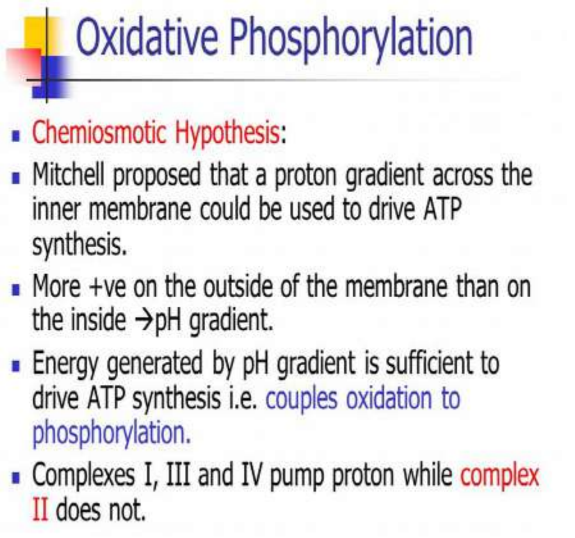
The ratio refer to the amount of ATP produced from movement of 2 electrons through a ETC, donated by reduction of an O atom by resting and active mitochondria. The oxidation of NADH and $FADH_2$ by O_2 is associated with synthesis of about 3 and 2 ATP respectively.

Oxidation and phosphorylation are closely coupled in well-functioning mitochondria, so electron transport can occur only if ADP is being phosphorylated.



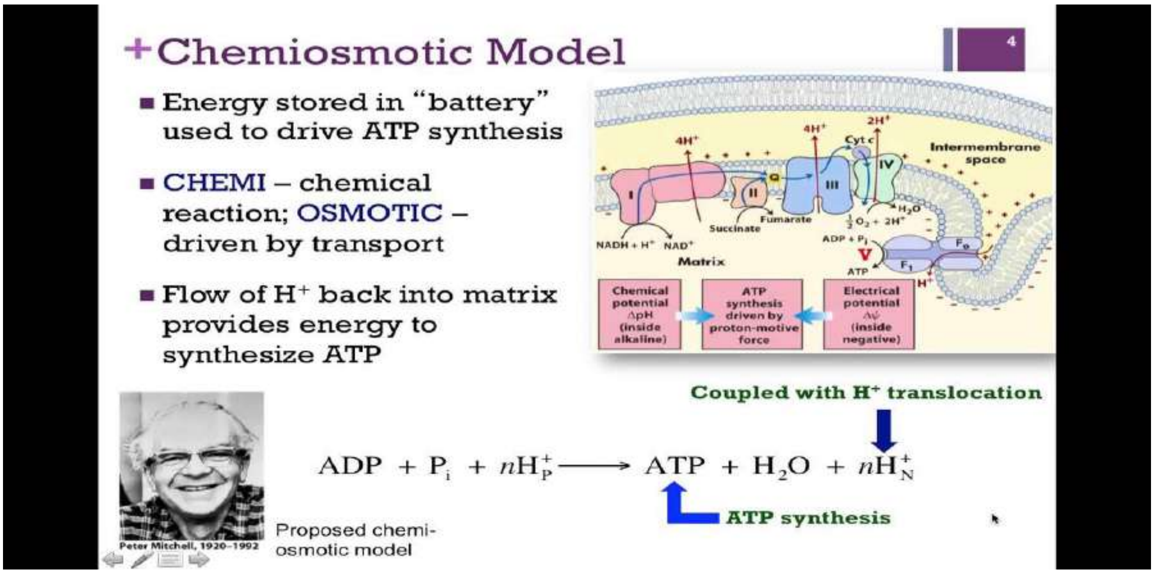
Chemiosmotic hypothesis

- is the most widely accepted hypothesis to explain oxidative phosphorylation
  - electron transport chain organized so protons move outward from the mitochondrial matrix as electrons are transported down the chain
  - proton expulsion during electron transport results in the formation of a concentration gradient of protons and a charge gradient
  - The combined chemical and electrical potential difference make up the proton motive force (PMF)



Oxidative Phosphorylation

- Chemiosmotic Hypothesis:
- Mitchell proposed that a proton gradient across the inner membrane could be used to drive ATP synthesis.
- More +ve on the outside of the membrane than on the inside →pH gradient.
- Energy generated by pH gradient is sufficient to drive ATP synthesis i.e. couples oxidation to phosphorylation.
- Complexes I, III and IV pump proton while complex II does not.



