Oxidative Phosphorylation

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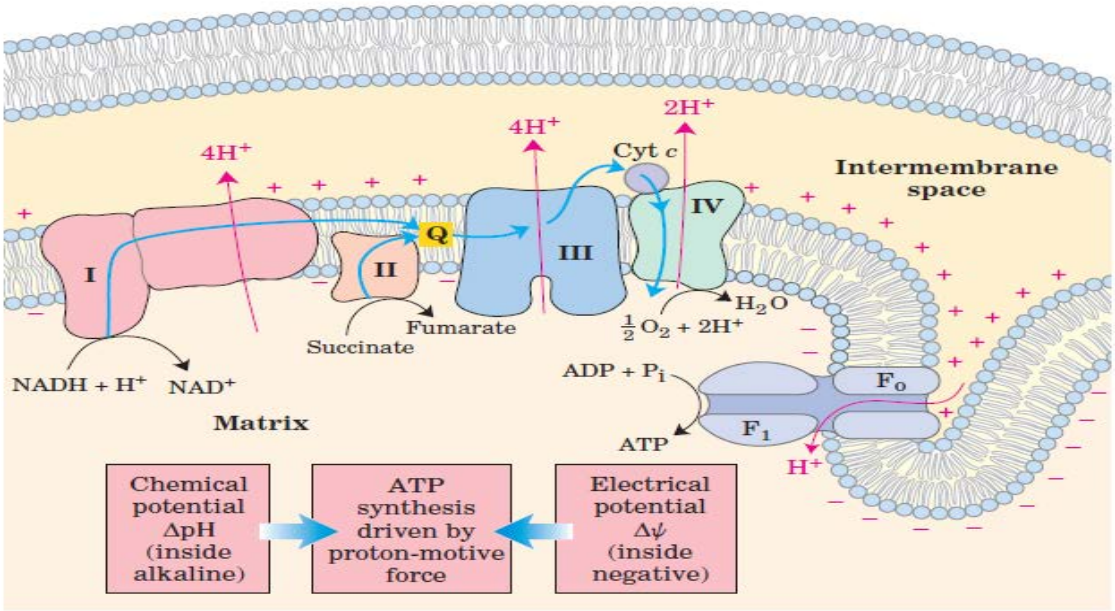
Oxidative Phosphorylation

- Currently the most widely accepted hypothesis about how oxidative phosphorylation occurs is the chemiosmotic hypothesis.
- The **chemiosmotic model,** proposed by Peter Mitchell, is the paradigm for this mechanism.
- According to the model, the electrochemical energy inherent in the difference in proton concentration and separation of charge across the inner mitochondrial membrane—the proton-motive force—drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore associated with **ATP synthase.**

$$\mathrm{ADP} + \mathrm{P_i} + n\mathrm{H^+_P} \longrightarrow \mathrm{ATP} + \mathrm{H_2O} + n\mathrm{H^+_N}$$

Chemiosmotic model

- In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane.
- Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient (Δψ).
- The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (Fo).
- The proton-motive force that drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F1 complex associated with Fo.



Oxidative phosphorylation in bacteria

- A similar process takes place in procaryotes, with electron flow causing the protons to move outward across the plasma membrane.
- ATP synthesis occurs when these protons diffuse back into the cell.
- The chemiosmotic hypothesis is accepted by most microbiologists.
- There is considerable evidence for the generation of proton and charge gradients across membranes.
- However, the evidence for proton gradients as the direct driving force for oxidative phosphorylation is not yet conclusive.
- In some halophilic marine bacteria, sodium ions may be used to drive ATP synthesis.
- Whatever the precise mechanism, ATP synthesis takes place at the F1F0 ATPase or **ATP synthase.**

