The Respiratory Chain

In the respiratory chain, also known as electron transport chain (ETC), the NADH and $FADH_2$ accumulated in the preceding degradative pathways, are finally disposed by reacting them with molecular oxygen. The free energy of this "cold combustion" is used to generate ATP.

Respiratory Chain Complexes:

Components of the respiratory chain are contained in 4 large protein complexes embedded in the inner mitochondrial membrane.

Complex I (NADH-Q oxidoreductase)

It is a **large protein** with many molecules of **FMN** and **iron sulfur centers**.

Complex I accepts hydrogen from NADH, thus it is oxidized back to NAD^+ and thereby prepared for the next round of glycolysis, citric acid cycle or by pyruvate dehydrogenase. The electrons are then passed via FMN then several Fe-S centers to the small carrier molecule ***ubiquinone***, also known as coenzyme Q. Four protons are expelled to the intermembrane space.

Complex II (Succinate dehydrogenase; Succinate-Q oxidoreductase)

This complex is actually the succinate dehydrogenase that catalyze the dehydrogenation of succinate to fumarate in the citric acid cycle. This enzyme is the only membrane bound enzyme of the enzymes catalyzing reactions of the citric acid cycle. $FADH_2$ formed during the conversion of succinate to fumarate is oxidized and electrons are then passed via several Fe-S centers to Q; but no proton extrusion occurs at complex II.

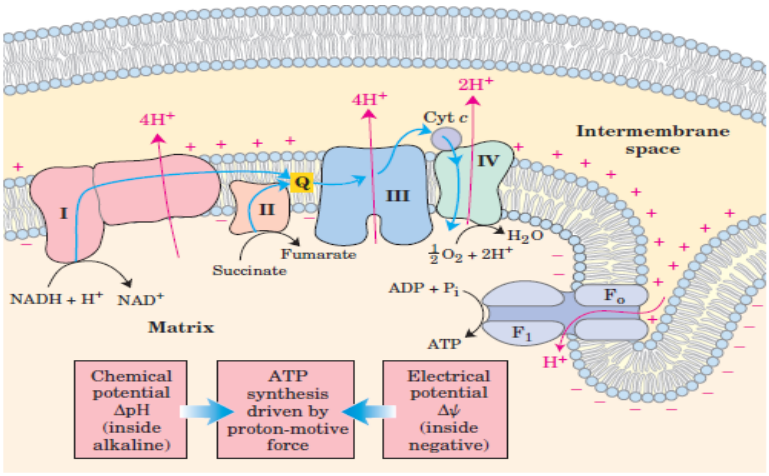
Complex III (Q-Cytochrome c oxidoreductase)

Electrons passed to ubiquinone (Q) make it reduced (QH_2 or ubqiuinol). Complex III is a multi-subunit structure that functions to accept electrons from ubiquinol and transfer them onto another electron carrier called cytochrome c. Complex III itself consists of three important groups: cytochrome c1, cytochrome b1, cytochrome b_H and a Fe-S center. The process by which the electrons are transferred from the ubiquinol to cytochrome c is known as the **Q cycle**. The considerable free energy associated with this electron transfer step is utilized to expel 4 protons from the mitochondrial matrix.

Complex IV (Cytochrome c oxidase)

Catalyzes the reoxidation of cytochrome c. The electrons are transferred from reduced cytochrome c to oxygen forming water molecules; the electron transfer generates sufficient free energy that is utilized to expel 2 protons from the mitochondrial matrix. This complex has many prosthetic groups that are: two heme groups (cytochrome a and cytochrome a3), and Cu centers (CuA and CuB). Electrons are passed initially to a Cu center (CuA), then in sequence to cytochrome a, cytochrome a 3, a second Cu center, CuB , and finally to O_2 .

The Chemiosmotic Theory



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

The ***chemiosmotic theory***, postulates that the two processes are coupled by a proton gradient across the inner mitochondrial membrane so that the ***proton motive force*** caused by the ***electrochemical potential difference*** (negative on the matrix side) drives the mechanism of ATP synthesis. As we have seen, Complexes I, III, and IV act as proton pumps. Since the inner mitochondrial membrane is impermeable to ions in general and particularly to protons, these accumulate in the intermembrane space, creating the proton motive force predicted by the chemiosmotic theory.