+Chemiosmotic Model

- Energy stored in "battery" used to drive ATP synthesis
- CHEMI – chemical reaction; OSMOTIC – driven by transport
- Flow of H^+ back into matrix provides energy to synthesize ATP

Coupled with H^+ translocation

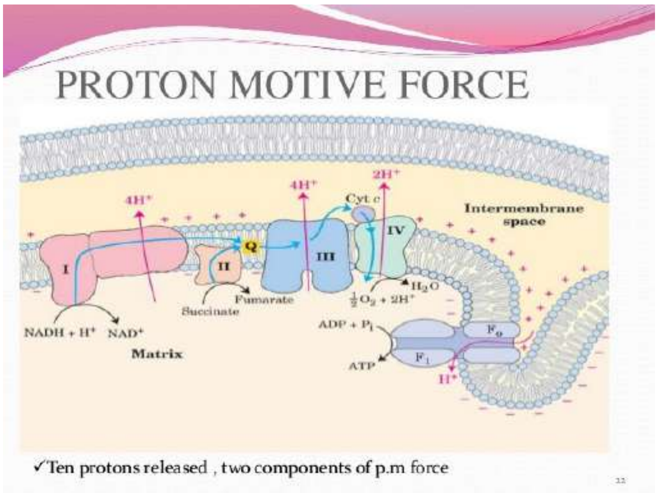
$$\mathrm{ADP} + \mathrm{P_i} + n\mathrm{H_P^+} \longrightarrow \mathrm{ATP} + \mathrm{H_2O} + n\mathrm{H_N^+}$$

ATP synthesis

Proposed chemi-osmotic model

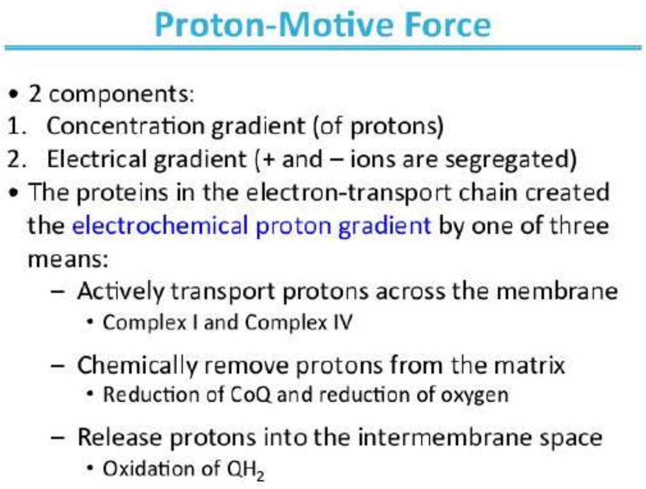
Several key observations are explained by the chemiosmotic hypothesis :

- Oxidative phosphorylation requires an intact inner mitochondrial membrane.
- The inner mitochondrial membrane is impermeable to ions such as H^+, OH^-, K^+ and Cl^-, whose free diffusion would discharge an electrochemical gradient.
- Electron transport results in the transport of H^+ out of intact mitochondria, thereby creating a measurable electrochemical gradient across the inner mitochondrial membrane.
- Compounds that increase the permeability of the inner mitochondrial membrane to protons, and thereby dissipate the electrochemical gradient, allow electron transport to continue but inhibit ATP synthesis; that is, they "uncouple" electron transport from oxidative phosphorylation.



