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Get Ready to Crack CSIR NET 2022 (Short notes on ATP)

ADENOSINE TRI PHOSPHATE (ATP) SYNTHESIS

Chemiosmotic theory:

The flow of electrons through the respiratory chain generates ATP by the process of oxidative phosphorylation.

- It was postulated by Peter Mitchell in 1961.
- This theory postulates that the two processes ; oxidation and phosphorylation are coupled by a proton gradient across the inner mitochondrial membrane
- The electrochemical potential difference(negative on the matrix side) results in a proton motive force that drives the process of ATP synthesis
- Oligomycin results in the blockage of flow of protons into the matrix through the proton channel of ATP synthase; and there is no path that exists for the return of protons to the matrix
- This results in the building up of proton-motive force until the cost (free energy) of pumping protons out of the matrix against this gradient equals or exceeds the energy released by the transfer of electrons from NADH to O_2
- Then the electron flow stops; the free energy for the overall process of electron flow coupled to proton pumping becomes zero.
- The system is ultimately at equilibrium

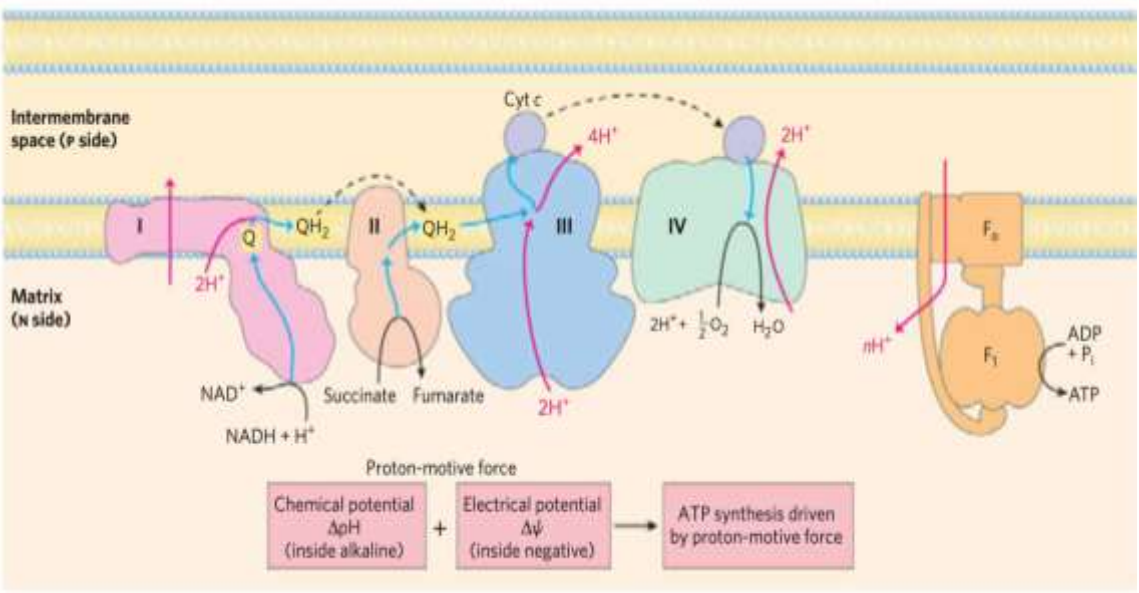

Fig 1: The chemiosmotic model

Experimental proof of chemiosmotic hypothesis:

- It was provided by Andre Jagendorf and Ernest Uribe in 1966
- Isolated chloroplast thylakoid vesicles containing F_0F_1 particles were equilibrated in the dark with a buffered solution at pH 4.0
- As the pH of the thylakoid lumen became 4.0; the vesicles were rapidly mixed with a solution at pH 8.0 containing ADP and Pi
- ATP synthesis was observed due to the transmembrane movement of protons driven by the electrochemical proton gradient
- An artificially generated membrane electric potential also resulted in ATP synthesis in inside-out preparations of submitochondrial vesicles

