

BIOLOGICAL OXIDATION

Electron transport chain

An **electron transport chain (ETC)** couples electron transfer between an electron donor (such as NADH) and an electron acceptor (such as O₂) with the transfer of H⁺ ions (protons) across a membrane. The resulting electrochemical proton gradient is used to generate chemical energy in the form of adenosine triphosphate (*ATP*). Electron transport chains are the cellular mechanisms used for extracting energy from sunlight in photosynthesis and also from redox reactions, such as the oxidation of sugars (respiration).

In chloroplasts, light drives the conversion of water to oxygen and NADP⁺ to NADPH with transfer of H⁺ ions across chloroplast membranes. In mitochondria, it is the conversion of oxygen to water, NADH to NAD⁺ and succinate to fumarate that are required to generate the proton gradient. Electron transport chains are major sites of premature electron leakage to oxygen, generating superoxide and potentially resulting in increased oxidative stress.

Electron transport chains in mitochondria

Most eukaryotic cells have mitochondria, which produce ATP from products of the citric acid cycle, fatty acid oxidation, and amino acid oxidation. At the mitochondrial inner membrane, electrons from NADH and succinate pass through the electron transport chain to oxygen, which is reduced to water. The electron transport chain comprises an enzymatic series of electron donors and acceptors. Each electron donor passes electrons to a more electronegative acceptor, which in turn donates these electrons to another acceptor, a process that continues down the series until electrons are passed to oxygen, the most electronegative and terminal electron acceptor in the chain. Passage of electrons between donor and acceptor releases energy, which is used to generate a proton gradient across the mitochondrial membrane by actively “pumping” protons into the intermembrane space, producing a thermodynamic state that has the potential to do work. The entire process is called oxidative phosphorylation, since ADP is phosphorylated to ATP using the energy of hydrogen oxidation in many steps.

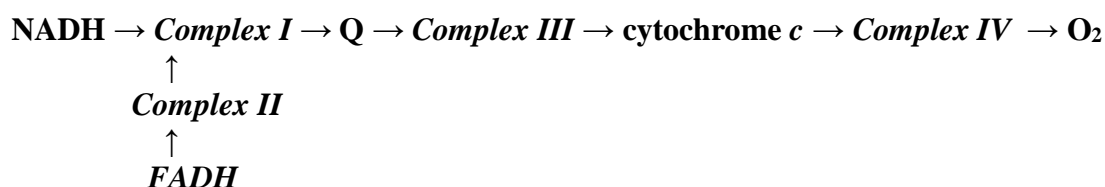
A small percentage of electrons do not complete the whole series and instead directly leak to oxygen, resulting in the formation of the free-radical superoxide, a highly reactive molecule that contributes to oxidative stress and has been implicated in a number of diseases and aging.

Mitochondrial redox carriers

Energy obtained through the transfer of electrons (black arrows) down the ETC is used to pump protons (red arrows) from the mitochondrial matrix into the intermembrane space, creating an electrochemical proton gradient across the mitochondrial inner membrane (IMM) called $\Delta\Psi$. This electrochemical proton gradient allows ATP synthase (ATP-ase) to use the flow of H⁺ through the enzyme back into the matrix to generate ATP from adenosine diphosphate (ADP)

and inorganic phosphate. Complex I (NADH coenzyme Q reductase; labeled I) accepts electrons from the Krebs cycle electron carrier nicotinamide adenine dinucleotide (NADH), and passes them to coenzyme Q (ubiquinone; labeled UQ), which also receives electrons from complex II (succinate dehydrogenase; labeled II). UQ passes electrons to complex III (cytochrome bc₁ complex; labeled III), which passes them to cytochrome *c* (cyt *c*). Cyt *c* passes electrons to Complex IV (cytochrome *c* oxidase; labeled IV), which uses the electrons and hydrogen ions to reduce molecular oxygen to water.

Four membrane-bound complexes have been identified in mitochondria. Each is an extremely complex transmembrane structure that is embedded in the inner membrane. Three of them are proton pumps. The structures are electrically connected by lipid-soluble electron carriers and water-soluble electron carriers. The overall electron transport chain:



Complex I

In *Complex I* (NADH dehydrogenase, also called NADH:ubiquinone oxidoreductase; EC 1.6.5.3) two electrons are removed from NADH and transferred to a lipid-soluble carrier, *ubiquinone* (Q). The reduced product, *ubiquinol* (QH₂) freely diffuses within the membrane, and Complex I translocates four protons (H⁺) across the membrane, thus producing a proton gradient. Complex I is one of the main sites at which premature electron leakage to oxygen occurs, thus being one of the main sites of production of harmful superoxide.