PROTON MOTIVE FORCE

✓Ten protons released , two components of p.m force



Proton-Motive Force

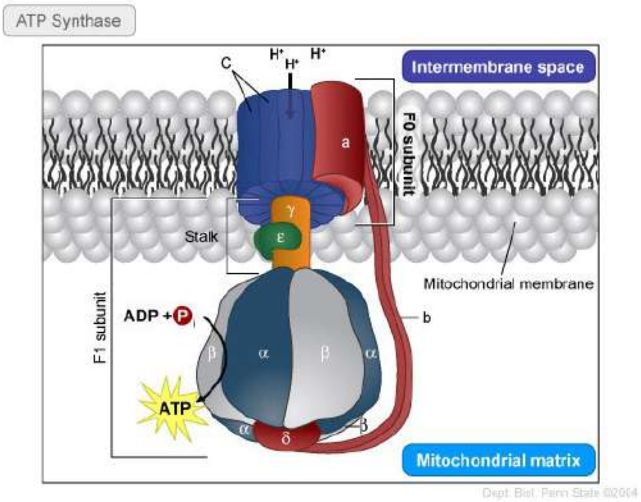
- 2 components:
1. Concentration gradient (of protons)
2. Electrical gradient (+ and – ions are segregated)
- The proteins in the electron-transport chain created the electrochemical proton gradient by one of three means:
  - Actively transport protons across the membrane
    - Complex I and Complex IV
  - Chemically remove protons from the matrix
    - Reduction of CoQ and reduction of oxygen
  - Release protons into the intermembrane space
    - Oxidation of QH_2

Proton Motive Force

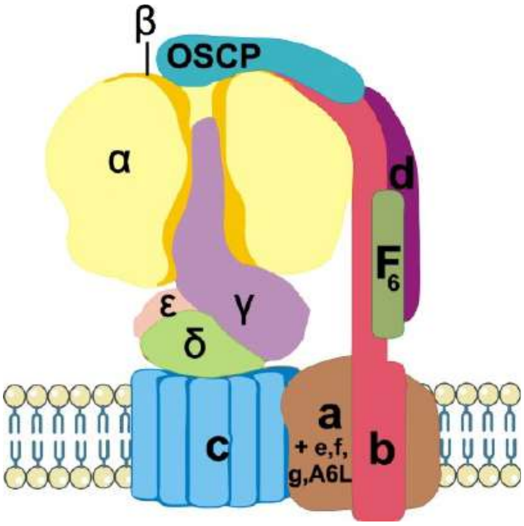
- Is an electrochemical gradient
- Two gradients drive the protons from the intermembrane space into the matrix
  - It is more negative inside the matrix than in the intermembrane space, so there is electric potential across the membrane.
  - H^+ is attracted to the opposite negative charge inside the matrix.
  - There is a chemical or pH gradient as well
  - Greater concentration of H^+ in the intermembrane space causes the protons to move to an area of lower concentration inside the matrix.
- About 85% of the proton motive force is derived from the electric or charge gradient while approximately 15% comes from the chemical gradient.

FoF1 ATP Synthase

ATP synthase is the very last enzyme in oxidative phosphorylation pathway that makes use of electrochemical energy to power ATP synthesis. The mitochondrial ATP synthase is a multi-subunit protein complex having an approximate molecular weight of 550 kDa. The human mitochondrial ATP synthase or F1/Fo ATPase or complex V (EC 3.6.3.14) is the fifth component of oxidative phosphorylation chain. The ATP synthase, also called Complex V, has two major subunits designated Fo and F_1. The Fo part, bound to inner mitochondrial membrane is involved in proton translocation, whereas the F_1 part found in the mitochondrial matrix is the water soluble catalytic domain. F_1 is the first factor recognized and isolated from bovine heart mitochondria and is involved in oxidative phosphorylation. It was named so from the term 'Fraction 1'. Fo was named so as it is a factor that conferred oligomycin sensitivity to soluble F_1.