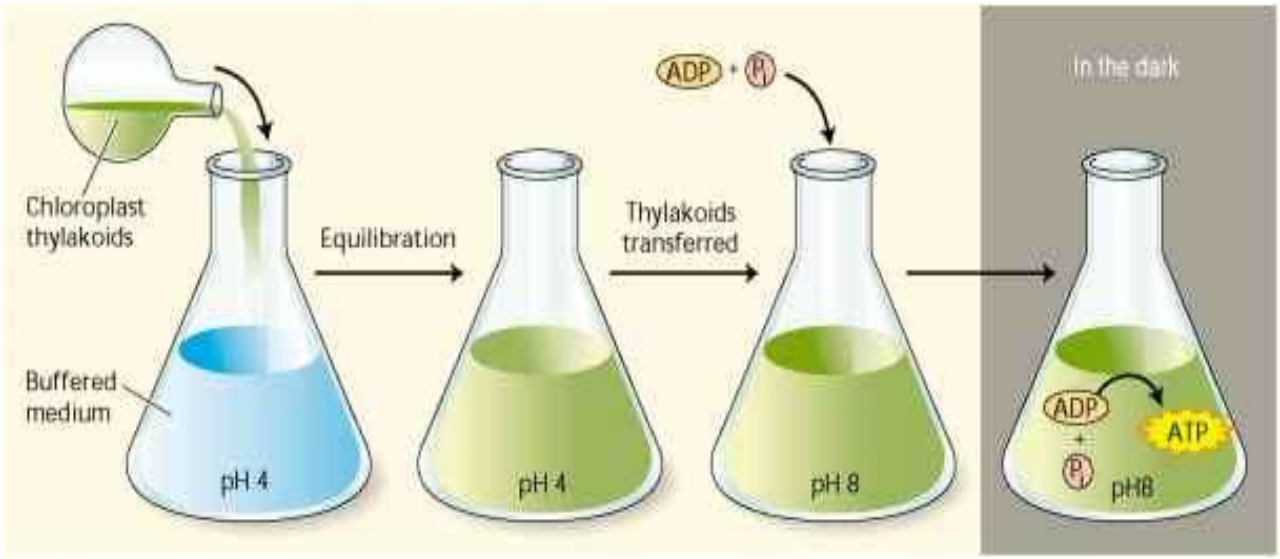

Fig 2: Proton transport and ATP synthesis in chloroplast

ATP synthase complex:

- The proton motive force helps in the process of ATP synthesis by the ATP synthase complex
- ATP is synthesized by ATP synthase or F_0F_1 complex or Complex V as protons flow back through the inner membrane down the electrochemical proton gradient.
- It consists of two components:
    A. F_0 component
    B. F_1 ATPase

A. F_0 component

- It is embedded in the inner mitochondrial membrane.
- It contains one 'a' subunit, two 'b' subunits and 9-12 'c' subunits.
- Two α helices are present in the c subunit that span the membrane.
- The second helix contains an aspartic acid residue on the center of the membrane.
- A regulated H^+ channel is formed by the F_0 complex
- The antibiotic- oligomycin completely blocks ATP synthesis by blocking the flow of protons through F_0 of ATP synthase

B. F_1 ATPase

- It is made up of 3α,3β,γ,δ and ε
- It is tightly bound to the F_0 and protrudes into the matrix
- It contains three β subunits that are sites of ATP synthesis
- At the centre of F_1 ATPase is the γ subunit
- The γ subunit extends through F_1 and interacts with F_0
- The γε and C_{9-12} ring complex is the rotor (moving unit) and the a, b_2 and $\alpha_3\beta_3\delta$ complex is the stator (stationary unit)
- Rotational motion is imparted to the rotor by the passage of protons

- ATP synthase synthesiszes ATP by harnessing the proton motive force.
- ATP synthase can also function in reverse direction to hydrolyze ATP and pump H^+ across the inner mitochondrial membrane
- It thus acts as a reversible coupling devise, interconverting electrochemical proton gradient and chemical bond energies and vice-versa
- Efraim Racker and his colleagues first purified and extracted F_1 ATPase from the mitochondrial inner membrane
- F_1 cannot synthesize ATP from ADP and P_i; because it can catalyze the hydrolysis of ATP
- Thus the enzyme was originally called F_1 ATPase
- The complete F_0F_1 complex, like isolated F_1, can hydrolyze ATP to ADP and P_i; but its biological function is to catalyze the condensation of ADP and P_i to form ATP
- The F_0F_1 complex is therefore more appropriately called ATP synthase

