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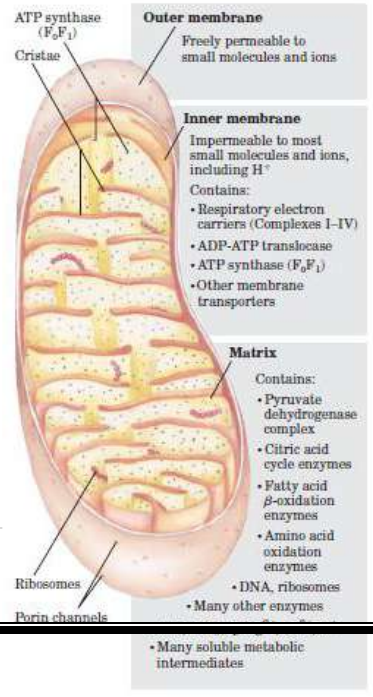
Oxidative Phosphorylation

"The aspect of the present position of consensus that I find most remarkable and admirable, is the altruism and generosity with which former opponents of the chemiosmotic hypothesis have not only come to accept it, but have actively promoted it to the status of a theory."
—Peter Mitchell, Nobel Address, 1978

- The enzymatic phosphorylation of ADP to ATP coupled to electron transfer from a substrate to molecular oxygen is called oxidative phosphorylation.
- Oxidative phosphorylation is the culmination of energy-yielding metabolism in aerobic organisms. 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. Photophosphorylation is the means by which photosynthetic organisms capture the energy of sunlight—the ultimate source of energy in the biosphere—and harness it to make ATP. Together, oxidative phosphorylation and photophosphorylation account for most of the ATP synthesized by most organisms most of the time.
- In eukaryotes, oxidative phosphorylation occurs in mitochondria, photophosphorylation in chloroplasts. Oxidative phosphorylation involves the reduction of O2 to H2O with electrons donated by NADH and FADH2; it occurs equally well in light or darkness. Photophosphorylation involves the oxidation of H2O to O2, with NADP———————————— as ultimate electron acceptor; it is absolutely dependent on the energy of light. Despite their differences,these two highly efficient energy-converting processes have fundamentally similar mechanisms.
- The current understanding of ATP synthesis in mitochondria and chloroplasts is based on the hypothesis, introduced by Peter Mitchell in 1961, that transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions.
- This chemiosmotic theory has been accepted as one of the great unifying principles of twentieth century biology. It provides insight into the processes of oxidative phosphorylation and photophosphorylation and into such apparently disparate energy transductions as active transport across membranes and the motion of bacterial flagella.
- Oxidative phosphorylation and photophosphorylation are mechanistically similar in three respects. (1) Both processes involve the flow of electrons through a chain of membrane-bound carriers. (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 (p. 390). (3) The



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transmembrane flow of protons 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.

- The discovery in 1948 by Eugene Kennedy and Albert Lehninger that mitochondria are the site of oxidative phosphorylation in eukaryotes marked the beginning of the modern phase of studies in biological energy transductions. Mitochondria, like gram-negative bacteria, have two membranes.

  - **Fig . 1 : Biochemical anatomy of a mitochondrion.** The convolutions (cristae) of the inner membrane provide a very large surface area. The inner membrane of a single liver mitochondrion may have more than 10,000 sets of electron-transfer systems (respiratory chains) and ATP synthase molecules, distributed over the membrane surface. The mitochondria of heart muscle, which have more profuse cristae and thus a much larger area of inner membrane, contain more than three times as many sets of electron-transfer systems as liver mitochondria. The mitochondrial pool of coenzymes and intermediates is functionally separate from the cytosolic pool. The mitochondria of invertebrates, plants, and microbial eukaryotes are similar to those shown here, but with much variation in size, shape, and degree of convolution of the inner membrane.

Figure 1 Biochemical anatomy of a mitochondrion.

- Oxidative phosphorylation means coupling of the electron transport in respiratory chain with phosphorylation of ADP to form ATP. It is a process by which the energy of biological oxidation is ultimately converted to the chemicalenergy of ATP.

Sites of ATP production by Oxidative Phosphorylation

- There are 3 sites. Starting through NAD+, 3 ATP are formed for each substrate molecule oxidized. Starting through FAD, 2 ATP are formed for each substrate molecule oxidized.