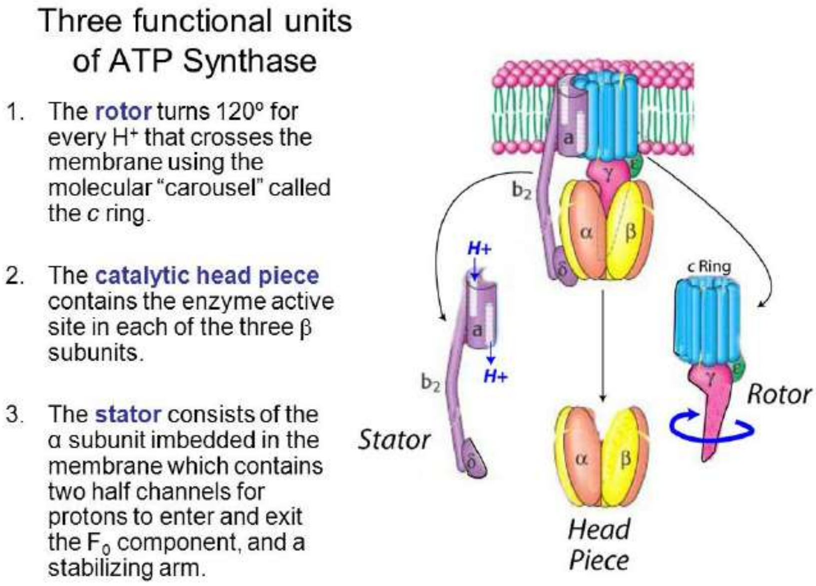
ATP synthase consists of two regions Fo and F_1. F_O causes rotation of F_1 and is made of c-ring and subunits a, two b, F6.



F_1 is made of α, β, γ, δ and ε subunits. F_1 has a water-soluble part that can hydrolyze ATP. Subunits α and β make a hexamer with 6 binding sites. Three of them are catalytically inactive and they bind ADP. Other three subunits catalyze the ATP synthesis. The other F_1 subunits γ, δ, ε are a part of a rotational motor mechanism (rotor/axle). γ subunit allows β to go through conformational changes (i.e., closed, half open, and open states) that allow for ATP to be bound and released once synthesized. F_O on the other hand has mainly hydrophobic regions. It consists of three main subunits, a, b, and c. Six c subunits make up the rotor ring, and subunit b makes up a stalk connecting to F_1 OSCP (Oligomycin sensitivity conferring protein) that prevents the αβ hexamer from rotating. Subunit a connects b to the c ring. Humans have six additional subunits, d, e, f, g, F6, and 8 (or A6L). This part of the enzyme is located in the mitochondrial inner membrane and couples proton translocation to the rotation the causes ATP synthesis in the F_1 region.

Mechanism of ATP synthesis

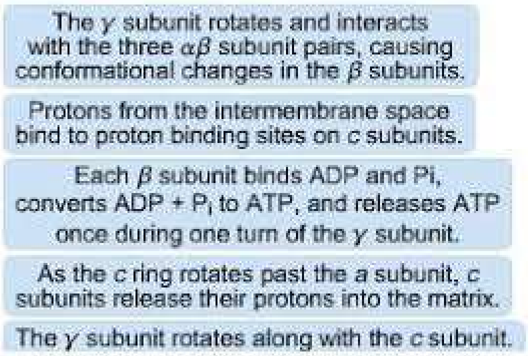
The F_oF_1 ATP synthase functions as a rotary motor where subunit rotation driven by a current of protons flowing through F_o drives the binding changes in F_1 that are required for net ATP synthesis.



Three functional units of ATP Synthase

1. The **rotor** turns 120° for every H^+ that crosses the membrane using the molecular "carousel" called the *c* ring.
2. The **catalytic head piece** contains the enzyme active site in each of the three β subunits.
3. The **stator** consists of the α subunit imbedded in the membrane which contains two half channels for protons to enter and exit the F_0 component, and a stabilizing arm.

ATP synthase uses the proton (H^+) gradient to drive ATP synthesis. There are the following steps :



The γ subunit rotates and interacts with the three αβ subunit pairs, causing conformational changes in the β subunits.

