Oxidative phosphorylation

The metabolic pathway in which cells use enzymes to oxidize nutrients, thereby releasing energy which is used to reform ATP. In most eukaryotes, this takes place inside mitochondria. Almost all aerobic organisms carry out oxidative phosphorylation.

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.

Ineukaryotes, these redox reactions are carried out by a series of protein complexes within the inner membrane of the cell's 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 protonsacross the inner mitochondrial membrane, in a process called electron transport.

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 process is known as chemiosmosis.

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 assuperoxide 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.

History

The field of oxidative phosphorylation began with the report in 1906 by Arthur Harden of a vital role for phosphate in cellular fermentation, but initially only sugar phosphates were known to be involved.

However, in the early 1940s, the link between the oxidation of sugars and the generation of ATP was firmly established by Herman Kalckar, confirming the central role of ATP in energy transfer that had been proposed by Fritz Albert Lipmann in 1941.

Later, in 1949, Morris Friedkin and Albert L. Lehninger proved that the coenzyme NADH linked metabolic pathways such as the citric acid cycle and the synthesis of ATP.

The term oxidative phosphorylation was coined by Volodymyr Belitser (uk) in 1939.

For another twenty years, the mechanism by which ATP is generated remained mysterious, with scientists searching for an elusive "high-energy intermediate" that would link oxidation and phosphorylation reactions.

This puzzle was solved by Peter D.

Mitchell with the publication of the chemiosmotic theory in 1961.

At first, this proposal was highly controversial, but it was slowly accepted and Mitchell was awarded a Nobel prize in 1978.

Subsequent research concentrated on purifying and characterizing the enzymes involved, with major contributions being made by David E.

Green on the complexes of the electron-transport chain, as well as Efraim Racker on the ATP synthase.

A critical step towards solving the mechanism of the ATP synthase was provided by Paul D.

Boyer, by his development in 1973 of the "binding change" mechanism, followed by his radical proposal of rotational catalysis in 1982.

More recent work has included structural studies on the enzymes involved in oxidative phosphorylation by John E. Walker, with Walker and Boyer being awarded a Nobel Prize in 1997

Overview of energy transfer by chemiosmosis

Oxidative phosphorylation works by using energy-releasing chemical reactions to drive energy-requiring reactions:

The two sets of reactions are said to be coupled. This means one cannot occur without the other.

The flow of electrons through the electron transport chain, from electron donors such as NADH to electron acceptors such as oxygen, is anexergonic process – it releases energy, whereas the synthesis of ATP is an endergonic process, which requires an input of energy.

Both the electron transport chain and the ATP synthase are embedded in a membrane, and energy is transferred from electron transport chain to the ATP synthase by movements of protons across this membrane, in a process called chemiosmosis.

In practice, this is like a simple electric circuit, with a current of protons being driven from the negative N-side of the membrane to the positive P-side by the proton-pumping enzymes of the electron transport chain.

These enzymes are like a battery, as they perform work to drive current through the circuit.

The movement of protons creates an electrochemical gradient across the membrane, which is often called the proton-motive force.

It has two components: a difference in proton concentration (a H+ gradient, ΔpH) and a difference in electric potential, with the N-side having a negative charge.

ATP synthase releases this stored energy by completing the circuit and allowing protons to flow down the electrochemical gradient, back to the N-side of the membrane.

This kinetic energy drives the rotation of part of the enzymes structure and couples this motion to the synthesis of ATP.

The two components of the proton-motive force are thermodynamically equivalent:

In mitochondria, the largest part of energy is provided by the potential; in alkaliphile bacteria the electrical energy even has to compensate for a counteracting inverse pH difference.

Inversely, chloroplasts operate mainly on ΔpH. However, they also require a small membrane potential for the kinetics of ATP synthesis.

At least in the case of the fusobacterium P. modestum it drives the counter-rotation of subunits a and c of the FO motor of ATP synthase

Electron and proton transfer molecules

The electron transport chain carries both protons and electrons, passing electrons from donors to acceptors, and transporting protons across a membrane.

These processes use both soluble and protein-bound transfer molecules.

In mitochondria, electrons are transferred within the intermembrane space by the water-soluble electron transfer protein cytochrome c.

This carries only electrons, and these are transferred by the reduction and oxidation of an iron atom that the protein holds within a heme group in its structure.

Cytochrome c is also found in some bacteria, where it is located within the periplasmic space.

Within the inner mitochondrial membrane, the lipid-soluble electron carrier coenzyme Q10 (Q) carries both electrons and protons by a redox cycle.

