The respiratory chain and oxidative phosphorylation are of utter importance for a cell's metabolism. Here, the energy won from ingested food is being converted into our body's energy unit (adenosine triphosphate). As the name respiratory chain suggests, oxygen also plays an important role. The following article will explain how exactly this complex mechanism works and which substances may disable it.

Function and Localisation of the Respiratory Chain

The goal of the respiratory chain is to recycle the reduction equivalents NADH/H⁺ and FADH₂ that have been used during various metabolite processes. Their electrons are therefore transformed to become oxygen.

\[ O_2 + 4H^+ + 4e^- = 2H_2O \]
The large amounts of energy that are released during this process are simultaneously used in form of a **proton gradient** in order to synthesize **adenosine triphosphate** (ATP) from **adenosine diphosphate** (ADP) and phosphate. This last step is known as **oxidative phosphorylation**.

The respiratory chain is subdivided into a total of at least four multienzyme complexes plus one for the oxidative phosphorylation. All complexes are localised in the inner mitochondrial membrane. The electron transfer is therefore separated into multiple reactions in order to avoid the detonating gas reaction typical for hydrogen-oxygen mixtures on the one hand and to be able to make full use of the energy produced by the exothermic reactions on the other hand.

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**Composition of Individual Complexes of the Respiratory Chain**

**Complex I: The NADH ubiquinone oxidoreductase**

The first complex of the respiratory chain is the NADH ubiquinone oxidoreductase. It is a **transmembrane protein** and oxidizes NADH/H⁺ into NAD⁺. Here NADH/H⁺ transfers both of its electrons and hydrogen atoms to the flavin. These are reduced at the same time.

The reduced form now passes the two electrons and hydrogen atoms on to the complex’s iron-sulphur centre, which then again reduces the coenzyme ubiquinone to ubiquinol with help of the electrons and hydrogen atoms. Ubiquinol can move freely in the inner mitochondrial membrane and later transfers the electrons and hydrogen atoms to complex II.

**Note:** Complex I transfers 2e⁻ and 2H⁺ from NADH/H⁺ to ubiquinol. 4H⁺ are thereby
pumped from the matrix into the inner membrane. Moreover, the mutations that occur within this complex have been attributed to the occurrence of numerous ailments such as Alzheimer’s disease, melas syndrome and leber hereditary optic neuropathy.

Complex II: The succinate ubiquinone oxidoreductase

The second complex of the respiratory chain is known as the succinate ubiquinone oxidoreductase. It enables the sudden entry of electrons from FADH2. Two electrons and hydrogen atoms from succinate and ubiquinone each are hereby transferred to ubiquinone. Succinate is being oxidized from FAD during this process.

This is in accordance with the citric acid cycle’s first step of regeneration. FADH2 now transfers the electrons and hydrogen atoms to ubiquinone. The same iron-sulphur centre from complex I takes part here as well.

The two electrons cause six protons to be exported through the inner mitochondrial membrane. In order to do so, they pass their energy onto the complexes III and IV, which both act as proton pumps. Complex II itself is a peripheral protein and can therefore not pump any protons over the inner mitochondrial membrane.

**Note:** Complex II transfers $2e^-$ and $2H^+$ from succinate to ubiquinone. Complex II is the only complex part of the respiratory chain that is no proton pump.

Complex III: The ubiquinone cytochrome-C oxidoreductase

The third complex of the respiratory chain is a transmembrane protein called ubiquinone cytochrome-C oxidoreductase. Here, the electrons from ubiquinone from complexes I and II are adopted and transferred onto 2 cytochrome-C. In order for this to happen, ubiquinol is oxidised into ubiquinone. Simply the electrons are transferred. These two electrons are given off to the 2 cytochrome-c either via an iron-sulphur centre or the q-cycle.

During this procedure cytochrome changes its valence by transforming from a Fe$^{3+}$-state into a Fe$^{2+}$-state. Cytochrome-C is located in the inter membrane space of the mitochondrion and can therefore move from complex III to complex IV.

The protons that become free during the oxidation of ubichinol are pumped into the inter membrane space immediately, just like the two protons that become free during the q-cycle. In total, two electrons are transferred per ubichinol and four protons are pumped through the inner mitochondrial membrane.

