M.Sc. Botany
Semester-II (2018-20)
MBOTCC-7: Physiology & Biochemistry

Unit –V
OXIDATIVE PHOSPHORYLATION
&
ELECTRON TRANSPORT SYSTEM (ETS)

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OXIDATIVE PHOSPHORYLATION & ELECTRON TRANSPORT SYSTEM (ETS)

Oxidative phosphorylation Is the process by which ATP is formed as electrons are transferred from the reduced forms of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH2) to molecular oxygen (O_2) by a series of electron transporters (i.e. the electron transport chain). All oxidative steps in the degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP.
In eukaryotes, oxidative phosphorylation occurs in mitochondria and involves huge protein complexes embedded in the mitochondrial membranes.
The mechanism of oxidative phosphorylation has three defining components.
(1) Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential (molecular oxygen, O2).

(2) The free energy made available by this ″downhill″ (exergonic) electron flow is coupled to the ″uphill″ transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a transmembrane electrochemical potential .
(3) The transmembrane flow of protons back down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP, in a process called **chemiosmosis** (introduced by Peter Mitchell in 1961, that transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions).

The Mitochondrial Respiratory Chain (ETS)

Oxidative phosphorylation begins with the entry of electrons into the series of electron carriers called the respiratory chain. Most of these electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors—nicotinamide nucleotides or flavin nucleotides.

Electron Carriers Function in Multienzyme Complexes

The electron carriers of the respiratory chain are organized into membrane embedded supramolecular complexes. Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II).
Complex III carries electrons from reduced ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O_2.

1. **Complex I**: NADH to Ubiquinone In mammals, Complex I, also called **NADH:ubiquinone oxidoreductase** or **NADH dehydrogenase**, is a large enzyme composed of 45 different polypeptide chains, including an FMN containing flavoprotein and at least 8 iron-sulfur centers. Complex I is L shaped, with one arm embedded in the inner membrane and the other extending into the matrix. Complex I catalyzes two simultaneous and obligately coupled processes:

(i) the exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by

$$\mathbf{NADH + H^+ + Q \rightarrow NAD^+ + QH_2}$$

(ii) the endergonic transfer of four protons from the matrix to the intermembrane space. Protons are moved against a transmembrane proton gradient in this process. Complex I is therefore a proton pump driven by the energy of electron transfer, and the reaction it catalyzes is vectorial.

$$\mathbf{NADH + 5H^{+}_{N} + Q \rightarrow NAD^{+} + QH_2 + 4H^{+}_{P}}$$

- Amytal (a barbiturate drug), rotenone (a plant product commonly used as an insecticide), and piericidin A (an antibiotic) inhibit electron flow from the Fe- S centers of Complex I to ubiquinone and therefore block the overall process of oxidative phosphorylation.

2. **Complex II**: Succinate to Ubiquinone - **succinate dehydrogenase**, the only membrane-bound enzyme in the citric acid cycle.

i. Complex II couples the oxidation of succinate at one site with the reduction of ubiquinone at another site about 40 Å away.

ii. Although smaller and simpler than Complex I, Complex II contains five prosthetic groups of two types and four different protein subunits .

iii. Subunits C and D are integral membrane proteins, each with three transmembrane helices. They contain a heme group, heme b, and a binding site for Q, the final electron acceptor in the reaction catalyzed by Complex II.

iv. Subunits A and B extend into the matrix; they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate.

v. Electron transfer through Complex II is not accompanied by proton pumping across the inner membrane, although the QH_2 produced by succinate oxidation will be used by Complex III to drive proton transfer.

3. **Complex III**: Complex III (also called **cytochrome bc1** complex or **ubiquinone:cytochrome c oxidoreductase**) couples the transfer of electrons from ubiquinol to cytochrome c with the vectorial transport of protons from the matrix to the intermembrane space.

i. The functional unit of Complex III is a dimer. Each monomer consists of three proteins central to the action of the complex: cytochrome b, cytochrome c1, and the Rieske iron sulfur protein.

ii. To account for the role of Q in energy conservation, Mitchell proposed the "Q cycle".

The net equation for the redox reactions of the Q cycle is

$$QH_2 + 2\text{cyt c (oxidized)} + 2H^{+}_{N} \rightarrow Q + 2\text{cyt c (reduced)} + 4H^{+}_{P}$$

The Q cycle results in

i. the uptake of two protons on the matrix side and the release of four protons on the intermembrane side

ii. two QH_2 are oxidized into Q, one Q is reduced into QH_2 (recycling step).