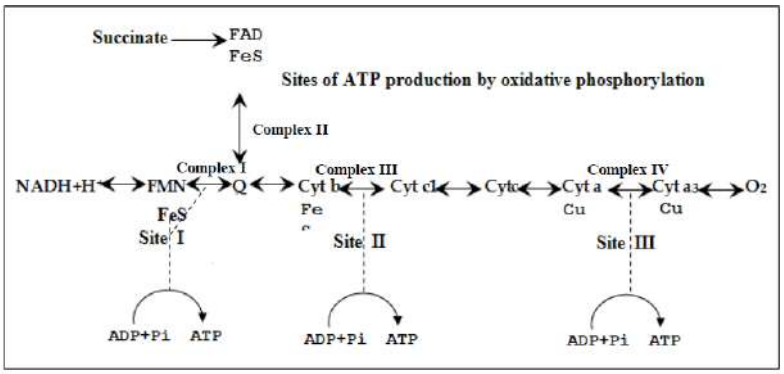

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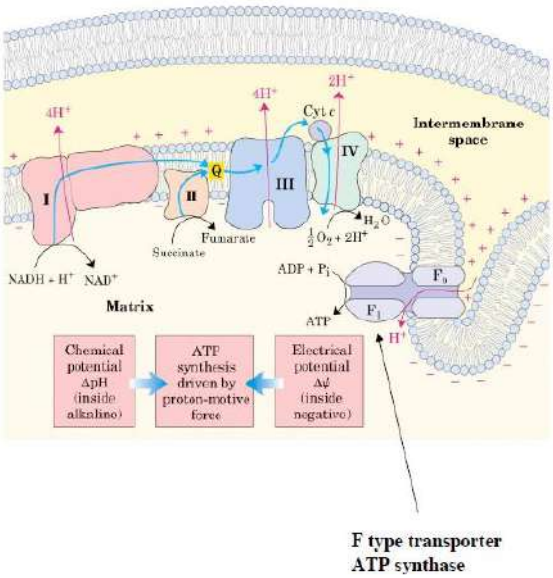
Figure 2 Sites of ATP production by Oxidative Phosphorylation

- P/O Ratio : It is ratio of the number of molecules of ADP converted to ATP to the number of oxygen atoms utilized by respiratory chain. It is a measure to the efficiency of oxidative phosphorylation. It is 3/1 if NADH+H+ is used and 2/1 if FADH2 is used.



Mechanism of oxidative phosphorylation

There are 2 theories: 1.The chemical theory. & 2.The chemiosmotic theory.

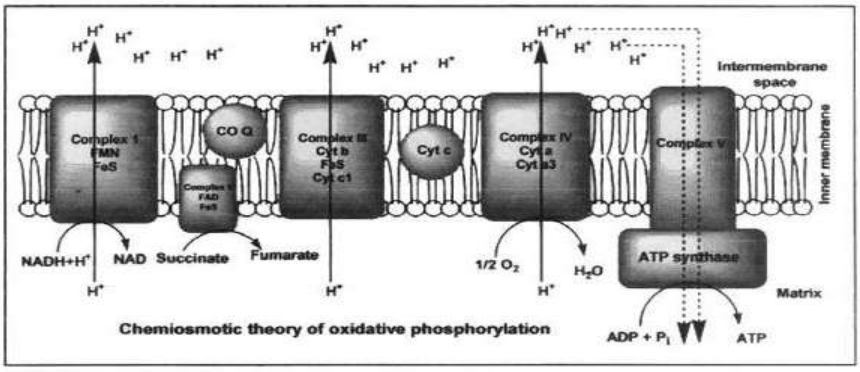
- Chemical theory :

It suggests that there is a direct chemical coupling of oxidation and phosphorylation through high-energy intermediate compounds. This theory is not accepted, as postulated high-energy intermediate compounds were never found.

- **Chemiosomotic theory :**

It suggest that the transfer of electrons through the electrons transport chain causes protons to be translocated (pumped out) from the mitochondrial matrix to the intermembrane space at the three sites of ATP production (i.e. it acts as a proton pump) resulting in an electrochemical potential difference across the inner mitochondrial membrane.

Figure 3 Oxidative Phosphorylation in brief



- The electrical potential difference is due to accumulation of the positively charged hydrogen ions outside the membrane. The chemical potential difference is due to the difference in pH, being more acidic outside the membrane. This difference forces ATP synthase to generate ATP from ADP and inorganic phosphate.

Figure 4 The Chemiosomotic theory of Oxidative phosphorylation

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- ATP synthase complex consists of: 1. Fo (multiple C-protein subunits act as proton channels) 2. F1 is formed of 3α & 3β subunits. 3. γ-subunits fits inside the F1.• Fo spans the inner mitochondrial membrane and is attached to F1 by γ- subunit.

