CC4: Cell Biology
Unit 3: Cytoplasmic organelles II
Chemi-osmotic hypothesis

Ruksa Nur
Assistant Professor
Department of Zoology
Dinabandhu Andrews College
Kolkata-700 084

Chemi-osmotic hypothesis

It is explained that the primary event in oxidative phosphorylation is the translocation of protons (H^+) to the exterior of a coupling membrane driven by oxidation in the respiratory chain.

The membrane is impermeable to protons which accumulate outside the membrane creating an electrochemical potential difference across the membrane. This electrochemical potential difference is utilized to drive a membrane-located ATP synthetase which, in the presence of P_i+ A DP, forms ATP (Fig. 12.28).

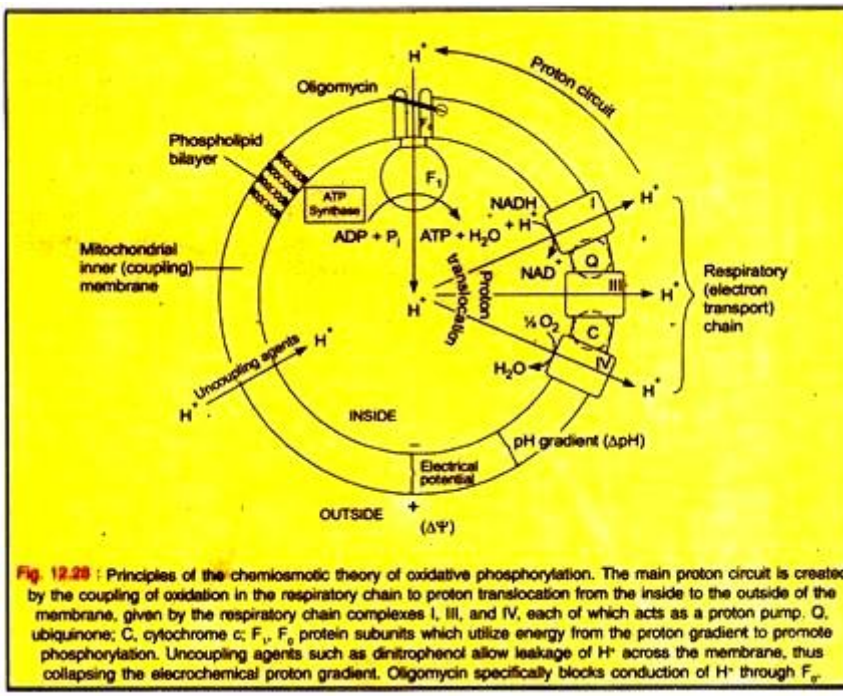


Fig. 12.28 : Principles of the chemiosmotic theory of oxidative phosphorylation. The main proton circuit is created by the coupling of oxidation in the respiratory chain to proton translocation from the inside to the outside of the membrane, given by the respiratory chain complexes I, III, and IV, each of which acts as a proton pump. Q, ubiquinone; C, cytochrome c; F_1, F_0 protein subunits which utilize energy from the proton gradient to promote phosphorylation. Uncoupling agents such as dinitrophenol allow leakage of H^+ across the membrane, thus collapsing the elecrochemical proton gradient. Oligomycin specifically blocks conduction of H^+ through F_0.

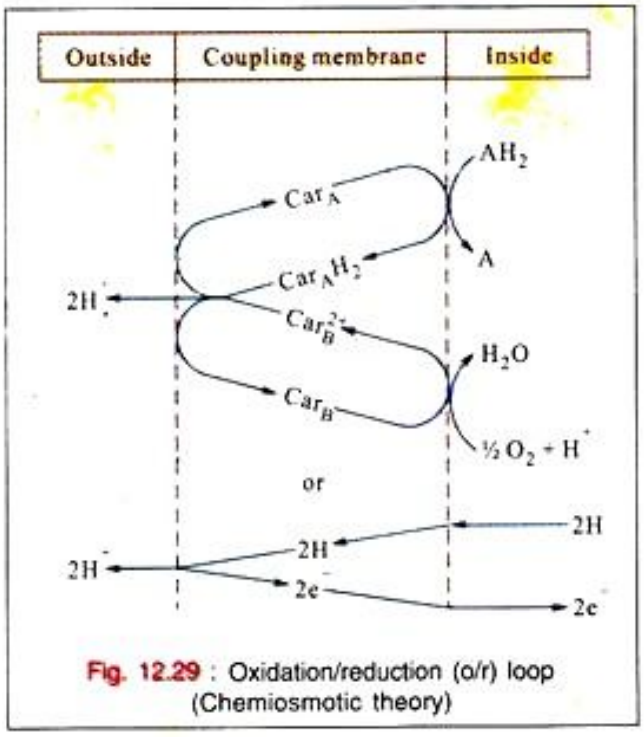


Fig. 12.29 : Oxidation/reduction (o/r) loop (Chemiosmotic theory)

It is suggested that the respiratory chain is folded into three oxidation/reduction (o/r) loops in the membrane, each loop functionally corresponds to site I, site II, and site III of the respiratory chain. A single loop consists of a hydrogen carrier and an electron carrier shown in Fig. 12.29.