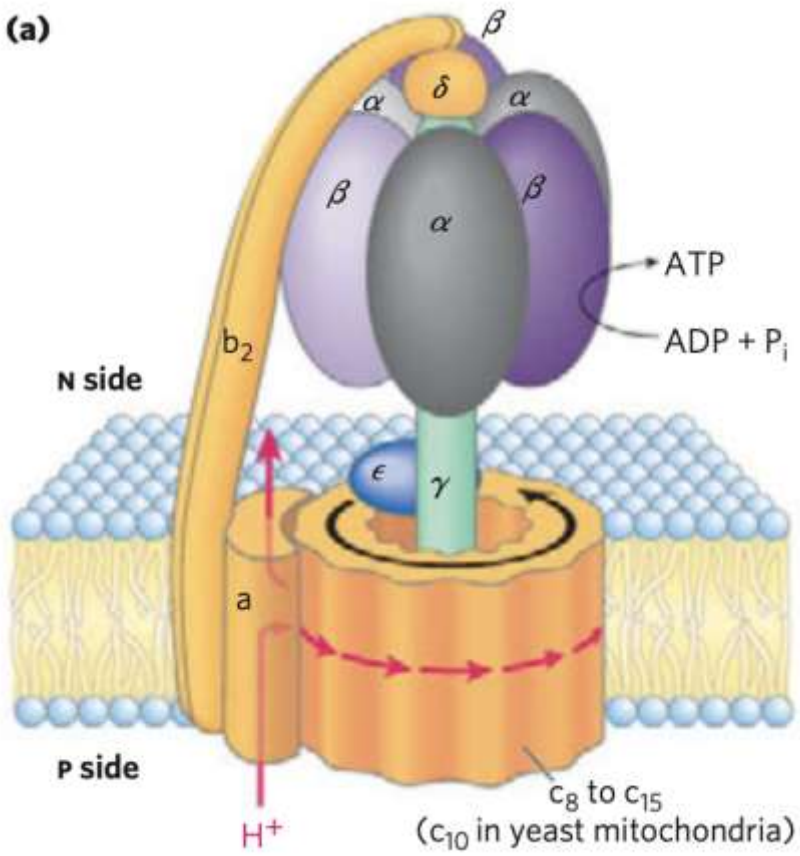

Fig 3: The ATP synthase complex

Process of ATP synthesis:

- The binding change mechanism is the most widely accepted model of ATP synthesis
- The binding change or flip-flop mechanism developed by Paul Boyer postulated that ATP synthesis is coupled with a conformational change in the ATP synthase generated by rotation of the gamma subunit
- Proton translocation through F_0 powers the rotation of the γ-subunit of F_1 ATPase, leading to changes in the conformation of the nucleotide-binding sites in the F_1 β-subunits
- Through this binding-change mechanism, the F_0F_1 complex harnesses the proton-motive force to power ATP synthesis
- The three F_1 β-subunits alternate between three conformational states that differ in their binding affinities for ATP, ADP and P_i
    - The O state (open state) binds ATP, ADP and P_i very weakly
    - The L state (loose state) binds ADP and P_i loosely
    - The T state (tight state) that binds ADP and P_i very tightly and gives ATP

- The phosphoanhydride bond of ATP is synthesized only in the T-state and ATP is released only in the O-state
- The free energy released on proton translocation is harnessed to interconvert three states
- The change in conformation occurs due to rotation of γ- subunit
- The 120° rotation of γ- subunit in counterclockwise direction helps in changing one conformation state to another
- Rotation of γ-subunit relative to fixed $\alpha_3\beta_3$ occurs in discrete 120° steps and conformation of each β-subunit changes in the order :

