Electron transport and Oxidative phosphorylation or

**Introduction:**

The complete oxidation of glucose carbons by glycolysis and the citric acid cycle can be written as

\[ C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \]

The reducing equivalents (electrons) are captured in the form of reduced co-enzymes (NADH and FADH\(_2\)) which eventually transfer electrons to molecular oxygen

\[ 6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \]

This process regenerates NAD\(^+\) and FAD and generates a proton gradient across the inner mito membrane, whose dissipation provides the free energy for ATP synthesis. known as oxidative phosphorylation.

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**Overall rxn:**

\[ 3 \text{NAD}^+ + \text{FAD} + \text{GDP} + P_i + \text{acetyl-CoA} + 2H_2O \rightarrow 3\text{NADH} + 3H^+ + \text{FADH}_2 + \text{GTP} + \text{CoA} + 2\text{CO}_2 \]

**Citric Acid Cycle**

A multistep catalytic process that converts acetyl groups derived from carbohydrates, fatty acids, and amino acids to CO\(_2\), and produces NADH, FADH\(_2\), and GTP.

The citric acid cycle acts as a multistep catalyst that can oxidize an unlimited number of acetyl groups.

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As the electron turns
Oxidative phosphorylation - the combined actions of:
- Electron transport - ETS - (the transport of e\textsuperscript{-} from reducing equivalents to O\textsubscript{2})
  - Harnessing the chemical and electrical potential produced by the ETS
  - O\textsubscript{2} is ultimate e- acceptor and drives ATP formation thus oxphos

Where do the equivalents come from?
- glycolysis
- TCA
- β oxidation of fatty acids

ETS and Oxphos tightly coupled via the H\textsuperscript{+} gradient - can be uncoupled by poisons and inefficient coupling leads to heat

Mitochondria
- Outer membrane is very porous
- Inner membrane very tight. Transfer into and out of matrix is controlled - important in H\textsuperscript{+} and shuttling reducing equivalents.
- membranes are topologically sided - different charge, lipids and proteins

NADH shuttle
Much of the reducing equivalents is produced in the cytosol and needs to be shuttled into the mitochondria - this happens by one of two means

- The malate-aspartate shuttle allows NADH to be indirectly transported into the mitochondrion by reducing OAA to malate and transporting malate across the inner mitochondrial membrane. OAA is then transaminated to asparate and then shuttled back to the cytosol
The glycerophosphate shuttle first reduces cytosolic dihydroxyacetone phosphate (DHAP) to 3-phosphoglycerate and NAD+. The 3-phosphoglycerate is oxidized by an inner mitochondrial membrane enzyme, flavoprotein dehydrogenase, which introduces electrons directly into the ETS via FADH₂.

**ATP Translocation**

Most of the ATP generated in the mitochondria is used in the cytosol. The ADP-ATP translocator exports ATP out of the matrix while importing ADP.

- ATP has one more negative charge than ADP thus the transport is based on membrane potential, electrogenic transport.
- Transport is driven by the electrochemical potential of the proton concentration gradient (positive outside).
Redox potential / free energy changes:
In an electron transfer reaction electrons flow from a substance with a lower reduction potential to a substance with a higher reduction potential.
- Thus redox potential is a measure of a molecule’s affinity for electrons

Redox potential give a measure of oxidizing and reducing strengths of the different electron carriers
- Oxidized form NADH -> Reduced form NAD⁺
- The reaction potential for a reaction is the sum of the voltage potentials
  \[ \Delta E^{o'} = E^{o'} (\text{e}^- \text{ acceptor}) - E^{o'} (\text{e}^- \text{ donor}) \]
- The voltage potential for the electron transfer from NADH (-0.315) to O₂ (0.815) is:
  \[ \Delta E^{o'} = \]
  \[ \text{Or for two half reactions} \]
  \[ \frac{1}{2} \text{O}_2 + \text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O} \]
  \[ \text{NAD}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADH} \]
  \[ \Delta E^{o'} = \text{MDH reaction: Malate + NAD}^+ \rightarrow \text{OAA} + \text{NADH} + \text{H}^+ \]
  \[ \text{NAD}^+ + 2\text{e}^- \rightarrow \text{NADH}^+ + \text{H}^+ \quad E^{o'} = -0.32 \text{ V} \]
  \[ \text{OAA} + 2\text{e}^- \rightarrow \text{Malate} \quad E^{o'} = -0.17 \text{ V} \]