Mechanism of ATP production by ATP synthase :

- Flow of H+ through F0 causes it to rotate. Rotation of F0 causes γ-subunits to rotate. F1 subunit is fixed and don't rotate and ADP+P are taken by β subunits of F1 to form ATP. ATP is expelled as γ –subunit squeezes β subunit and F1. • Each NADH oxidized translocate 4 H+ through complex I and 6 H+ through complex III and IV (10 H+). As 4 H+ taken give 1 ATP so oxidation of NADH gives 2.5 ATP.

Inhibitors of oxidative phosphorylation

- They are classified into: A. Specific-site inhibitors: They block the oxidation process at specific sites on the respiratory chain (at one of the 3 sites of ATP production B. Non specific-site inhibitors: inhibit phosphorylation.
- A- Specific-site inhibitors
- 1. Site 1 inhibitors : These are substances that inhibit electron transport from reduced FMN to coenzyme Q. I. Rotenone (insecticide and fish poisoning), II. Chloropromazine (tranquilizer), III. Barbiturates (hypnotic), IV. Alkyl guanidine (hypotensive).

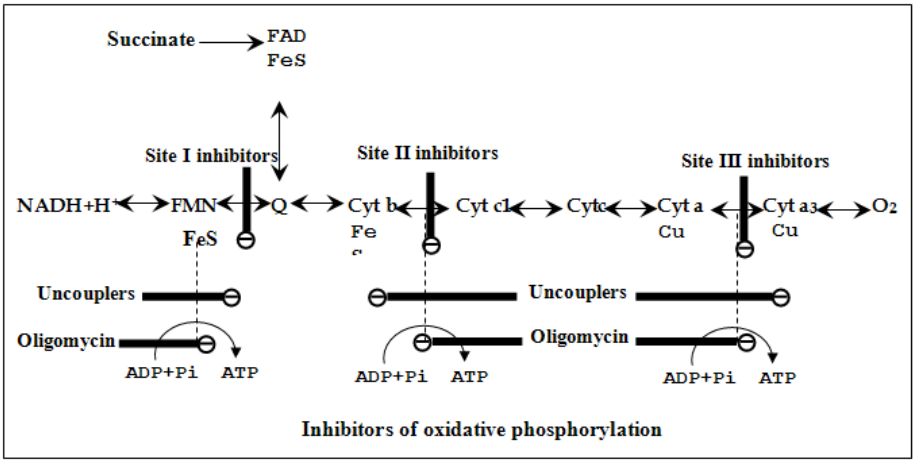

Figure 5 Inhibitors of Oxidative Phosphorylation

- 2- Site II inhibitors : These are substances that inhibit electron transport from reduced cyt b to cyt c1. I. Antimycin A (antibiotic), II. BAL (British Anti Lewisite), III. Phenformine (hypoglycemic), IV. Dimercaprol, V. Napthoquinone.
- 3- Site III inhibitors : These are substances that inhibit electron transport from reduced cyt a to cyt a3. I. Cyanide, II. Carbon monoxide (CO), III. Hydrogen sulphide (H2S), IV. Sodium azide.
- B- Non specific-site inhibitors :
- They block phosphorylation, but they prevent the whole process of oxidative phosphorylation e.g. I. Oligomycin (antibiotic) inhibits ATP synthase enzyme, II. Atractyloside (herbicide) inhibits ADP/ ATP transporter which is responsible for the transport of ADP into the mitochondria and the transport ATP out of the mitochondria

Uncouplers

They dissociate oxidation from phosphorylation leading to loss of the resulting energy as heat. There is normal oxygen consumption without ATP generation and P/O ratio becomes zero. They are amphipathic; increasing membrane permeability to protons which leads to transport of protons into the mitochondrial matrix reducing the electrochemical potential difference, so, ATP will not be generated.

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Examples of uncouplers : I. 2,4 dinitrophenol. II. Dinitrocrisol. III. Pentachlorophenol. IV. Calcium injection. V. Thyroid hormones. VI. Progesterone. N.B. Uncoupling proteins (Thermogenin) -They are responsible for fatty acid oxidation & heat production in brown adipose tissue of mammals. -Uncoupling proteins (UCP1, UCP2 & UCP3) have been found in humans but their exact function is controversial.

