**Lecture 33: Electron transport, ATP synthesis (Oxidative Phosphorylation), Anaerobic Metabolism**

**Electron Transport:**

* The energy captured in glycolysis, TCA cycle, and fatty acid oxidation on NADH and FADH2 is converted to a **proton gradient** across the inner mitochondrial membrane.
* *The energy stored in this gradient is used to produce ATP*.

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| **Pathway** | **NAD+/NADH** | **FAD/FADH2** |
| Glycolysis | Glyceraldehyde 3-phosphate dehydrogenase |  |
| TCA cycle | Pyruvate dehydrogenaseIsocitrate dehydrogenaseα-ketoglutarate dehydrogenaseMalate dehydrogenase | Succinate dehydrogenase |
| Fatty Acid Ox, | hydroxyacyl-CoA dehydrogenase | Acyl-CoA dehydrogenase |
| Within above pathways |  |  |
| Electron Transport |  |  |

* In most organisms the electrons from these compounds are deposited on oxygen, reducing it to water. Note that the oxygen only serves as a final acceptor of electrons in this process.
* In many organisms other compounds besides oxygen can serve as electron sinks, allowing organisms to perform 'oxidative' phosphorylation in the absence of O2.
* The actual **synthesis of ATP** is from a **proton gradient** across the inner membrane that is generated during the transfer of electrons to oxygen.

The oxidation of NADH releases a lot of energy:

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| Oxidation of NADH | NADH → NAD+ + 2 e- + 2H+ | ΔG = -60 kJ/m. |
| Reduction of oxygen | 2e- + 2H+ + (1/2) O2 → H2O | ΔG= - 156 kJ/m. |
| **Tot. Reaction** | **NADH + (1/2) O2 → H2O + NAD+** | **-200KJ/mol** |

Key Components in Electron Transfer:

**1. Inorganic carriers of electrons**

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| 1. Iron-sulfur centers (e.g. succinate dehydrogenase)

 | b) Fe in heme – e.g. cytochrome C |

**2. Organic Carriers of electrons:**

a) Coenzyme Q. Coenzyme Q is a non-polar electron carrier that diffuses freely in the ***fluid*** mitochondrial membrane. R group is non-polar.

* Can participate in one or two electron redox transactions, two electron reduction shown on the right

**Electron Transport: Gibbs Energy & Flow**

***Complex I*: NADH-CoQ oxidoreductase**

* **Four protons/NADH** are pumped from the inside (matrix) to the intermembrane space.

***Complex II*: Succinate-CoQ oxidoreductase**

* Succinate dehydrogenase of the citric acid cycle is part of this complex.

* Two electrons from FADH2 are transferred to CoQ via Fe-S clusters, generating CoQH2.
* **Does not pump any protons.**

***Complex III*: CoQH2-cytochrome c oxidoreductase**

* Transfers electrons from CoQH2 to cytochrome c one electron at a time.
* **Four protons are pumped/NADH or FADH2**

***Cytochrome C****:* Shuttles one electron from III to IV.

***Complex IV*: Cytochrome c oxidase**

* Accepts 4 *e-* , one at at a time from cytochrome c.
* Donates a total of *four* electrons/O2.
* Site of oxygen reduction to water.

i) Produces 2 water molecules/O2 molecule.

ii) Pumps an additional **two protons/NADH or FADH*2****.*

**Energy Stored in the Proton Gradient**

The energy 'stored' in a concentration gradient can be considered to consist of two parts: 

Defining the reaction direction from inter-membrane space (out) to the matrix (in):

i) The Gibbs energy due to a concentration difference across a sealed membrane. The Gibbs energy is:



Since the standard chemical potential (μ0) for the species ([X]) is the same on both the inside and the outside of the membrane:

This is the amount of energy that is released when the concentration gradient moves towards equilibrium.

ii) Movement of a charged particle through a voltage difference. The free energy associated with moving a particle of charge Z, through a voltage difference ΔΨ(=ΔV), is:

ΔGELEC = ZFΔΨ

* Z = the charge on the transported ion (+1 in the case of the proton)
* F is Faraday's constant, 96,494 C/mol. C=coulomb
* ΔΨ is the voltage difference across the membrane, in volts. This voltage difference is often referred to as the membrane potential: ΔΨ = VIN – VOUT.

The total Gibbs free energy is the sum of these two terms:

**Example Calculation:** Typical values across the inner mitochondrial membrane are:

[H+]IN/[H+]out = 0.1 (pH=6.5 outside, 7+ on the outside of the membrane:



**ATP Synthesis:**

*ATP synthesis is attained by coupling the free energy of a proton gradient to the chemical synthesis of ATP. The enzyme that accomplishes this coupling is called* ***ATP-synthase*** *(also known as FoF1 ATPase)*

***3 H+ = 1 ATP synthesized***

**Structural Features:**

1. The Fo Complex

* Membrane-spanning, multi-protein complex.
* Responsible for coupling the movement of three protons to 120° rotations of the **γ-subunit**.

2. The F1 Complex

* Attached to Fo, it protrudes into the mitochondrial matrix.

* Composed of five different subunits: α3β3γδε
* The γ subunit is the shaft at the center of the α3β3 disk. **γ rotates 120o every time 3 protons pass through the complex.**
* The β subunits are asymmetric due to their interactions with the γ-subunit.
1. One conformation of the β subunit has very **low affinity** for both ADP and ATP. Everything is released.
2. One conformation of the β subunit has **high affinity for ADP and Pi**.
3. One conformation of the β subunit makes **ATP lower in energy than ADP+Pi.**

**How the motor works**:

* Every time three proton move through the complex, the γ subunit rotates 120°.
* The rotation of γ subunit changes the conformation of the β-subunits such that the Gibbs energy of the bound ADP + Pi becomes higher than the energy of ATP, thus ATP forms spontaneously from the bound ADP and Pi.
* The newly-formed ATP is released with the transport of three additional protons.
* The actual synthesis, or formation of the bond between ADP and PI, is catalyzed by conformational changes of the β-subunit that occur as a consequence of the rotation.
* Since all three β subunits are functioning at the same time, the transport of 9 protons in a complete cycle produces 3 ATP.

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| **NADH** | **~10 protons pumped** | **~ 3 ATP** |
| **FADH2** | **~6 protons pumped** | **~2 ATP** |

**Anaerobic Metabolism and the Fate of Pyruvate.**

**Anaerobic Metabolism:**

NAD+ is required as the electron acceptor in glycolysis.

*Which step in glycolysis does this occur?*

*How is NAD+ regenerated?*

*What happens to glycolysis if NAD+ cannot be regenerated?*

**Other Fates of Pyruvate**

i) Pyr can be converted to Acetyl CoA, a **one way reaction in humans.**

a) acetyl CoA can be oxidized by the TCA cycle.

b) acetyl CoA can be used to synthesize fatty acids (via citrate), which are then used to make triglycerides.

ii) Pyruvate can be converted to alanine in a one-step transaminase reaction.

iii) Pyruvate can be used to make oxaloacetate, to replace the carbons that are removed from the citric acid cycle by anabolic processes (this reaction is the first step in gluconeogenesis).

**Cooperation between muscle and liver cells during active exercise (Cori cycle).**

a) During intense exercise muscle tissue cannot get sufficient oxygen to process the NADH produced in metabolism.

b) Pyruvate is reduced to lactate, to regenerate NAD+ for glycolysis.

c) The lactate travels to the liver, where it is oxidized to pyruvate, used to make more glucose, which is then returned to the muscle.