\[ \Delta E^{o'} = \text{change in redox potential} = \text{oxidant } E^{o'} - \text{reductant } E^{o'} \]
\[ \Delta E^{o'} = 0.15 \text{ V} \quad \rightarrow \text{from this we can determine the spontaneity of the reaction} \]

\[ \Delta G^{o'} = -nF\Delta E^{o'} \]
\[ n = \# \text{ of electrons transferred} \]
\[ F = \text{faraday's constant} = 23.06 \text{ kcal/volt} \cdot \text{mol} \]
\[ \Delta G^{o'} = -2 \times 23.06 \text{ kcal/volt} \cdot \text{mol} \times -0.15 \text{ V} \]
\[ \Delta G^{o'} = +6.92 \text{ kcal/mol} \]

- A reaction will proceed spontaneously when \[ \Delta E^{o'} > 0 \]
- Just like the Gibbs function, changes in actual concentration will shift the reaction

Physical contact need not occur for redox reactions to take place

Transfer of e⁻ starts at NADH (or FADH₂) and ends with O₂ → H₂O
\[ \Delta E^{o'} = +1.13 \text{ V} \quad \Delta G^{o'} = -56.52.6 \text{ kcal/mol} \]

Electron Transport Chain II
The ETS is composed of four large protein complexes in the inner mitochondrial membrane and are involved in transferring electrons from reduced carriers (coenzymes) to to O₂. Complexes I and II transfer electrons to the lipid-soluble electron carrier coenzyme Q, which transfers electrons to complex III. From there, electrons pass to cytochrome c, a peripheral membrane protein with a heme prosthetic group, which then transfers electrons to complex IV.

Cytochromes - There are 7 cytochromes (heme proteins; heme = iron + porphyrin) in the ETS. All have a reddish-brown tint caused by the presence of iron. Each cytochrome has a distinct
absorbance spectra which represent a structural feature of the cytochrome and is designated as a member of a, b, or c family. Why hemes? You must recognize the differences with the function of hemes in myoglobin and hemoglobin and the cytochromes. The metals (iron for most copper of a and a3) are used for there ability to accept and donate electrons easily. Differences in the redox state is due to the total environment of the heme/cytochrome complex.

**Iron-sulfur centers** – A characteristic of the ETS is to have components with different oxidative potential placed strategically along the chain. The proteins with iron-sulfur centers are needed to provide a low oxidation potential. Thus they are present in complexes I, II and III but not in IV.

![Complex I](image)

* large complex (850 kDa) with over 30 subunits some from mitochondrial DNA.
* mediates transfer of e- from NADH to ubiquinone
* NADH is oxidized and in doing so transfer of e- to FMN
* There are several Fe-S centers held in place by cys residues (2Fe-2S, or 4Fe-4S)
* Coenzyme Q is ultimate acceptor of electrons in this complex
* Q and FMN can adopt 3 oxidation states
* protein changes conformation redox state - most likely leads to proton pumping - resulting pKa changes in aas in complex I probably leads to loss and gain of protons
* 2 e- are transferred from NADH and 4 H+ are pumped

**Complex II**

* Succinate dehydrogenase and other FADH producing enzymes are linked to the complex - direct transfer of e- from the TCA
* FADH is part of the complex and 2e- electrons are donated here
* Transfer of 1 e- to the Fe-S center to Q
* is not the second part of the chain, rather a another entry point into the chain
* no H+ pumped, so there is a reduced potential of H+ gradient formed and ATP formation potential