A configuration of the respiratory chain folded into three functional o/r loops is shown in Fig. 12.30.

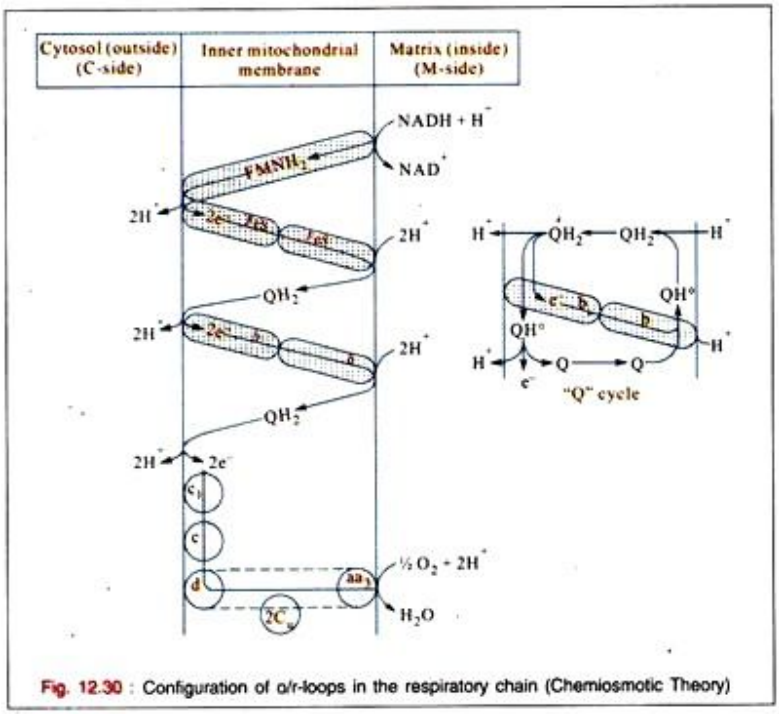
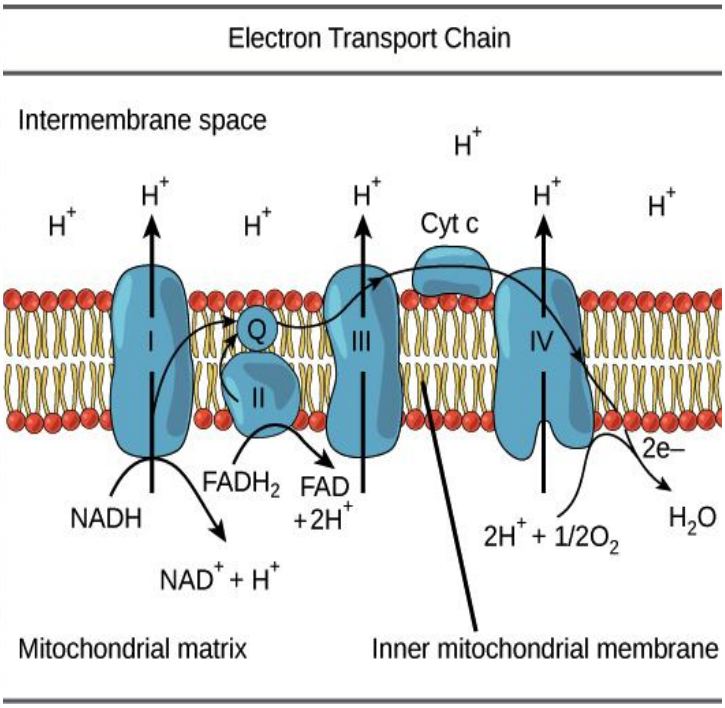


Fig. 12.30 : Configuration of o/r-loops in the respiratory chain (Chemiosmotic Theory)



Each electron pair transferred from NADH to oxygen causes six protons to be trans-located from the inside to the outside of the mitochondrial membrane. NADH first donates one proton and two electrons, which, together with another proton from the internal medium, reduce FMN to $FMNH_2$. FMN is considered to extend the full width of the membrane and enables it to release two protons to the outside of the membrane and then to return two electrons to the inside surface via FeS proteins which is reduced.

Each reduced FeS complex donates one electron to an ubiquinone (Q) molecule which, upon taking up a proton from inside the membrane, forms QH_2. QH_2, being lipid-soluble and a small molecule, is free to move to the outside of the membrane where it discharges a proton pair into the cytosol and donates two electrons to two molecules of the next carrier in the respiratory chain, cytochrome b.