$$O \longrightarrow L \longrightarrow T \longrightarrow O$$

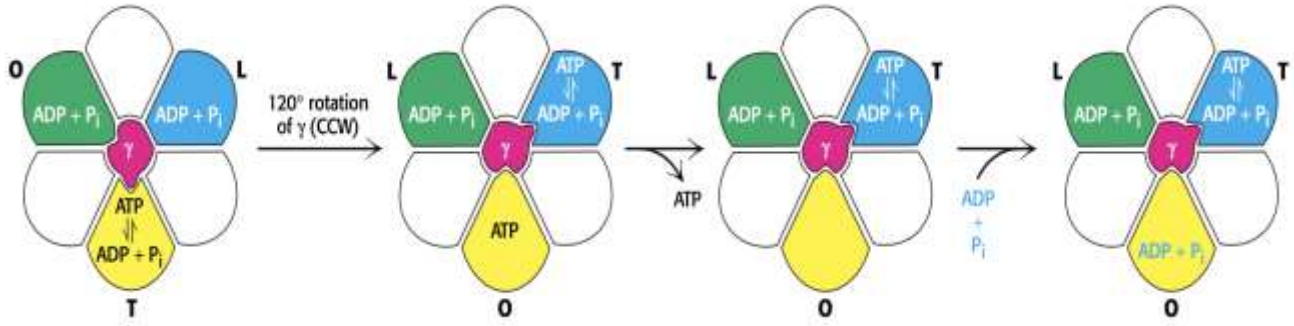

Fig 4: Mechanism of ATP synthesis

Calculation of free energy change:

Nernst equation helps us to calculate the standard free energy change for the movement of protons across the membrane along the electrochemical proton gradient

$$\Delta G \text{ (cal/mole)} = -n F \Delta E$$

We can calculate the amount of free energy released by the passage of one mole of protons down an electrochemical gradient of 220mV from the above equation:

$$\Delta G = -n F \Delta E \qquad \text{where, } n = 1$$
$$= -23{,}062 * 0.22V = -5074 \text{ cal/mol or } -5.1 \text{ Kcal/mole}$$

- Three H^+ are required to synthesize one ATP by ATP synthase
- However, the most widely accepted theory for the number of protons required for the synthesis of one ATP molecule is four.
- Hence, if 10 protons are pumped out per NADH, four must flow in to produce one ATP

Uncoupling agents and ionophores:

A. Uncouplers-

- Uncoupling agents uncouple oxidation from phosphorylation
- They allow the oxidation of NADH and $FADH_2$ and reduction of O_2 to continue at high levels but do not permit ATP synthesis
- Thus, electron transport continues; but ATP synthesis stops
- The common uncoupling agents are 2,4-dinitrophenol (DNP), dicoumarol and carbonyl cyanide-p-(trifluoromethoxy) phenylhydrazone (FCCP)
- DNP, which is a weak acid is soluble in lipid bilayer both in their protonated neutral forms and in their anionic state
- DNP in the anionic state picks up protons in the inter-membrane space and diffuse readily across the mitochondrial membrane
- After entering the matrix in the protonated form, they release a proton, thus dissipating the proton gradient and inhibiting ATP synthesis
- Heat is produced as energy is released by NADH oxidation in presence of DNP.
- Both, Dicoumarol and FCCP act in the same way
- Similarly, thermogenin is a physiological uncoupler found in brown adipose tissue that functions to generate body heat. It is particularly for the new born and during hibernation in animals

B. Ionophores-

- They are lipophilic molecules that bind specific cations and facilitate their transport through the membrane
- Ionophore uncouple electron transfer from oxidative phosphorylation by dissipating the electrochemical gradient across the mitochondrial membrane
- Valinomycin, an antibiotic, is an example of ionophore
- Its addition makes inner mitochondrial membrane permeable for K^+
- It causes the movement of K^+ along the concentration gradient from cytosol into the matrix
- It decreases the membrane potential component of pmf (without a direct effect on the pH gradient) and thus ATP synthesis

Importance of ATP in biological systems:

1. ATP is the immediate energy source as its energy store is not long lasting due to instability of the phosphate bonds.
2. ATP is the energy currency of the cell; that provides energy to drive many cellular processes like muscle contraction, nerve impulse propagation etc.
3. The hydrolysis of ATP to ADP is a single reaction releasing energy immediately whereas the process for glucose is much longer.
4. It is also a precursor to DNA and RNA, and is used as a coenzyme

5. It is involved in amino acid activation in protein synthesis- The aminoacyl-tRNA synthetase enzyme consume ATP in the attachment of tRNA to amino acids, forming aminoacyl-tRNA complexes.
6. ATP binding cassette transporter- The transport of molecules out of a cell against a gradient is often associated with ATP hydrolysis. This transport is mediated by ATP binding cassette transporter

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