**Coenzyme Q** -

* mobile part of ETS - long hydrophobic isoprenoid chain allows movement in hydrophobic membrane core
* three different states
* transfers one or two electrons between complexes
* part of several portions of chain
* Q cycle is important in transfer single e- instead of two
Complex III
* Accepts two one e- transfers from QH2 and eventually transferred to cytochrome C
* Fe-S center and two similar hemes B and c1 involved
* reduction potential of the b hemes is different due to membrane topology
* Important that it is two individual transfers of e- to cyto C
* Two protons are pumped - partially due to lower reduction potential at this point in the chain
- Hemes
  * same porphyrin ring as in hemoglobin
  * cytochromes b and c are covalently attached through cysteines via thioester linkages
  * Heme A has a long hydrophobic side chain
How are these held in place differently than hemoglobin heme?
Q cycle
- mobile part of ETS - long hydrophobic isoprenoid chain movement in hydrophobic core
- three different states
- transfers one or two electrons complexes
- part of several portions of ETS
- QH- is anchored at each while Q and QH2 are mobile
- Q cycle is important in transfer instead of two

Cytochrome C
* Only water soluble cytochrome
* loosely associated with inner mitochondrial membrane (cytosolic side)
* migrates in the reduced carrying 1e- to complex IV
* highly conserved through evolution

Complex IV
* Differs in copper ions in e- transfer not Fe-S.
* e- transferred from cytochrome C to molecular oxygen, one at a time
* one proton pumped per e- transferred - two for O2, two cytosolic side
* Cytochrome C to CuA-Hemea -> Hemeb2-CuB -> O2
* This complicated system is to prevent the formation of oxygen radicals and superoxide anions
* Controlled by transfer of e- to oxygen while bound to Fe and Cu complex

Electron Transport Chain III
ATP synthase (F1:F0) - also known as the ATPase for the reverse direction. Without a proton gradient, the reverse reaction is spontaneous. Also called complex V in some books.

ATP synthase phosphorylates ADP by a mechanism driven by the free energy of electron transport, which is conserved in the formation of an electrochemical proton gradient across the inner mitochondrial membrane.

The protonmotive force results from the difference in pH and the difference in charge on both sides of the inner mitochondrial membrane.

\[ \Delta G = 2.3 \, RT \, \left[ \text{pH(in)} - \text{pH(out)} \right] + ZF \Delta \Psi \]

The controversy comes down to thermodynamics – the free energy of ATP synthesis from ADP is about 51.6
kJ/mol. Yet the actual ΔG for one H+ returned to the matrix is much less.

Mitchel chemiosmotic theory - oxidative phosphorylation
ADP + Pi -> ATP =⇒ ATPase found on inner mitochondria
the proton motive force -> that force generated by the unbalance of hydrogen ions across the inner mitochondrial membrane
• combination of pH and membrane potential (0.14 V and 1.4 pH units) drive ATP synthesis = 0.224V
Evidence for the theory - bacteriorhodopsin, artificial pH gradient, broken mitochondria, uncouplers

H+ pumped per 2 e- transferred from NADH or FADH₂ is not certain
• anywhere from 6 to 10 total protons pumped when starting from site I and four less than that when electrons enter from site II
• about 2.5 to 3 protons / ATP generation – controversial
• Some leakage of the proton gradient creates inefficient coupling - thus a more realistic interpretation is that there are 2.5 ATP produced for each NADH

ATP synthase has two functional units
several subunits.
• two complexes - F₁ and F₀
  F₀ complex
• transmembrane pore or channel for protons to move through
• H⁺ ions build up at junction of the subunits
  • increases in H⁺ concentration may lead to protonization of critical amino acids (asp)
  • Asp-H shifts rotor to open position and new aa interactions ionizes the asp and releases proton out of matrix
  • This is shown by the inhibition of a reactive glutamate residues with the compound dicyclohexylcarbodiimide.