**Note:** Complex III gets $2e^-$ from ubichinol out of complexes I and II. It passes these on to 2 cytochrom-C, during which $4H^+$ are being pumped from the matrix into the inter membrane space.

Complex IV: The cytochrome-C-O$_2$ oxidoreductase

The fourth complex of the respiratory chain is a transmembrane protein and is known as cytochrome-C-O$_2$ oxidoreductase. Here, electrons from both cytochrome-c’s from complex III are transferred onto 1/2 O$_2$. In order for this process to work, cytochrome-c is oxidised from 1/2 O$_2$. An O$^-$ develops in consequence, which connects with two H$^+$-ions in the mitochondrial matrix to form water.
Complex IV is copper dependent. Copper A and B collect the electrons and for an electron transport chain in order to be able to simultaneously transfer these onto oxygen. Again two protons are being pumped into the inter membrane space, but this time only two.

**Note:** Complex IV gets $2e^-$ via 2 cytochrom-C from complex III. It transfers these onto oxygen. During this process $2H^+$ are being pumped from the matrix into the inner membrane space. Complex IV is the only complex of the respiratory chain containing an iron-sulphur centre.

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**Structure of the Oxidative Phosphorylation**

**Complex V: The ATP synthase**

The fifth complex is in charge of the oxidative phosphorylation and is called ATP synthase. The ATP synthase uses the **proton gradient** build up by the four complexes of the respiratory chain, which causes an electrochemical potential difference. The protons therefore try to reach the matrix via their concentration gradient. The ATP synthase enables this through a **proton canal**, which is contained in the so-called **F-part**. The protons can therefore diffuse from the inter membrane space through the F-part into the matrix space.

The $F_1$-part extends into the matrix space and is similarly shaped like a mushroom. The
actual ATP synthase happens here. The proton flow through the F-part causes a rotation in the F\textsubscript{1}-part. Around three protons are needed for a full rotation and therefore also the synthesis of an ATP.

\[
\text{ADP} + \text{phosphate} = \text{ATP}
\]

The F\textsubscript{1}-part is divided into three alpha and three beta units that mutually take turns. The gamma unit is located in the middle. The beta unit possesses a high affinity for ADP and phosphate in the **Loose-position**.

If it turns a third further into the **Tight-position**, the affinity for ATP rises until it is higher than ADP’s and phosphate’s affinity. This leads to the creation of ATP. The beta unit turns into the **Open-position** with the next turn by a third. Now the complete ATP is pulled out of the binding pocket. The F\textsubscript{1}-part then again turns by a third and the cycle is initiated from the beginning.

**Energy Balance of the Respiratory Chain**

Roughly three protons are needed for the synthesis of one ATP. The oxidation of NADH/H\textsuperscript{+} causes ten protons to be pumped from the matrix into the inner membrane space. This means that roughly three ATP can be formed per NADH/H\textsuperscript{+}.

The oxidation of FADH\textsubscript{2} makes it possible that six protons are pumped through the inner mitochondrial membrane. This results in the fact that two ATP can be produced per FADH\textsubscript{2}. Often, however, lower scores are declared in books. This is because some protons are used for other reasons. Not all of the protons that are being pumped by complexes I, III and IV flow into the proton canal of the ATP synthase.

**Inhibitors and Isolators of the Respiratory Chain**

The many substances that decrease or even stop the productivity of the respiratory chain and oxidative phosphorylation are divided into inhibitors and isolators. Inhibitors block certain complexes so that the transport of electrons is either inhibited or completely blocked. A further consequence is that oxygen reduction is drastically decreased and may also even stop.

**Inhibitors of the respiratory chain**

Barbiturates count as inhibitors. They inhibit the first complex of the respiratory chain by blocking the hydrogen transfer from flavin to ubiquinone. This leads to the fact that only the electrons from complex II reach complex III and can then be converted into oxygen in complex IV. The respiratory chain is therefore inhibited but not completely stopped. The in Germany illegal insecticide Rotenon operates in a similar matter.

Malonat inhibits complex II of the respiratory chain. It works similarly to the barbiturates: Since the electron feed from complex I is still active the respiratory chain is only restricted, however not completely stopped.