The pathway of electrons occurs as follows:

NADH is oxidized to NAD⁺, by reducing Flavin mononucleotide to FMNH₂ in one two-electron step. FMNH₂ is then oxidized in two one-electron steps, through a semiquinone intermediate. Each electron thus transfers from the FMNH₂ to an Fe-S cluster, from the Fe-S cluster to ubiquinone (Q). Transfer of the first electron results in the free-radical (semiquinone) form of Q, and transfer of the second electron reduces the semiquinone form to the ubiquinol form, QH₂. During this process, four protons are translocated from the mitochondrial matrix to the intermembrane space.

Complex II

In *Complex II* (succinate dehydrogenase; EC 1.3.5.1) additional electrons are delivered into the quinone pool (Q) originating from succinate and transferred (via FAD) to Q. Complex II consists of four protein subunits: SDHA, SDHB, SDHC, and SDHD. Other electron donors (e.g., fatty acids and glycerol 3-phosphate) also direct electrons into Q (via FAD).

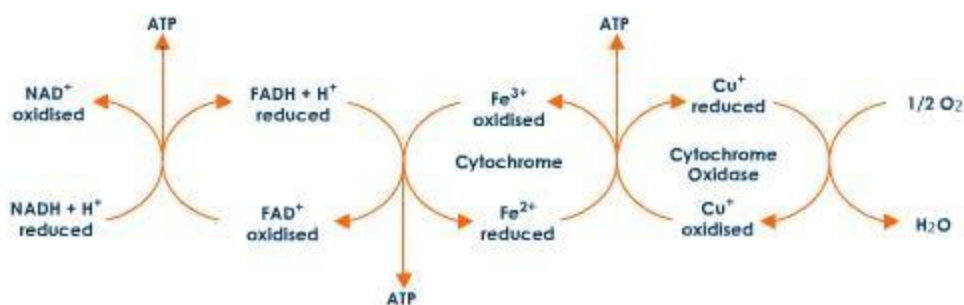
Complex III

In *Complex III* (cytochrome *bc₁* complex; EC 1.10.2.2), the Q-cycle contributes to the proton gradient by an asymmetric absorption/release of protons. Two electrons are removed from QH₂ at the Q_O site and sequentially transferred to two molecules of cytochrome *c*, a water-soluble electron carrier located within the intermembrane space. The two other electrons sequentially pass across the protein to the Q_i site where the quinone part of ubiquinone is reduced to quinol. A proton gradient is formed by two quinol (4H+4e⁻) oxidations at the Q_o site to form one quinol (2H+2e⁻) at the Q_i site. (in total six protons are translocated: two protons reduce quinone to quinol and four protons are released from two ubiquinol molecules).

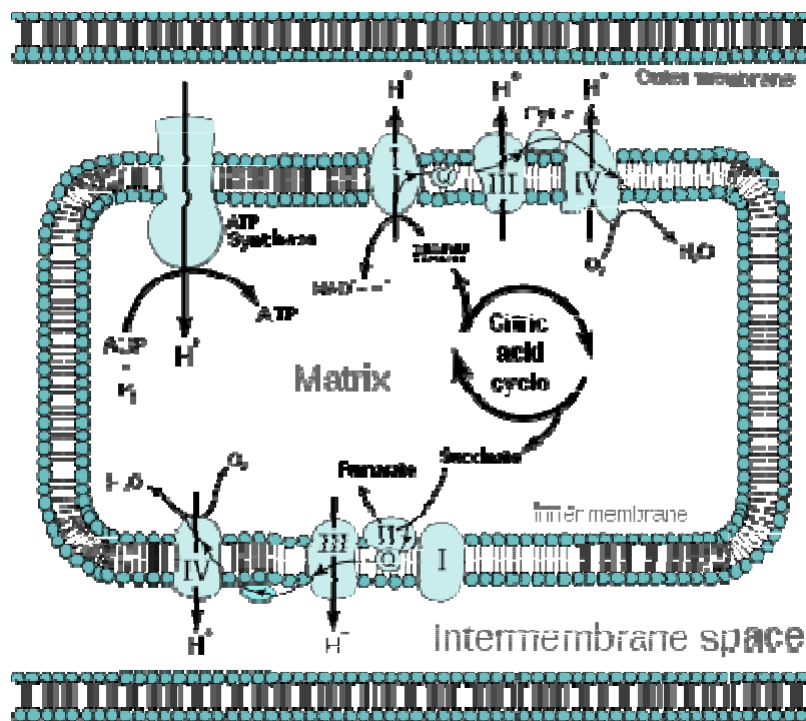
When electron transfer is reduced (by a high membrane potential or respiratory inhibitors such as antimycin A), Complex III may leak electrons to molecular oxygen, resulting in superoxide formation.