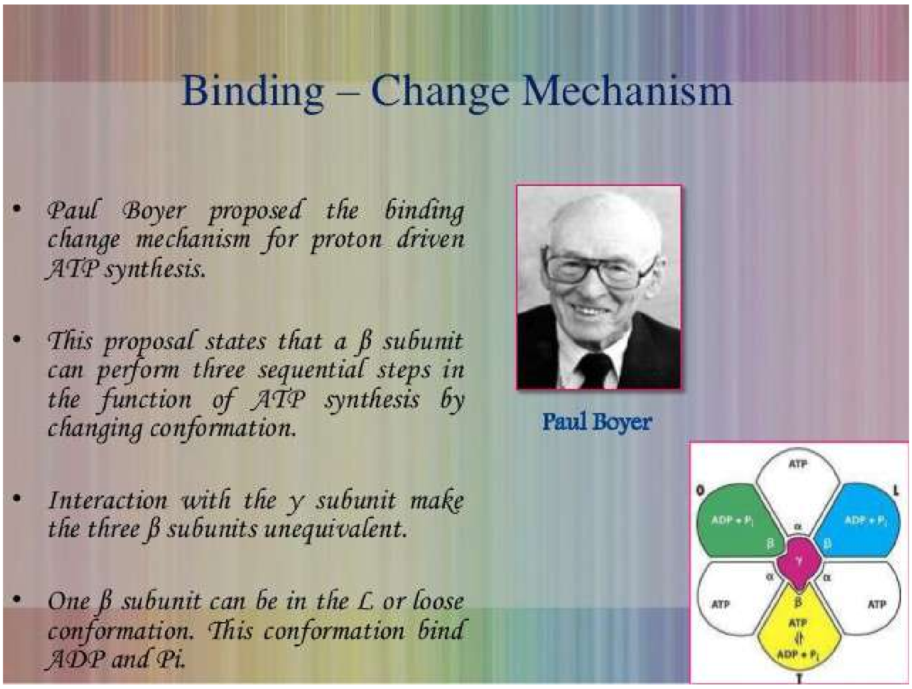
Protons from the intermembrane space bind to proton binding sites on *c* subunits.

Each β subunit binds ADP and Pi, converts ADP + P_i to ATP, and releases ATP once during one turn of the γ subunit.

As the *c* ring rotates past the *a* subunit, *c* subunits release their protons into the matrix.

The γ subunit rotates along with the *c* subunit.

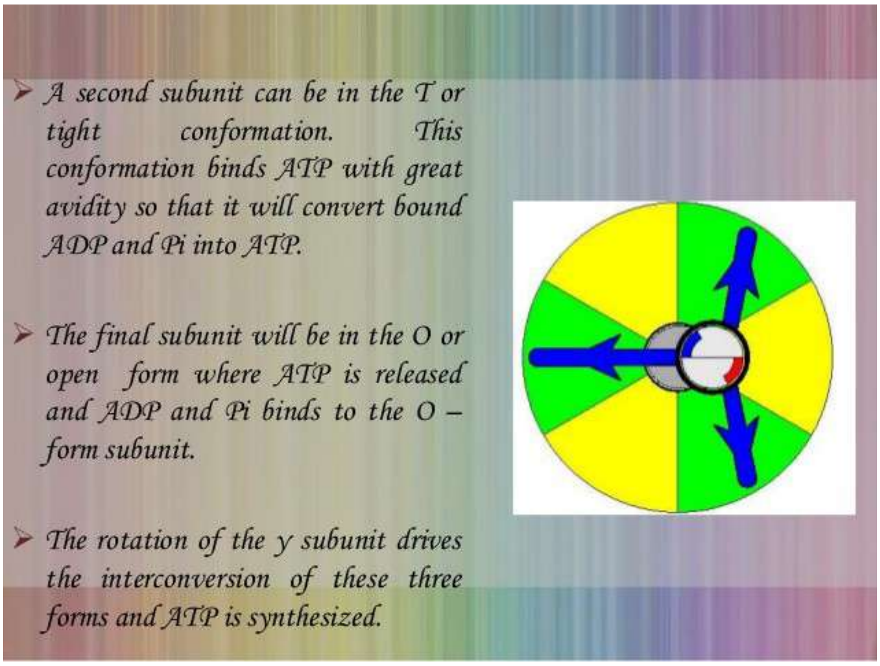
Each β subunit binds ADP and Pi, converts ADP + Pi to ATP and releases ATP once during one turn of the γ subunit.