ATP Synthase

The proton motive force drives a membrane-located ATP synthase that forms ATP in the presence of P_i + ADP. ATP synthase is embedded in the inner membrane, together with the respiratory chain complexes. Several sub-units of the protein form a ball-like shape arranged around an axis known as F1, which projects into the matrix and contains the phosphorylation mechanism. F1 is attached to a membrane protein complex known as F0, which also consists of several protein subunits. F0 spans the membrane and forms a proton channel. The flow of protons through F0 causes it to rotate, driving the production of ATP in the F1 complex. This is thought to occur by a binding change mechanism in which the

conformation of the β-subunits in F1 is changed as the axis rotates from one that binds ATP tightly to one that releases ATP and binds ADP and P_i so that the next ATP can be formed.

Amount of ATP produced by oxidative phosphorylation vs. that produced substrate level phosphorylation of 1 mole of glucose

There is a net direct capture of two high-energy phosphate groups in the glycolytic reactions. Two more high-energy phosphates per mole of glucose are captured in the citric acid cycle during the conversion of succinyl CoA to succinate. All of these phosphorylations occur at the substrate level. **Substrate-level phosphorylation** refers to the formation of ATP from ADP and a phosphorylated intermediate, rather than from ADP and inorganic phosphate, Pi, as is done in **oxidative phosphorylation**. For each mole of substrate oxidized via Complexes I, III, and IV in the respiratory chain (ie, via NADH), 2.5 mole of ATP are formed per 0.5 mole of O_2 consumed; ie, the P:O ratio = 2.5. Similarly, regarding $FADH_2$ P:O ratio =1.5.

Taking these values into account, it can be estimated that nearly 90% of the high-energy phosphates produced from the complete oxidation of 1 mole glucose is obtained via oxidative phosphorylation and less than 10% is obtained by substrate-level phosphorylation.

Inhibitors of The Respiratory Chain

They may be classified as inhibitors of the respiratory chain, inhibitors of oxidative phosphorylation, or uncouplers of oxidative phosphorylation.

Inhibitor	**Site of Action**
Inhibitors of the electron transport chain	
Rotenone and Amobarbital	Complex I
Malonate	Complex II
Antimycin A and Dimercaprol	Complex III
H_2S, carbon monoxide, and cyanide	Complex IV
Inhibitors of the oxidative phosphorylation or Uncouplers	
Atractyloside	Inhibiting the transporter of ADP into and ATP out of the mitochondrion
Oligomycin	Blocking the flow of protons through ATP synthase

2,4-dinitrophenol	Dissociate oxidation in the respiratory chain from phosphorylation

Note: by uncoupling oxidation in the respiratory chain from phosphorylation, the uncouplers cause respiration to become uncontrolled, since the rate is no longer limited by the concentration of ADP or P_i.

Thermogenin (The uncoupling protein; UCP) is a physiological uncoupler protein found in the mitochondrial inner membrane of brown adipose tissue that functions to generate body heat, particularly for the newborn and during hibernation in animals

Respiratory Chain Control and the Action of Uncouplers

ATP synthase activity is controlled by its substrates concentration that are ADP and P_i. In case of high energy state, ADP level is low so the rate of ATP synthase will be low. Since electron transport in the respiratory chain is coupled to phosphorylation, then the slow ADP phosphorylation results in slow dehydrogenation of NADH and $FADH_2$.

The chemiosmotic theory can account for the respiratory control and the action of uncouplers. The electrochemical potential difference across the inner mitochondrial membrane, once established as a result of proton translocation, inhibits further transport of reducing equivalents through the respiratory chain unless discharged by back-translocation of protons across the membrane through the ATP synthase. This in turn depends on availability of ADP and P*i*.

Uncouplers (eg, dinitrophenol) are amphipathic (having both hydrophilic and hydrophobic parts) and increase the permeability of the lipoid inner mitochondrial membrane to protons, thus reducing the electrochemical potential and short-circuiting the ATP synthase. In this way, oxidation can proceed without phosphorylation.