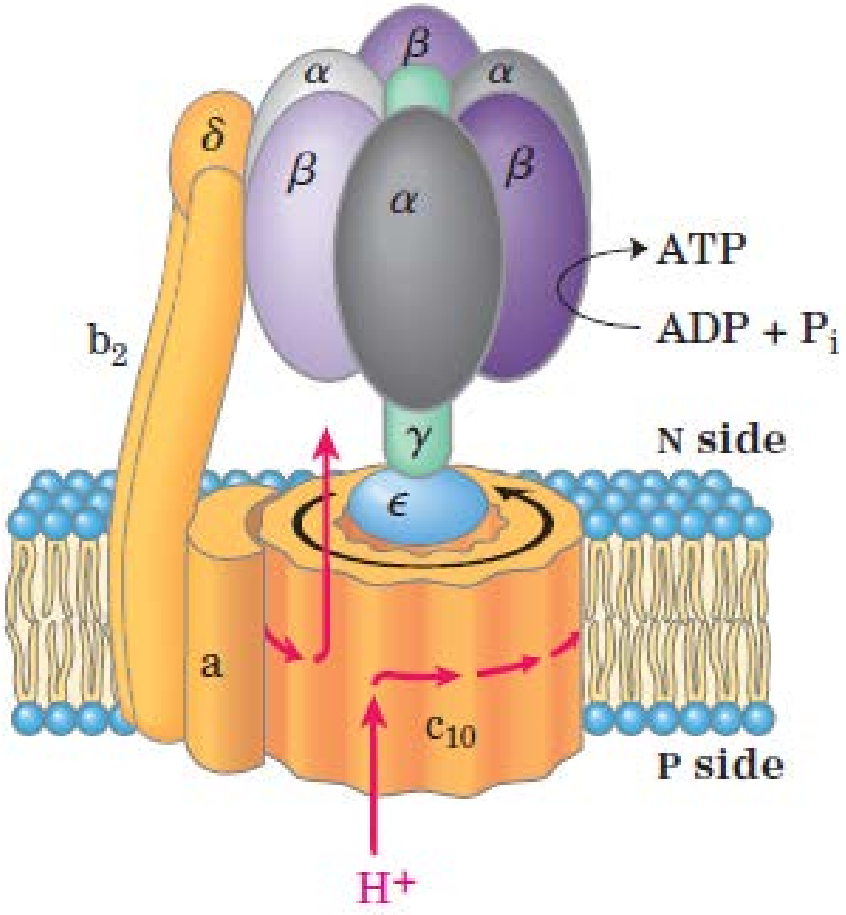
ATP Synthase

- Mitochondrial **ATP synthase is an F-type ATPase,** similar in structure and mechanism to the ATP synthases of chloroplasts and eubacteria**.**
- ATP synthase, also called Complex V, has two distinct components:
  - F1, a peripheral membrane protein,
  - and Fo (*o denoting* oligomycin-sensitive), which is integral to the membrane.
- Mitochondrial F1 has nine subunits of five different types, with the composition $\alpha_3\beta_3\gamma\delta\varepsilon$. Each of the three β subunits has one catalytic site for ATP synthesis.
- The knoblike portion of F1 is a flattened sphere, consisting of alternating α and β subunits arranged like the sections of an orange .
- The polypeptides that make up the stalk in the F1 crystal structure are asymmetrically arranged, with one domain of the single *γ subunit* making up a central shaft that passes through F1, and another domain of γ *associated primarily with one of* the three *subunits, designated -empty.*

ATP Synthase

- Although the amino acid sequences of the three *subunits* are identical, *their conformations differ, in part* because of the association of the γ *subunit with just one* of the three.
- The conformational differences among *subunits* extend to differences in their ATP/ADP-binding sites.
- The Fo complex making up the proton pore is composed of three subunits, a, b, and c, in the proportion $ab_2c_{10–12}$.
- The yeast complex has ten c subunits, each with two transmembrane helices roughly perpendicular to the plane of the membrane and arranged in two concentric circles.
- The inner circle is made up of the amino-terminal helices of each c subunit; the outer circle, about 55 Å in diameter, is made up of the carboxyl-terminal helices.
- The ε and *γ subunits of F1 form a leg-and-foot that projects* from the bottom (membrane) side of F1 and stands firmly on the ring of c subunits.

ATP Synthase



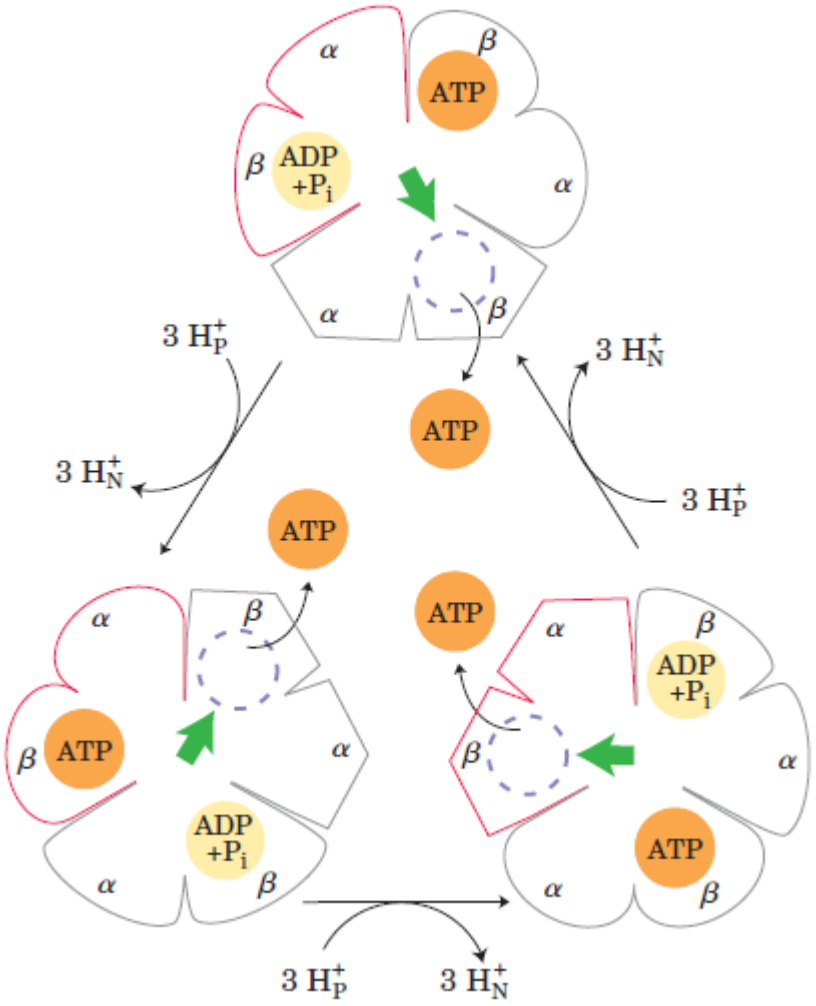
Rotational Catalysis: Key to the Binding-Change Mechanism for ATP Synthesis