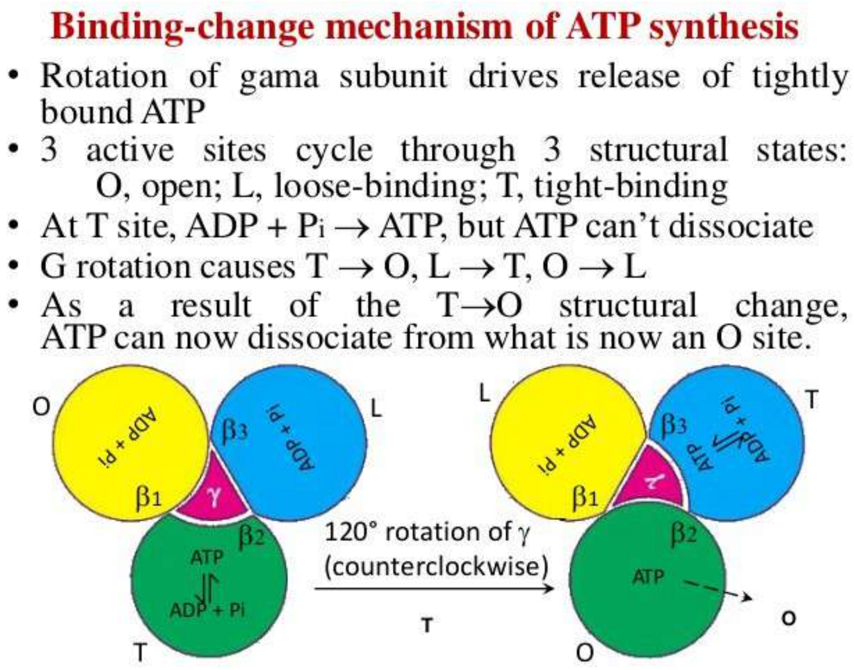
Binding – Change Mechanism

- *Paul Boyer proposed the binding change mechanism for proton driven ATP synthesis.*
- *This proposal states that a β subunit can perform three sequential steps in the function of ATP synthesis by changing conformation.*
- *Interaction with the γ subunit make the three β subunits unequivalent.*
- *One β subunit can be in the L or loose conformation. This conformation bind ADP and Pi.*

Paul Boyer

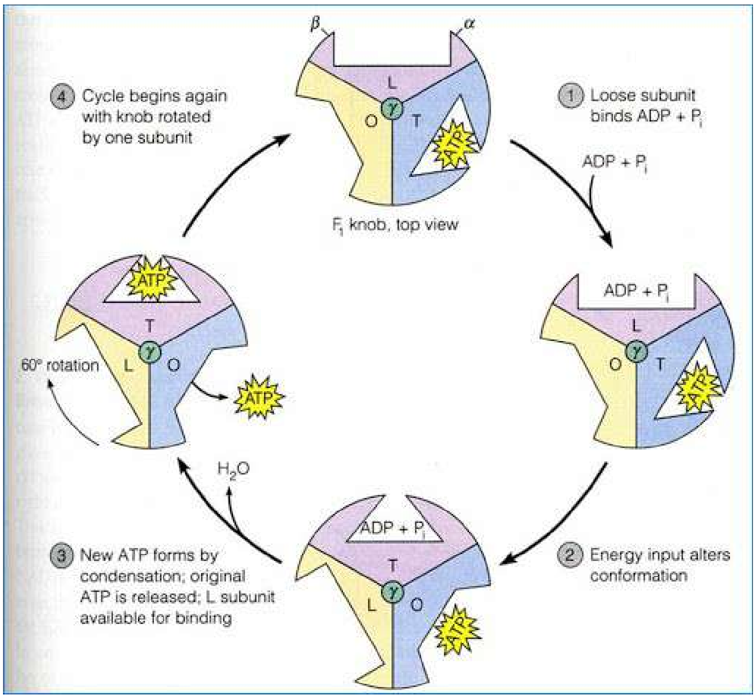


- *A second subunit can be in the T or tight conformation. This conformation binds ATP with great avidity so that it will convert bound ADP and Pi into ATP.*
- *The final subunit will be in the O or open form where ATP is released and ADP and Pi binds to the O – form subunit.*
- *The rotation of the γ subunit drives the interconversion of these three forms and ATP is synthesized.*