Some elements/ reactants/intermediates/facts of oxidative phosphorylation

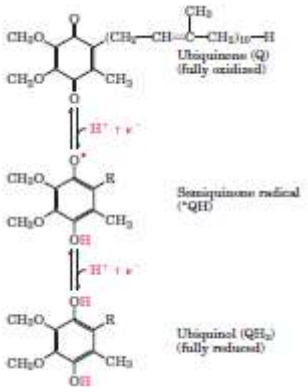


Figure 5 **Ubiquinone (Q, or coenzyme Q)**

- **Ubiquinone (Q, or coenzyme Q).** Complete reduction of ubiquinone requires two electrons and two protons, and occurs in two steps through the semiquinone radical intermediate.
- Ubiquinone (also called coenzyme Q, or simply Q) is a lipid-soluble benzoquinone with a long isoprenoid side chain (Fig. 19–2). The closely related compounds plastoquinone (of plant chloroplasts) and menaquinone (of bacteria) play roles analogous to that of ubiquinone, carrying electrons in membrane-associated electrontransfer chains. Ubiquinone can accept one electron to become the semiquinone radical (QH) or two electrons to form ubiquinol (QH2) (Fig. 19–2) and, like flavoprotein carriers, it can act at the junction between a two electron donor and a one-electron acceptor. Because ubiquinone is both small and hydrophobic, it is freely diffusible within the lipid bilayer of the inner mitochondrial membrane and can shuttle reducing equivalents between other, less mobile electron carriers in the membrane. And because it carries both electrons and protons, it plays a central role in coupling electron flow to proton movement.

Prosthetic groups of cytochromes. Each group consists of four five-membered, nitrogen-containing rings in a cyclic structure called a porphyrin. The four nitrogen atomsare coordinated with a central Fe ion, either Fe2+ or Fe3+.Iron protoporphyrin IX is found in b-type cytochromes and in hemoglobin and myoglobin. Heme c is covalently bound to the protein of cytochrome c through thioether bonds to two Cys residues. Heme a, found in atype cytochromes, has a long isoprenoid tail attached to one of the five-membered rings. The conjugated double-bond system (shaded pink) of the porphyrin ring accounts for the absorption of visible light by these heme

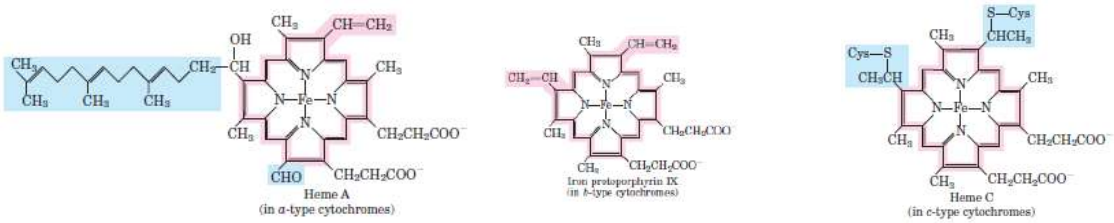
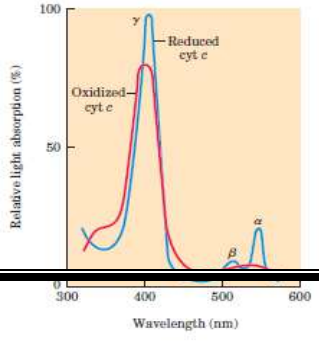


Figure 6 Prosthetic groups of cytochromes

Absorption spectra of cytochrome *c* (cyt *c*) in its oxidized (red) and reduced (blue) forms. Also labeled are the characteristic α, β and γ bands of the reduced form. The cytochromes are proteins with characteristic strong absorption of visible light, due to their iron-containing heme prosthetic groups , Mitochondria contain three classes of cytochromes, designated a b, and c, which are distinguished by differences in their



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light-absorption spectra. Each type of cytochrome in its reduced (Fe^{2+}) state has three absorption bands in the visible range (Fig. 19–4). The longest-wavelength band is near 600 nm in type a cytochromes, near 560 nm in type b, and near 550 nm in type c. To distinguish among closely related cytochromes of one type, the exact absorption maximum is sometimes used in the names, as in cytochrome b562.