- Paul Boyer proposed a rotational catalysis mechanism in which the three active sites of F1 take turns catalyzing ATP synthesis.
- A given *subunit* starts in the β-ADP conformation, which binds ADP and Pi from the surrounding medium.
- The subunit now changes conformation, assuming the β-ATP form that tightly binds and stabilizes ATP, bringing about the ready equilibration of ADP + Pi with ATP on the enzyme surface.
- Finally, the subunit changes to the β-empty conformation, which has very low affinity for ATP, and the newly synthesized ATP leaves the enzyme surface.
- Another round of catalysis begins when this subunit again assumes the β-ADP form and binds ADP and Pi.
- The conformational changes central to this mechanism are driven by the passage of protons through the Fo portion of ATP synthase.
- The streaming of protons through the Fo "pore" causes the cylinder of c subunits and the attached γ *subunit to rotate about the long axis* of γ, which is perpendicular to the plane of the membrane.
- The γ *subunit passes through the center of the* $\alpha_3\beta_3$ spheroid, which is held stationary relative to the membrane surface by the b2 and δ *subunits*.
- With each rotation of 120°, γ *comes into contact* with a different β *subunit, and the contact forces* that β *subunit into the* β*-empty conformation.*

Rotational Catalysis

- The three subunits interact in such a way that when one assumes the β-empty conformation, its neighbor to one side must assume the β-ADP form, and the other neighbor the β-ATP form.
- Thus one complete rotation of the β subunit causes each β subunit to cycle through all three of its possible conformations, and for each rotation, three ATP are synthesized and released from the enzyme surface.
- One strong prediction of this binding-change model is that the *subunit should rotate in one direction when* FoF1 is synthesizing ATP and in the opposite direction when the enzyme is hydrolyzing ATP.

Binding-change model for ATP synthase



Chemiosmotic Coupling and ATP Synthesis

- The consensus values for number of protons pumped out per pair of electrons are 10 for NADH and 6 for succinate.
- The most widely accepted experimental value for number of protons required to drive the synthesis of an ATP molecule is 4, of which 1 is used in transporting Pi, ATP, and ADP across the mitochondrial membrane.
- If 10 protons are pumped out per NADH and 4 must flow in to produce 1 ATP, the proton-based P/O ratio (the number of ATPs formed per oxygen atom reduced by 2 electrons in electron transport) is 2.5 for NADH as the electron donor and 1.5 (6/4) for succinate.
- Because bacterial electron transport systems often have lower P/O ratios than the eucaryotic system being discussed, bacterial aerobic ATP yields can be less.
- For example, *E. coli* with its truncated electron transport chains has a P/O ratio around 1.3 (4/3) when using the cytochrome *bo path at high oxygen* levels and only a ratio of about 0.67 (2/3) when employing the cytochrome *bd branch at low oxygen concentrations.*
- In this case ATP production varies with environmental conditions.

Inhibitors for Aerobic ATP Synthesis

- Many chemicals inhibit the aerobic synthesis of ATP and can even kill cells at sufficiently high concentrations.
- These inhibitors generally fall into two categories.
- **Some directly block the transport of electrons.**
- The antibiotic piericidin competes with coenzyme Q; the antibiotic antimycin A blocks electron transport between cytochromes *b* and *c;* and both cyanide and azide stop the transfer of electrons between cytochrome *a* and O_2 because they are structural analogs of O_2.
- **Another group of inhibitors known as uncouplers stops ATP synthesis without inhibiting electron transport itself.**
- Uncouplers disconnect oxidative phosphorylation from electron transport; therefore the energy released by the chain is given off as heat rather than as ATP.
- Many uncouplers like dinitrophenol and valinomycin may allow hydrogen ions, potassium ions, and other ions to cross the membrane without activating the F1F0 ATPase. In this way they destroy the pH and ion gradients.
- Valinomycin also may bind directly to the F1F0 ATPase and inhibit its activity.

Questions

- What are the different components of various complexes involve in eukaryotic respiratory electron transport chain?
- Write a short note on Q-cycle.
- Write an essay on respiratory electron transport system of eucaryotes.
- Explain respiratory electron transport process of bacteria.
  - *E. coli*
  - *Paracoccus denitrificans*
- What do you mean by oxidative phosphorylation? What are the steps of oxidative synthesis?
- Explain chemiosmotic model of ATP synthesis.
- Explain structure of ATP synthase.
- Explain binding change mechanism of ATP synthesis given by Paul D. Boyer.
- Differentiate between oxidative and substrate level phosphorylation.
- What is Pasteur effect? Briefly discuss.