iii. Two cytochrome c molecules are reduced

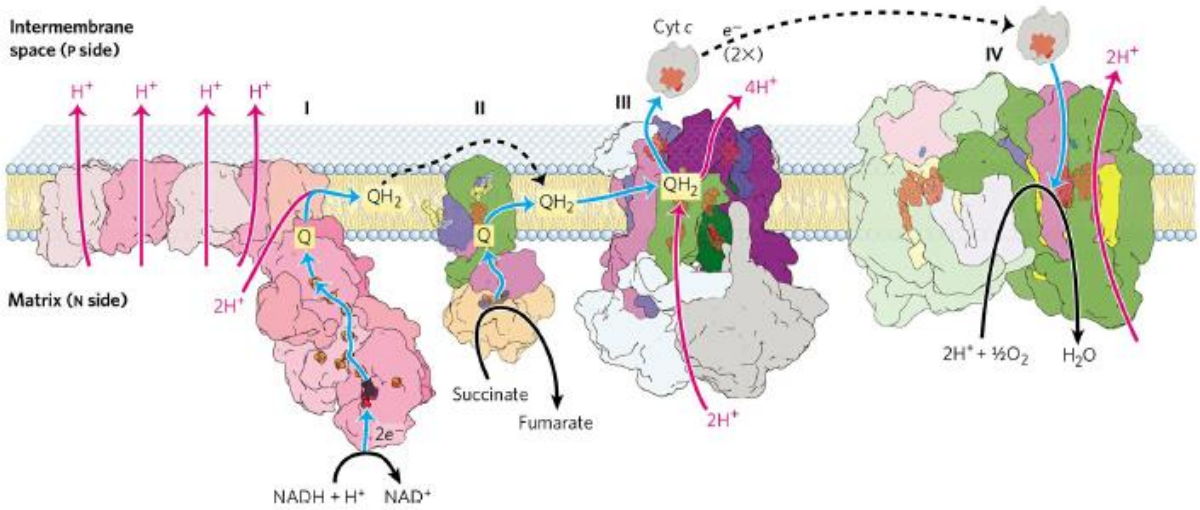


Fig: Flow of electrons and protons through the four complexes of the respiratory chain. Electrons reach Q through Complexes I and II (as well as through several other paths shown in Reduced Q (QH_2) serves as a mobile carrier of electrons and protons. It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c*. Complex IV then transfers electrons from reduced cytochrome *c* to O_2. Electron flow through Complexes I, III, and IV is accompanied by proton efflux from the matrix into the intermembrane space.

4. **Complex IV**: Cytochrome c to O_2 In the final step of the respiratory chain, Complex IV, also called **cytochrome oxidase**, carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O.

i. Complex IV is a large, dimeric enzyme of the inner mitochondrial membrane, each monomer having 13 subunits . The three proteins critical to electron flow are subunits I, II, and III.

ii. Subunit I has two heme groups, a and a3, near a single copper ion, CuB.

iii. Subunit II of Complex IV contains two Cu ions complexed with the —SH groups of two Cys residues in a binuclear center (CuA;) that resembles the 2Fe-2S centers of iron-sulfur proteins.

iv. Subunit III is essential for rapid proton movement through subunit II.

v. Electron transfer through Complex IV is from cytochrome c to the CuA center, to heme a, to the heme a3–CuB center, and finally to O_2.

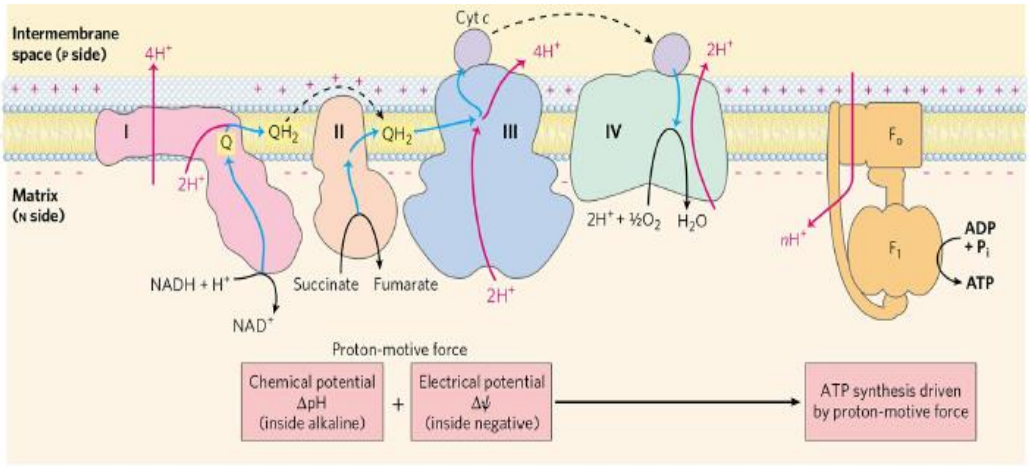
vi. The overall reaction catalyzed by Complex IV is

$$2\text{cyt c (reduced)} + 4H^+_N + \tfrac{1}{2}\,O_2 \rightarrow 2\text{cyt c (oxidized)} + 2H^+_P + H_2O$$

At Complex IV, O_2 is reduced at redox centers that carry only one electron at a time. Normally the incompletely reduced oxygen intermediates remain tightly bound to the complex until completely converted to water, but a small fraction of oxygen intermediates escape. These intermediates are reactive oxygen species that can damage cellular components unless eliminated by defense mechanisms.

ATP SYNTHESIS

1. The chemiosmotic model, proposed by Peter Mitchell, is the paradigm for energy coupling. According to the model , the electrochemical energy inherent in the difference in proton concentration and the 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 in ATP synthase.
2. The flow of electrons through Complexes I, III, and IV results in pumping of protons across the inner mitochondrial membrane, making the matrix alkaline relative to the intermembrane space. This proton gradient provides the energy, in the form of the proton-motive force, for ATP synthesis from ADP and Pi by ATP synthase (FoF1 complex) in the inner membrane.
3. 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.



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 ($\Delta\psi$), which, combined, create the proton-motive force.

Chemiosmotic theory readily explains the dependence of electron transfer on ATP synthesis in mitochondria.