The antibiotic **antimycin A** inhibits the third complex of the respiratory chain. Therefore the transfer of electrons is inhibited in complex IV and the reduction of oxygen can no longer take place.

**Hydrocyanic acid HCN** inhibits the fourth complex of the respiratory chain. It blocks the electron transfer of cytochrome C to oxygen by making cytochromoxidase inoperable.
Therefore the electrons cannot be used and through their accumulation all complexes of the respiratory chain soon come to rest. Protons are consequently not being pumped any longer which leads to a termination of ATP generation. The cell respiration is completely blocked and results in a deadly inner suffocation.

Cyanide intoxication is also a significant inhibitor of the respiratory chain as it is characterized in patients reporting smoke inhalation following the occurrence of industrial or residential fires or in persons who professionally deal with cyanide. In essence, the Fe $^{+++}$ from cyanide bonds with the cytochrome oxidase thus causing detrimental effects in the Electron Transport Chain. The result would be the cessation of redox reactions which would ultimately impede the functionality of proton pumps hence energy will not be produced.

Decoupler of the respiratory chain

Issue that decouples energy generation from energy flow is called a decoupler. This means that the proton gradient built through the electron flow is directly reduced again and therefore cannot be used by ATP synthase. The energy that is now not converted by ATP synthase is released in form of heat. The electron transport however continues unobstructed, resulting in a same or even higher level of oxygen consumption through the reduction, as the regulation of the respiratory chain via the ADP concentration is no longer effective.

**Note:** Decouplers raise the oxygen reduction and stop ATP synthesis.

The proton canal Thermogenin is a physiological substitute of the decouplers and is integrated in the inner mitochondrial membrane when it’s cold. It appears in brown fat tissue and contributes to heat generation.

Dinitrophenol on the other side is a pathological substitute of the decouplers. This matter concerns a liposoluble molecule stored in the inner mitochondrial membrane, which transports protons of the inter membrane area to the matrix area. The proton gradient is therefore reduced.

The transportation of Calcium ($\text{Ca}^{++}$) is essential for electron transportation. Nonetheless, energy is required is required to pump the $\text{Ca}^{++}$ into the mitochondrial structures hence no energy is stored in the form of ATP. Additionally, there are other physiological un-couplers such as valinomycin which may be defined as an ionophore of oxidative phosphorylation which is produced by a certain species of Streptomyces. Valinomycin is comprised of four different types of residues namely L-lactate, L-Valine, D-Valine and D-hydroxyisovalarate. Essentially, valinomycin transports $\text{K}^+$ from cytosol into matrix and $\text{H}^+$ in the reverse direction and in the process diminishes the proton gradient.

**Popular Exam Questions Concerning the Respiratory Chain and Oxidative Phosphorylation**

The answers can be found underneath the list of references.

1. **Which of the following statements about complexes of the respiratory chain is true?**
   
   A. The first complex of the respiratory chain is the only one that doesn’t function as a proton pump.
   
   B. The second complex of the respiratory chain pumps 4 protons in the inner
membrane area.
C. The third complex of the respiratory chain oxidates NADH/H+ into NAD+.
D. The fourth complex of the respiratory chain is the only one that doesn't have an iron-sulphur centre.
E. The fifth complex of the respiratory chain reduces oxygen to water.

2. The ATP synthase...
   A. ...consists of one F1- and one F2-part.
   B. ...contains a proton canal in its F0-part.
   C. ...needs ca. 4 protons per turn.
   D. ...produces ATP from AMP and pyrophosphate.
   E. ...extends into the inter membrane area with its F1-part.

3. Which of the following statements concerning inhibitors and decouplers is true?
   A. Barbiturates inhibit complex II of the respiratory chain.
   B. Rotenon inhibits complex I of the respiratory chain.
   C. Antimycin A is an inhibitor.
   D. Cyanid inhibits complex III of the respiratory chain.
   E. Inhibitors lower the reduction of oxygen.

References
Eggemann, I. (sechste Auflage). Medi-Learn Skriptenreihe Biochemie 1 Energiestoffwechsel. MEDI-LEARN.
Atmungskette via Wikipedia.

Correct Answers: 1D, 2B, 3B

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