➢CHEMIOSMOTIC THEORY

➢INHIBITORS AND UN-COUPLERS OF ELECTRON TRANSPORT SYSTEM

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CHEMIOSMOTIC COUPLING HYPOTHESIS

Energy released by the oxidation reactions in mitochondrial electron transport chain is used to drive the synthesis of ATP. The coupling between electron transport and oxidative phosphorylation has been explained successfully by the British scientist Peter Mitchell in 1961. Mitchell's theory is also known as **Chemiosmotic Coupling Hypothesis**. The salient features of Mitchell's hypothesis are as follows:

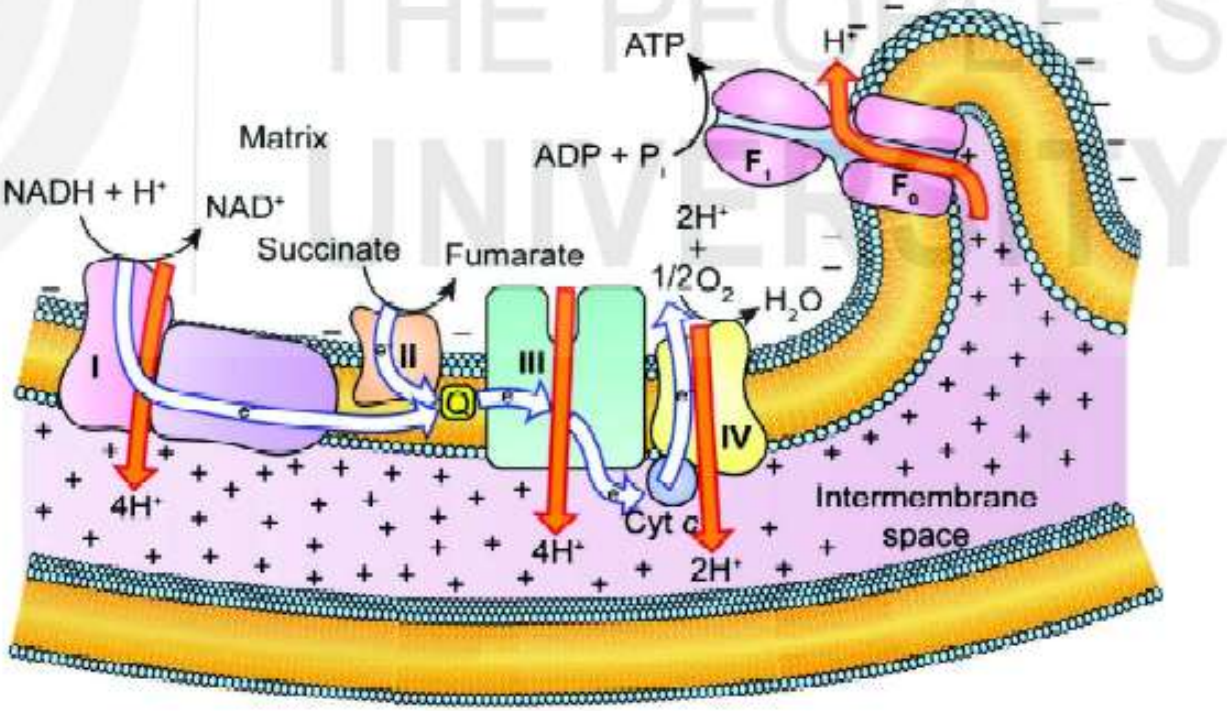
- Mitochondrial electron transport leads to proton pumping from mitochondrial matrix into the intermembrane space, thereby generating an electrochemical H^+ gradient (also known as proton motive force) across the inner mitochondrial membrane.
- The pH in the intermembrane space becomes lower than that in the matrix, and this difference in pH is called ΔpH. In addition to the pH gradient, membrane potential also contributes to the electrochemical H^+ gradient as expressed in the equation below:

$$\Delta\mu_H = \Delta\psi - 2.3RT\Delta pH/F$$

$\Delta\mu_H$	$\Delta\psi$	$2.3RT\Delta pH/F$
Electrochemical H^+ gradient	Membrane potential	pH gradient

- In other words, the free energy spent in H^+ pumping is stored in the form of proton motive force. As the H^+ concentration in the intermembrane space is higher, the protons have a tendency to flow back into the matrix, but this is not possible since the inner mitochondrial membrane is impermeable to H^+. This is the reason that generation and maintenance of proton motive force requires the inner mitochondrial membrane to be intact (Fig. 9.8).
- The F_0F_1 ATP synthase complex, which is located in the vicinity of the electron transport complexes, provides an opportunity for the protons to flow back from the intermembrane space to the mitochondrial matrix. As the protons flow back to the matrix, the energy stored as the proton motive force is dissipated. A part of this energy so released is utilized for synthesis of ATP from ADP and P_i. Fig. 9.8 shows the electron transport and generation of electrochemical gradient across mitochondrial membrane.