  F₁ complex catalyzes ATP synthesis
  • five subunits - on the matrix side of the inner membrane
  • alpha and beta are nearly identical but the beta subunit contains the active site
  • when separated the F₁ complex is an ATPase - hydrolysis of the gamma phosphate of ATP

The binding change mechanism - Paul Boyer - U of M and John Walker determined much of the ATP synthase - Nobel Prize winners 1997 for this work
• H+ gradient leads to conformational changes of the F₁ complex
• Both ATP and ADP bind to the three beta subunits
• Three conformation changes for the whole enzyme (F₁F₀) the open (O), loose (L) and tight (T) binding sites
• Proton flux through pore shifts beta subunit conformations
• As the conformation of each of the subunits change a phosphoanhydride bond is formed with Pi and ADP
• The membrane potential helps to create high concentration gradient within the F_{0} pore.
• The free energy of the proton concentration gradient converts the T state to the O state, thereby releasing ATP.

The amount of ATP produced to the amount of O_{2} consumed is the P/O ratio (atomic O not molecular O_{2}).

**ETS Poisons and Inhibitors** - Specific inhibitors and uncouplers change the electron transfer and ATP production - respiration - O_{2} + H^{+} -> H_{2}O

- Think of this as a pipeline, if the middle is blocked no ATP production and O_{2} used. If the end is blocked and a hole is punched in the hose you lose O_{2} but no ATP production. Uncouplers “punch holes” in the ets. Some inhibitors act as blockers in the hose itself or at the end of the hose.

- Rate of system depends on oxygen to accept electrons
- Without ADP - ATP synthase is stopped and electrons do not flow back into mitochondrial matrix and respiration stops
- Uncouplers, degrade proton gradient. Transfer though membrane reversing H^{+} gradient. No ATP produced but lots of electrons transferred to try and restore H^{+} gradient - heat is produced - thus oxidation (e- transfer) without phosphorylation
  - 2,4 DNP is an uncoupling agent that can transverse the inner mitochondria membrane dissipating the H^{+} gradient

- Respiration poisons - block at complex I, III and IV. Effect is to stop the flow of e- through the chain. - when added to the effect of uncouplers can lead to interesting studies
  - Carbon monoxide (CO), cyanide, and hydrogen disulfide (H_{2}S) – inhibit cytochrome C oxidase (site IV)
  - The fish poison and insecticide rotenone and barbiturates inhibit the oxidation of NADH (site I)
  - Antimycin A inhibits the cytochromes b and C (site III)
- If site 1 is blocked site II can still input electrons, ATP is formed and oxygen consumed. However if site two is blocked then no e- are transferred and O_{2} is not consumed.
- Think of what happens if various combinations of these inhibitors are used.
**Brown fat (thermogenesis)** - regulated by fatty acids, leads to uncoupling. High amounts of thermogenin (uncoupling protein) are found in babies and some women (lower back). The protein thermogenin is responsible for the uncoupling and is under the control of free fatty acids and nucleotides. It acts as a channel to allow $H^+$ to re-enter the mito.

Hormone sensitive lipase
- Uncoupling protein - inhibited by adenine and guanine nucleotides.
- Norepinephrine $\rightarrow$ [cAMP] and activates hormone sensitive lipase
- Increase in [FA] reverse nucleotide inhibition of uncoupling channel

**Oxygen Radicals**
- Addition of electrons to oxygen $\rightarrow$ superoxide $O_2^-$

- Highly reactive oxidants - results in damage of mitochondria and surrounding tissue - example of this in Parkinson’s, Alzheimers and Huntington’s disease.
- Apoptosis (programed cell death) can be activated by leaking damaged mitos
- Antioxidants limit damage SOD reduces superoxide
- Mutations associated with Lou Gehrig’s disease