Complex IV

In *Complex IV* (cytochrome *c* oxidase; EC 1.9.3.1), sometimes called cytochrome A3, four electrons are removed from four molecules of cytochrome *c* and transferred to molecular oxygen (O₂), producing two molecules of water. At the same time, four protons are translocated across the membrane, contributing to the proton gradient. The activity of cytochrome *c* oxidase is inhibited by cyanide.



Oxidative phosphorylation



The electron transport chain in the mitochondrion is the site of oxidative phosphorylation in eukaryotes. The NADH and succinate generated in the citric acid cycle are oxidized, releasing energy to power the ATP synthase.

Oxidative phosphorylation (or OXPHOS in short) is a metabolic pathway that uses energy released by the oxidation of nutrients to produce adenosine triphosphate (ATP). Although the many forms of life on earth use a range of different nutrients, almost all aerobic organisms carry out oxidative phosphorylation to produce ATP, the molecule that supplies energy to metabolism. This pathway is probably so pervasive because it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis.

During oxidative phosphorylation, electrons are transferred from electron donors to electron acceptors such as oxygen, in redox reactions. These redox reactions release energy, which is used to form ATP. In eukaryotes, these redox reactions are carried out by a series of protein complexes within the cells intermembrane wall mitochondria, whereas, in prokaryotes, these proteins are located in the cells' intermembrane space. These linked sets of proteins are called electron transport chains. In eukaryotes, five main protein complexes are involved, whereas in prokaryotes many different enzymes are present, using a variety of electron donors and acceptors.

The energy released by electrons flowing through this electron transport chain is used to transport protons across the inner mitochondrial membrane, in a process called *chemiosmosis*. This generates potential energy in the form of a pH gradient and an electrical potential across this

membrane. This store of energy is tapped by allowing protons to flow back across the membrane and down this gradient, through a large enzyme called ATP synthase. This enzyme uses this energy to generate ATP from adenosine diphosphate (ADP), in a phosphorylation reaction. This reaction is driven by the proton flow, which forces the rotation of a part of the enzyme; the ATP synthase is a rotary mechanical motor.

Although oxidative phosphorylation is a vital part of metabolism, it produces reactive oxygen species such as superoxide and hydrogen peroxide, which lead to propagation of free radicals, damaging cells and contributing to disease and, possibly, aging (senescence). The enzymes carrying out this metabolic pathway are also the target of many drugs and poisons that inhibit their activities.

Inhibitors

There are several well-known drugs and toxins that inhibit oxidative phosphorylation. Although any one of these toxins inhibits only one enzyme in the electron transport chain, inhibition of any step in this process will halt the rest of the process. For example, if oligomycin inhibits ATP synthase, protons cannot pass back into the mitochondrion. As a result, the proton pumps are unable to operate, as the gradient becomes too strong for them to overcome. NADH is then no longer oxidized and the citric acid cycle ceases to operate because the concentration of NAD^+ falls below the concentration that these enzymes can use.

Compounds	Use	Effect on oxidative phosphorylation
Cyanide Carbon monoxide Azide	Poisons	Inhibit the electron transport chain by binding more strongly than oxygen to the Fe–Cu center in cytochrome c oxidase, preventing the reduction of oxygen.
Oligomycin	Antibiotic	Inhibits ATP synthase by blocking the flow of protons through the F_o subunit.
CCCP 2,4-Dinitrophenol	Poisons	Ionophores that disrupt the proton gradient by carrying protons across a membrane. This ionophore uncouples proton pumping from ATP synthesis because it carries protons across the inner mitochondrial membrane.
Rotenone	Pesticide	Prevents the transfer of electrons from complex I to ubiquinone by blocking the ubiquinone-binding site.
Malonate oxaloacetate	anc	Competitive inhibitors of succinate dehydrogenase (complex II).

Not all inhibitors of oxidative phosphorylation are toxins. In brown adipose tissue, regulated proton channels called uncoupling proteins can uncouple respiration from ATP synthesis.^[89] This rapid respiration produces heat, and is particularly important as a way of maintaining body temperature for hibernating animals, although these proteins may also have a more general function in cells' responses to stress.