- In other words the H^+ pumping, that generates proton motive force, is coupled to ATP synthesis. This is known as **Chemiosmotic coupling** as proposed by Peter Mitchell.



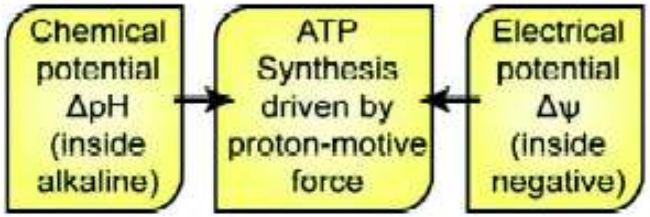


Fig. 9.8: Proton and electrochemical gradient across mitochondrial membrane. (Adapted from Garret and Grisham).

INHIBITORS OF ETC AND UNCOUPLERS

Oxidative phosphorylation is susceptible to inhibition at any point in the process. Several inhibitors have been identified which can block specific carriers within the electron transport chain or in oxidative phosphorylation to disrupt the whole process of electron transport and ATP synthesis. Use of these inhibitors under laboratory conditions has yielded valuable information on the sequence of organization of electron carriers in the respiratory chain, and the process of oxidative phosphorylation.

Inhibitors of Electron Transport Chain

Although a number of inhibitors of electron transport chain are known, but the following are worth mentioning:

i. **Rotenone:** It is a toxic plant product that was historically used by South American Indians as a fish poison, and now commonly used as an insecticide. It blocks the electron flow from NADH to CoQ.

ii. **Amytal:** It is a barbiturate drug, and acts at the same site as rotenone.

iii. **Antimycin A:** it is an antibiotic produced by *Streptomyces griseus* which blocks electron transport from cytochrome *b* to cytochrome c_1.

iv. **Cyanide, azide, hydrogen sulfide and carbon monoxide:** They function as cytochrome oxidase inhibitors. Cyanide and azide react with the oxidized form whereas carbon monoxide reacts with the reduced form of the cytochrome target.

It is important to note that if an inhibitor of electron transport binds its target electron carrier, it disrupts the continuity of electron flow. As the electron transport was continuing, all the electron carriers upstream of the target site get into reduced state, whereas all the carriers which are situated downstream of the target site remain in the oxidized state. In fact this difference in the redox state of carriers has allowed identification of the site of action of inhibitors.

Uncouplers

Uncouplers are chemical compounds which inhibit phosphorylation without any disruption in mitochondrial electron transport chain. So the electron transport continues right up to the terminal electron acceptor O_2, but ATP synthesis does not take place. The uncouplers function by dissipating the H^+ gradient across the inner mitochondrial membrane which is generated due to electron transport. In other words, **they disrupt the coupling between electron transport and oxidative phosphorylation.** This loss of respiratory control leads to increased oxygen consumption and oxidation of NADH. As a result, metabolic fuel is continued to be consumed. Energy is not conserved in the form of ATP, but it is released as heat. In fact, controlled uncoupling of oxidative phosphorylation is the biological means of heat generation. The process of uncoupling is reversible, and ATP synthesis resumes as the uncoupler is removed. **2, 4-dinitrophenol (DNP)** is a common example of uncouplers which functions by dissipation of the H^+ gradient.

The antibiotic **oligomycin** is another phosphorylation inhibitor which binds to the F_0 unit of the F_0F_1-ATP synthase complex and prevents the passage of H^+ flow through it. This prevents the dissipation of proton gradient and, therefore, energy of H^+ gradient could not be harnessed for ATP synthesis. The prevention of H^+ gradient dissipation makes it increasingly more and more difficult for the electron transport chain to pump protons into the intermembrane space. And eventually as a consequence, H^+ pumping also ceases.

Inhibitors and Uncouplers

Table 1. Inhibitors of Respiration and Oxidative Phosphory

Site-Specific	Target Complex
Carbon monoxide	IV
Cyanide	IV
Sodium Azide	IV
Rotenone	I
Antimycin A	III
Amytal	I
Phosphorylation	
Oligomycin	F_o
Uncouplers	
2,4-Dinitrophenol (DNP)	Proton gradient
Trifluorocarbonylcyanide Phenylhydrazone (FCCP)	Proton gradient

Any compound that stops electron transport will stop respiration…this means you stop breathing

Electron transport can be stopped by inhibiting ATP synthesis

An uncoupler breaks the connection between ATP synthesis and electron transport