The Selective Permeability of the Inner Mitochondrial Membrane

Exchange diffusion systems involving transporter proteins that span the membrane are present in the membrane for exchange of anions against OH ions and cations against H ions. Such systems are necessary for uptake and output of ionized metabolites while preserving electrical and osmotic equilibrium.
The inner mitochondrial membrane is freely permeable to uncharged small molecules, such as oxygen, water, CO_2 , NH_3, and to monocarboxylic acids, such

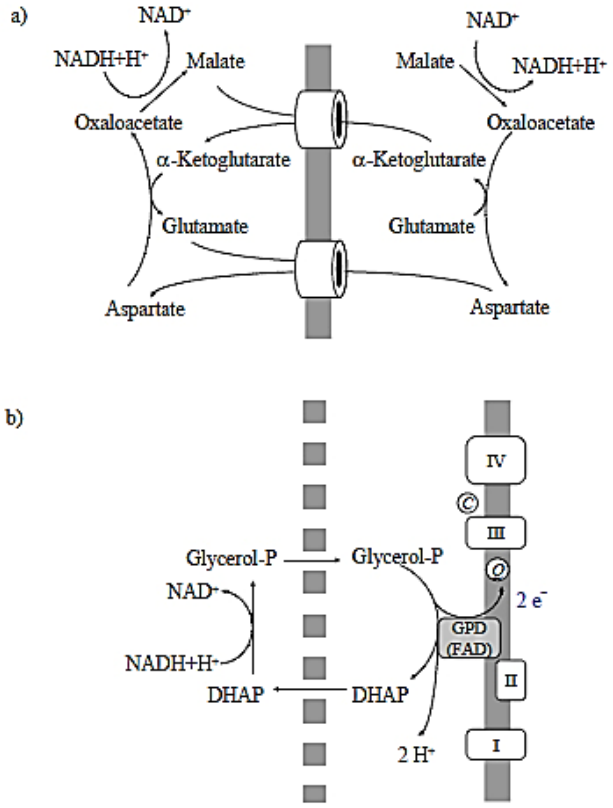
as 3-hydroxybutyric, acetoacetate, and acetate, especially in their undissociated, more lipid soluble form.
In glycolysis, it was mentioned that the NAD^+ converted to NADH by glyceraldehyde-3-phosphate dehydrogenase is re-oxidized in the respiratory chain. However, NADH cannot pass the inner mitochondrial membrane, and in fact not even the more porous outer membrane.
The mechanism of transferring of reducing equivalents through the inner mitochondrial membrane involves the use of two auxiliary shuttle systems:

Malate-Oxaloacetate shuttle

One enzyme that regenerates cytosolic NAD^+ is ***cytosolic malate dehydrogenase***, which reduces oxaloacetate to malate. Cytosolic malate is then exchanged for mitochondrial α-ketoglutarate by a specific transporter and dehydrogenated back to oxaloacetate inside the mitochondrion using ***mitochondrial malate dehydrogenase***.
Oxaloacetate is transaminated using mitochondrial glutamate and the resulting aspartate exchanged for cytosolic glutamate. In the cytosol, transamination is reversed, which closes the cycle.

Glycerolphosphate shuttle

In the glycerolphosphate shuttle, the hydrogen is never actually transported to the mitochondrion. Dihydroxyacetonephosphate (DHAP) serves as the intermediate hydrogen acceptor and is reduced in the cytosol to glycerolphosphate by glycerolphosphate dehydrogenase. Glycerolphosphate traverses the outer mitochondrial membrane and reaches the surface of the inner one, where it is converted back to DHAP by a second dehydrogenase, which abstracts the electrons and feeds them into the respiratory chain at the level of ubiquinone. This is similar to the activity of succinate dehydrogenase, and as with the latter, FAD is the coenzyme employed by the mitochondrial glycerolphosphate dehydrogenase.
Although this shuttle is present in some tissues (eg, brain, muscle), in others (eg, heart muscle) it is deficient. It is therefore believed that the malate shuttle system is of more universal utility.



Shuttle systems for the transfer of NADH equivalents from the cytosol to the mitochondrion.
a: *The malate-oaloacetate shuttle.*
b: *The glycerophoshate shuttle.*

Where:
DHAP= dihydroxyacetone phosphate
GPD= glycerolphosphate dehydrogenase.

Continuous gray bars represent the inner mitochondrial membrane; the broken bar in b represents the outer mitochondrial membrane.