This small benzoquinone molecule is very hydrophobic, so it diffuses freely within the membrane.

When Q accepts two electrons and two protons, it becomes reduced to the ubiquinol form (QH2); when QH2 releases two electrons and two protons, it becomes oxidized back to the ubiquinone (Q) form.

As a result, if two enzymes are arranged so that Q is reduced on one side of the membrane and QH2 oxidized on the other, ubiquinone will couple these reactions and shuttle protons across the membrane.

Some bacterial electron transport chains use different quinones, such as menaquinone, in addition to ubiquinone.

Within proteins, electrons are transferred between flavin cofactors,[3][11] iron–sulfur clusters, and cytochromes.

There are several types of iron–sulfur cluster.

The simplest kind found in the electron transfer chain consists of two iron atoms joined by two atoms of inorganic sulfur; these are called [2Fe–2S] clusters.

The second kind, called [4Fe–4S], contains a cube of four iron atoms and four sulfur atoms.

Each iron atom in these clusters is coordinated by an additional amino acid, usually by the sulfur atom of cysteine.

Metal ion cofactors undergo redox reactions without binding or releasing protons, so in the electron transport chain they serve solely to transport electrons through proteins.

Electrons move quite long distances through proteins by hopping along chains of these cofactors.

This occurs by quantum tunnelling, which is rapid over distances of less than 1.4×10−9 m

Eukaryotic electron transport chains

Many catabolic biochemical processes, such as glycolysis, the citric acid cycle, and beta oxidation, produce the reduced coenzyme NADH.

This coenzyme contains electrons that have a high transfer potential; in other words, they will release a large amount of energy upon oxidation.

However, the cell does not release this energy all at once, as this would be an uncontrollable reaction.

Instead, the electrons are removed from NADH and passed to oxygen through a series of enzymes that each release a small amount of the energy.

This set of enzymes, consisting of complexes I through IV, is called the electron transport chain and is found in the inner membrane of the mitochondrion.

Succinate is also oxidized by the electron transport chain, but feeds into the pathway at a different point.

In eukaryotes, the enzymes in this electron transport system use the energy released from the oxidation of NADH to pump protons across the inner membrane of the mitochondrion.

This causes protons to build up in the intermembrane space, and generates an electrochemical gradient across the membrane.

The energy stored in this potential is then used by ATP synthase to produce ATP.

Oxidative phosphorylation in the eukaryotic mitochondrion is the best-understood example of this process.

The mitochondrion is present in almost all eukaryotes, with the exception of anaerobic protozoa such as Trichomonas vaginalis that instead reduce protons to hydrogen in a remnant mitochondrion called a hydrogenosome.

Prokaryotic electron transport chains

In contrast to the general similarity in structure and function of the electron transport chains in eukaryotes, bacteria and archaea possess a large variety of electron-transfer enzymes.

These use an equally wide set of chemicals as substrates.

In common with eukaryotes, prokaryotic electron transport uses the energy released from the oxidation of a substrate to pump ions across a membrane and generate an electrochemical gradient.

In the bacteria, oxidative phosphorylation in Escherichia coli is understood in most detail, while archaeal systems are at present poorly understood.

The main difference between eukaryotic and prokaryotic oxidative phosphorylation is that bacteria and archaea use many different substances to donate or accept electrons.

This allows prokaryotes to grow under a wide variety of environmental conditions.

In E. coli, for example, oxidative phosphorylation can be driven by a large number of pairs of reducing agents and oxidizing agents, which are listed below.

The midpoint potential of a chemical measures how much energy is released when it is oxidized or reduced, with reducing agents having negative potentials and oxidizing agents positive potentials.

ATP synthase (complex V)

ATP synthase, also called complex V, is the final enzyme in the oxidative phosphorylation pathway. This enzyme is found in all forms of life and functions in the same way in both prokaryotes and eukaryotes.

The enzyme uses the energy stored in a proton gradient across a membrane to drive the synthesis of ATP from ADP and phosphate (Pi).

Estimates of the number of protons required to synthesize one ATP have ranged from three to four, with some suggesting cells can vary this ratio, to suit different conditions

Reactive oxygen species

Molecular oxygen is an ideal terminal electron acceptor because it is a strong oxidizing agent. The reduction of oxygen does involve potentially harmful intermediates.

Although the transfer of four electrons and four protons reduces oxygen to water, which is harmless, transfer of one or two electrons produces superoxide or peroxide anions, which are dangerously reactive.

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.