Enzyme complex/protein	Mass (kDa)	Number of subunits*	Prosthetic group(s)
I NADH dehydrogenase	850	43 (14)	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone:cytochrome *c* oxidoreductase	250	11	Hemes, Fe-S
Cytochrome c^1	13	1	Heme
IV Cytochrome oxidase	160	13 (3–4)	Hemes; Cu

Figure 7 Absorption spectra of cytochrome c (cyt c)

Figure 8 The Protein Components of the Mitochondrial Electron-Transfer Chain

Path of electrons from NADH, succinate, fatty acyl–CoA, and glycerol 3-phosphate to ubiquinone.

- Electrons from NADH pass through a flavoprotein to a series of iron-sulfur proteins (in Complex I) and then to Q. Electrons from succinate pass through a flavoprotein and several Fe-S centers (in Complex II) on the way to Q. Glycerol 3-phosphate donates electrons to a flavoprotein (glycerol 3-phosphate dehydrogenase) on the outer face of the inner mitochondrial membrane, from which they pass to Q.

- Acyl-CoA dehydrogenase (the first enzyme of β oxidation) transfers electrons to electron-transferring flavoprotein (ETF), from which they pass to Q via ETF:ubiquinone oxidoreductase.

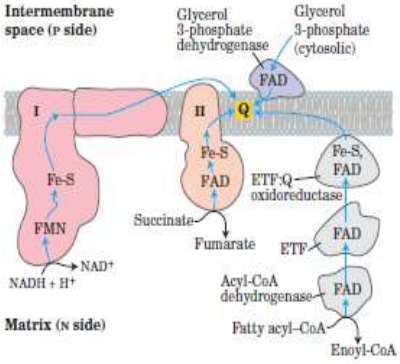

Figure 9 **Path of electrons from NADH, succinate, fatty acyl–CoA, and glycerol 3-phosphate to ubiquinone.**

Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

Type of interference	Compound*	Target/mode of action
Inhibition of electron transfer	Cyanide, Carbon monoxide	Inhibit cytochrome oxidase
	Antimycin A	Blocks electron transfer from cytochrome *b* to cytochrome c_1
	Myxothiazol, Rotenone, Amytal, Piericidin A	Prevent electron transfer from Fe-S center to ubiquinone
	DCMU	Competes with Q_B for binding site in PSII
Inhibition of ATP synthase	Aurovertin	Inhibits F_1
	Oligomycin, Venturicidin	Inhibit F_o and CF_o
	DCCD	Blocks proton flow through F_o and CF_o
Uncoupling of phosphorylation from electron transfer	FCCP, DNP	Hydrophobic proton carriers
	Valinomycin	K^+ ionophore
	Thermogenin	In brown adipose tissue, forms proton-conducting pores in inner mitochondrial membrane
Inhibition of ATP-ADP exchange	Atractyloside	Inhibits adenine nucleotide translocase

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Figure 10 Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

Note :

- Chemiosmotic theory provides the intellectual framework for understanding many biological energy transductions, including oxidative phosphorylation and photophosphorylation. The mechanism of energy coupling is similar in both cases: the energy of electron flow is conserved by the concomitant pumping of protons across the membrane, producing an electrochemical gradient, the proton-motive force.
- In mitochondria, hydride ions removed from substrates by NAD-linked dehydrogenases donate electrons to the respiratory (electron-transfer) chain, which transfers the electrons to molecular O2, reducing it to H2O. Shuttle systems convey reducing equivalents from cytosolic NADH to mitochondrial NADH.
- Reducing equivalents from all NAD-linked dehydrogenations are transferred to mitochondrial NADH dehydrogenase (Complex I). Reducing equivalents are then passed through a series of Fe-S centers to ubiquinone, which transfers the electrons to cytochrome b, the first carrier in Complex III. In this complex, electrons take two separate paths through two b-type cytochromes and cytochrome c1 to an Fe-S center. The Fe-S center passes electrons, one at a time, through cytochrome c and into Complex IV, cytochrome oxidase. This copper-containing enzyme, which also contains cytochromes a and a3, accumulates electrons, then passes them to O2, reducing it to H2O.
- Some electrons enter this chain of carriers through alternative paths. Succinate is oxidized by succinate dehydrogenase (Complex II), which contains a flavoprotein that passes electrons through several Fe-S centers to ubiquinone. Electrons derived from the oxidation of fatty acids pass to ubiquinone via the electron-transferring flavoprotein.
- Potentially harmful reactive oxygen species produced in mitochondria are inactivated by a set of protective enzymes, including superoxide dismutase and glutathione peroxidase.
- Plants, fungi, and unicellular eukaryotes have, in addition to the typical cyanide-sensitive path for electron transfer, an alternative, cyanide-resistant NADH oxidation pathway.

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