This electron carrier is thought to span the mitochondrial membrane, enabling the electrons to join another molecule of ubiquinone together with two more protons from the internal medium.

The resulting QH_2 shuttles to the outer surface where two protons are liberated and two electrons passed to two molecules of cytochrome C.

These electrons then pass through the remainder of the cytochrome chain, traversing the membrane to cytochrome aa_3 which lies on the inside of the membrane. At this site, two electrons combine with two H^+ from the internal medium and an oxygen atom to form water.

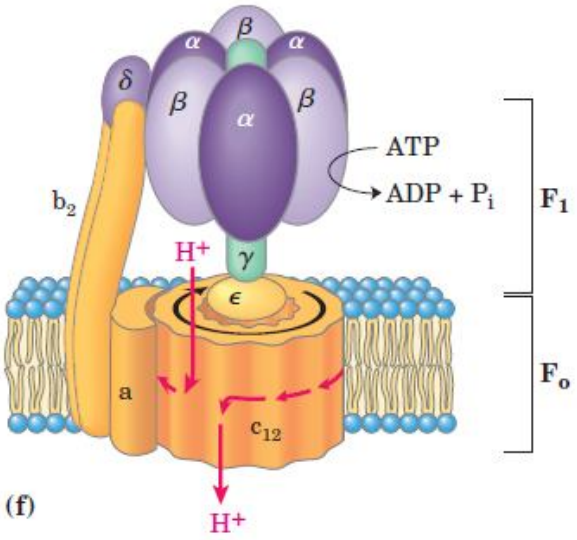


FIGURE 11-39 Structure of the F_oF_1 ATPase/ATP synthase. F-type ATPases have a peripheral domain, F_1, consisting of three α subunits, three β subunits, one δ subunit (purple), and a central shaft (the γ subunit, green). The integral portion of F-type ATPases, F_o (yellow), has multiple copies of c, one a, and two b subunits. F_o provides a transmembrane channel through which about four protons are pumped (red arrows) for each ATP hydrolyzed on the β subunits of F_1. The remarkable mechanism by which these two events are coupled is described in detail in Chapter 19. It involves rotation of F_o relative to F_1 (black arrow). The structures of V_oV_1 and A_oA_1 are essentially similar to that of F_oF_1, and the mechanisms are probably similar, too.

The phosphorylating subunits responsible for the production of ATP are scattered over the surface of the inner membrane. These consist of several proteins collectively known as an F, subunit which projects into the matrix and which contains the ATP synthetase (Fig. 12.28).

These subunits are attached, possibly by the stalk, to a membrane protein subunit known as F_0, which probably extends through the membrane. For every proton pair passing through the F_0 – F_1 complex, one ATP molecule is formed from ADP and P_i.

A model was suggested which is shown in Fig. 12.31. A proton pair attacks one oxygen of P_i to form H_2O and an active form of P_i which immediately combines with ADP to form ATP. Other studies have suggested that ATP synthesis is not the main energy-requiring step-rather it is

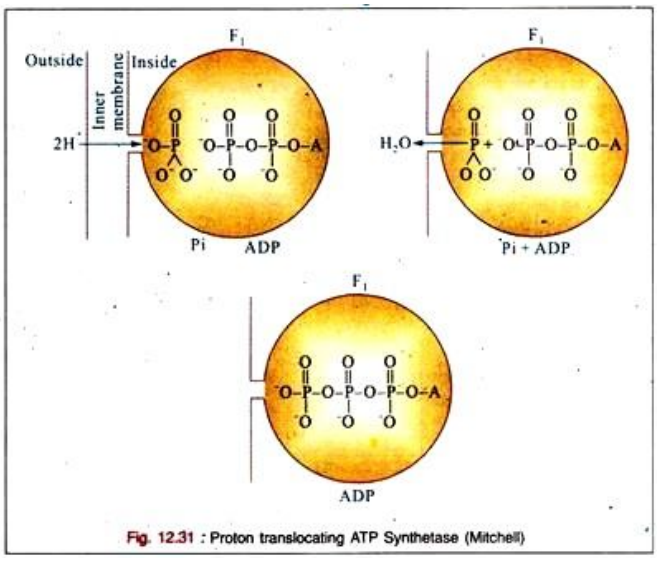


Fig. 12.31 : Proton translocating ATP Synthetase (Mitchell)

the release of ATP from the active site. This may involve conformational changes in the F_1 subunit.

The ion leakage would have to be balanced by extrusion of ions against the electric gradient to prevent swelling and lysis. It was, therefore, postulated that the coupling membrane contains exchange diffusion systems for exchange of anions against OH^- ions and of cations against H^+ ions. Such system would be necessary for uptake of ionized metabolites through the membrane.