Binding-change mechanism of ATP synthesis

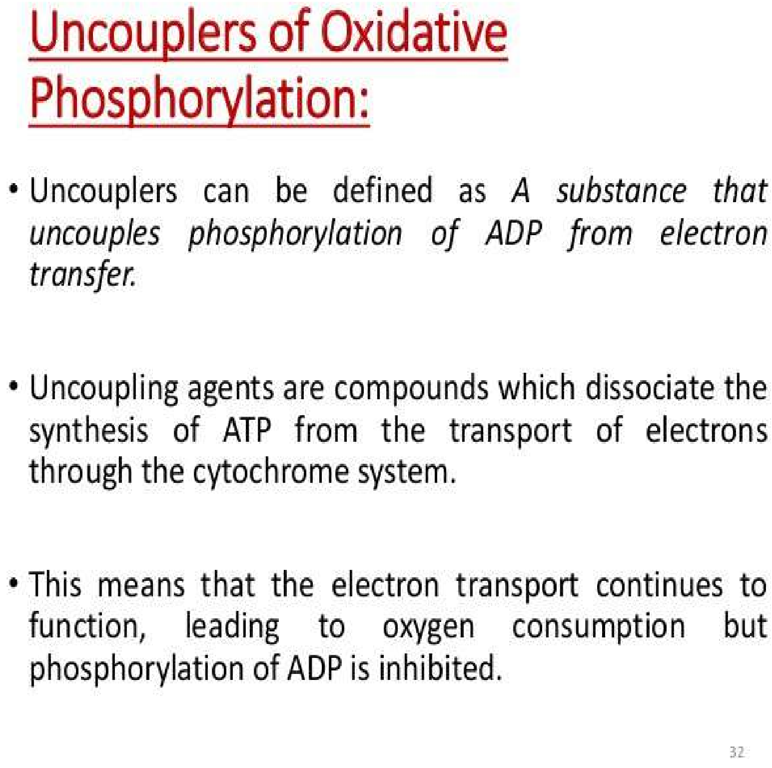
- Rotation of gama subunit drives release of tightly bound ATP
- 3 active sites cycle through 3 structural states: O, open; L, loose-binding; T, tight-binding
- At T site, ADP + Pi → ATP, but ATP can't dissociate
- G rotation causes T → O, L → T, O → L
- As a result of the T→O structural change, ATP can now dissociate from what is now an O site.



A model for energy coupling by F_oF_1 ATP synthases that has gained widespread support is called the binding change mechanism. According to this proposal, the major energy-requiring step is not the synthesis of ATP at catalytic sites, but rather the simultaneous and highly cooperative binding of substrates to, and release of products from, these sites. Furthermore, it is proposed that these affinity changes are coupled to proton transport by the rotation of a complex of subunits that extends through F_oF_1. Rotation of the γ subunit in the centre of F_1 is thought to deform the surrounding catalytic subunits to give the required binding changes, whereas rotation of the c subunits relative to the single a subunit in F_o is believed to be required for completion of the proton pathway. The binding change mechanism stipulates that ADP and P_i must bind at a catalytic site on F_1 before protons can be transported through F_o down an electrochemical gradient.

Regulation of Oxidative Phosphorylation

- Overall rate of oxidative phosphorylation depends upon <u>substrate availability</u> and <u>cellular energy demand</u>.
- Important substrates : **NADH, O_2, ADP**
- Electron transport is tightly coupled to phosphorylation. ATP can not be synthesised by oxidative phosphorylation unless there is energy from electron transport.
- Electrons do not flow through the ETC to O_2 unless ADP is phosphorylated to ATP.
- In eukaryotes intra mitochondrial ratio ATP/ADP is a secondary control mechanism. High ratio inhibits oxidative phosphorylation as ATP allosterically binds to a subunit of complex IV.



Uncouplers of Oxidative Phosphorylation:

- Uncouplers can be defined as *A substance that uncouples phosphorylation of ADP from electron transfer.*
- Uncoupling agents are compounds which dissociate the synthesis of ATP from the transport of electrons through the cytochrome system.
- This means that the electron transport continues to function, leading to oxygen consumption but phosphorylation of ADP is inhibited.

For example : 2,4 dinitrophenol (DNP)

References :

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3. www.google.com