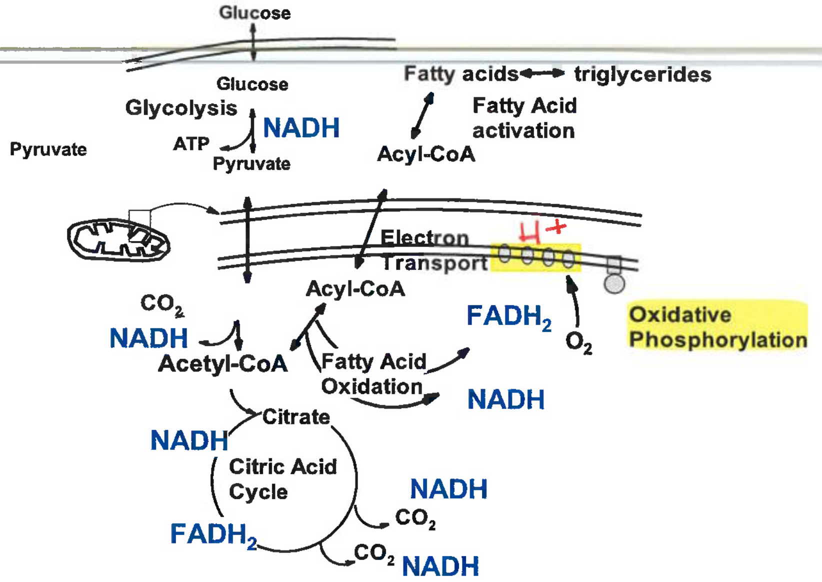
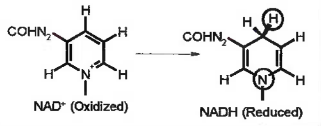
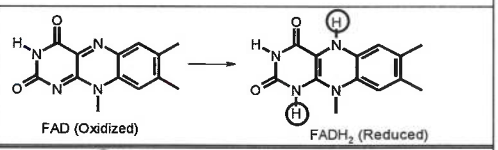
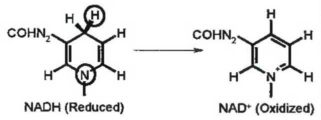
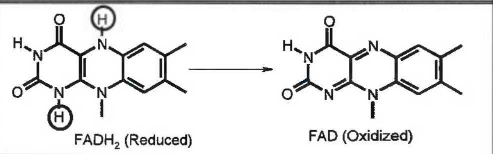
Lecture 33: Electron transport, ATP synthesis

Electron Transport:



- The energy captured in glycolysis, TCA cycle, and fatty acid oxidation on NADH and $FADH_2$ is converted to a **proton gradient** across the inner mitochondrial membrane.
- *The energy stored in this gradient is used to produce ATP* by ATP synthase.
- In most organisms the electrons from NADH and $FADH_2$ are deposited on oxygen, reducing it to water, oxygen serves as a final acceptor of electrons in this process.
- In many organisms other compounds besides oxygen can serve as electron sinks, allowing these organisms to perform 'oxidative' phosphorylation in the absence of O_2.

Pathway	NAD^+/NADH	FAD/$FADH_2$
Glycolysis	Glyceraldehyde 3-phosphate dehydrogenase	
TCA cycle	Pyruvate dehydrogenase Isocitrate dehydrogenase α-ketoglutarate dehydrogenase Malate dehydrogenase	Succinate dehydrogenase
Fatty Acid Ox,	hydroxyacyl-CoA dehydrogenase	Acyl-CoA dehydrogenase
Within above pathways		
Electron Transport		

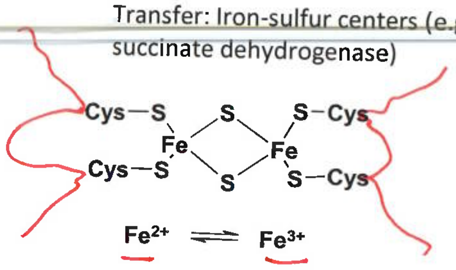
The oxidation of NADH releases a lot of energy:

Oxidation of NADH	NADH $\rightarrow NAD^+ + 2e^- + 2H^+$	ΔG = -60 kJ/m.
Reduction of oxygen	$2e^- + 2H^+ + (1/2) O_2 \rightarrow H_2O$	ΔG= - 156 kJ/m.
Tot. Reaction	**NADH + $(1/2) O_2 \rightarrow H_2O + NAD^+$**	**-200KJ/mol**

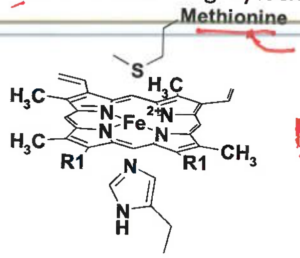
Heme

1. Inorganic carriers of electrons

a) Key Components in Electron Transfer: Iron-sulfur centers (e.g. succinate dehydrogenase)



b) Fe in heme – e.g. cytochrome C



blocks O_2 access to Fe

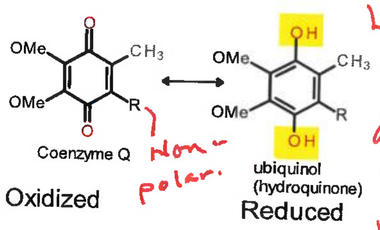
Fe^{2+} ⇌ Fe^{3+}

1 electron.