The electrochemical potential difference across the membrane would inhibit further transport of reducing equivalents through the o/r loops unless it was discharged by back translocation of protons across the membrane through the vectorial ATP synthetase system. This depends on the availability of ADP and P_i.

Several points arise from the chemiosmotic theory that have experimental support.

These are:

1. Mitochondria are generally impermeable to protons and other ions. The specific transport systems enable ions to penetrate the inner mitochondrial membrane.

2. Un-couplers like dinitrophenol increase the permeability of mitochondria to protons reducing the electrochemical potential for the generation of ATP.

3. Addition of acid to the external medium leads to the generation of ATP.

4. The P/H^+ (transported out) quotient of the ATP synthetase is 1/2 and the H^+/O quotients for succinate and β- hydroxybutyrate oxidation are 4 and 6, respectively, conforming with the expected P/O ratios of 2 and 3, respectively. These ratios are compatible with the postulated existence of o/r loops in the respiratory chain.

5. Oxidative phosphorylation does not occur in soluble systems where there is no possibility of a vectorial ATP synthetase.

The Creatine phosphate shuttle facilitates transport of high energy phosphate from mitochondria:

1. An isoenzyme of creatine kinase is found in the mitochondria catalysing the transfer of high energy phosphate to creatine from ATP. The creatine phosphate is transported into the cytosol via protein pores in the outer mitochondrial membrane.

2. Different isoenzymes of creatine kinase cause transfer of high-energy phosphate to and from the various systems that utilize it (e.g., muscle contraction, glycolysis).

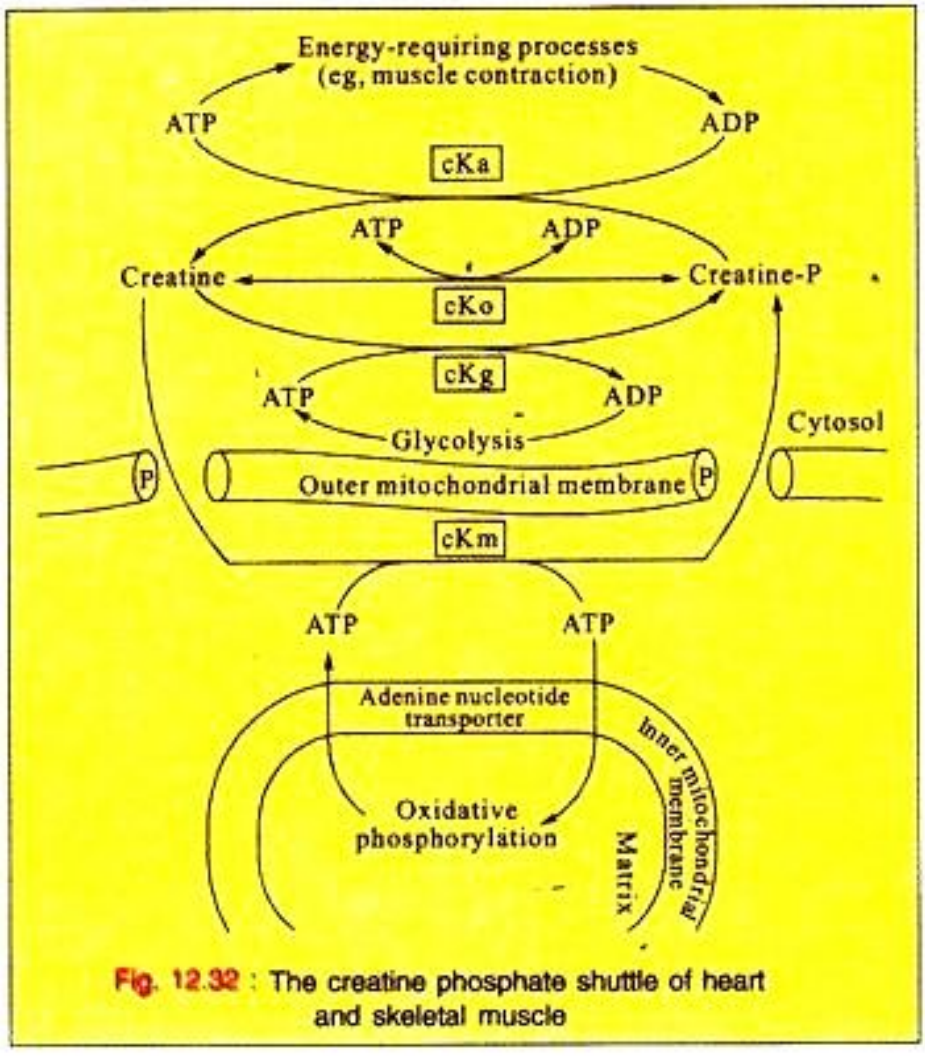


Fig. 12.32 : The creatine phosphate shuttle of heart and skeletal muscle