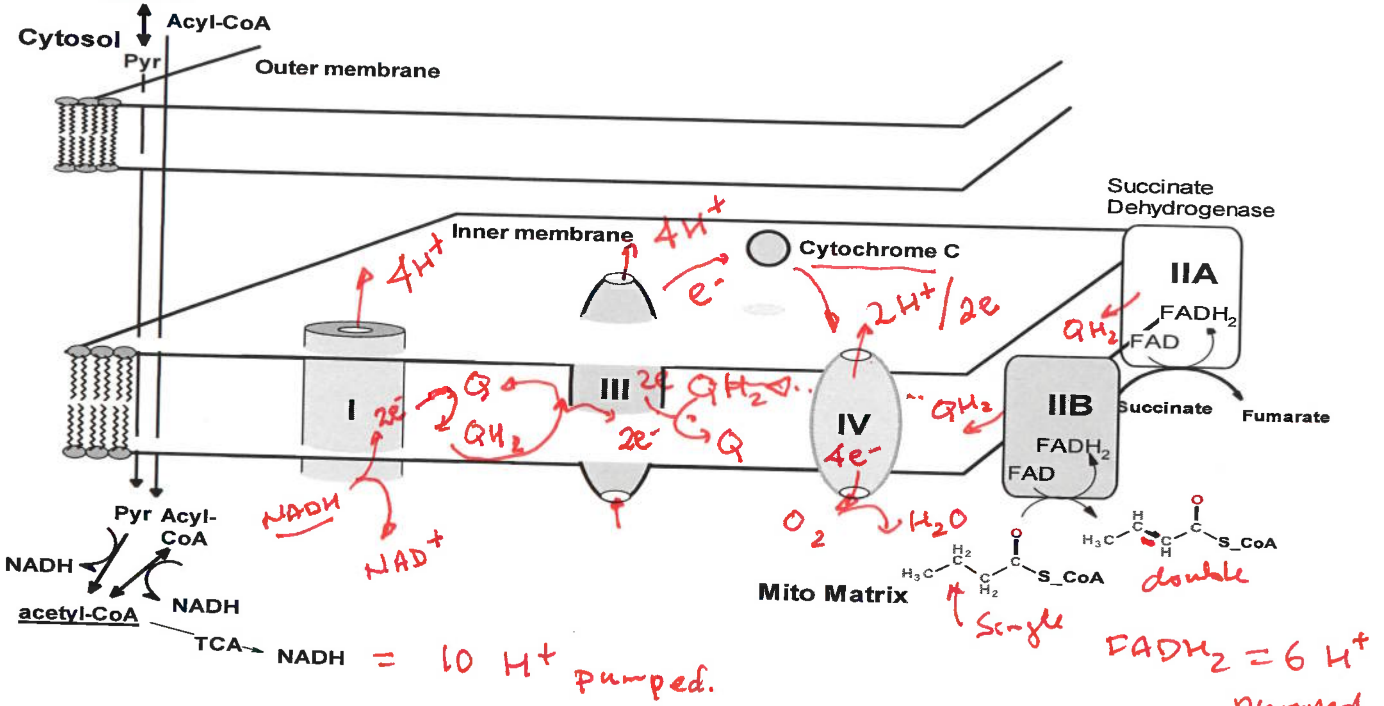
2. Organic Carriers of electrons:

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



Lipid soluble diffuse in the membrane



Electron Transport: Gibbs Energy & Flow – As with all pathways, $\Delta G < 0$ for each step

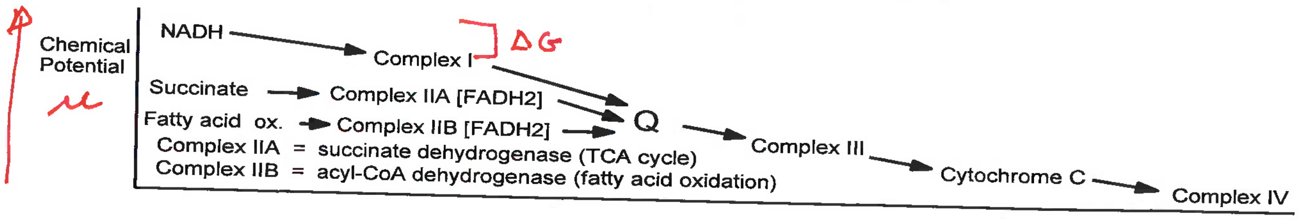
NADH Oxidation: - Electrons from NADH through complexes I,Q,III, IV involved. A total of ~10 H^+ are moved across the membrane.

$FADH_2$ Oxidation: Complex II, Q, III, CytoC, IV. A total of ~6 H^+ are moved across the membrane.

$FADH_2$ is produced at two sites:

i) Succinate dehydrogenase in the TCA cycle.

ii) acyl-CoA dehydrogenase, the first oxidation in β-oxidation of fatty acids.



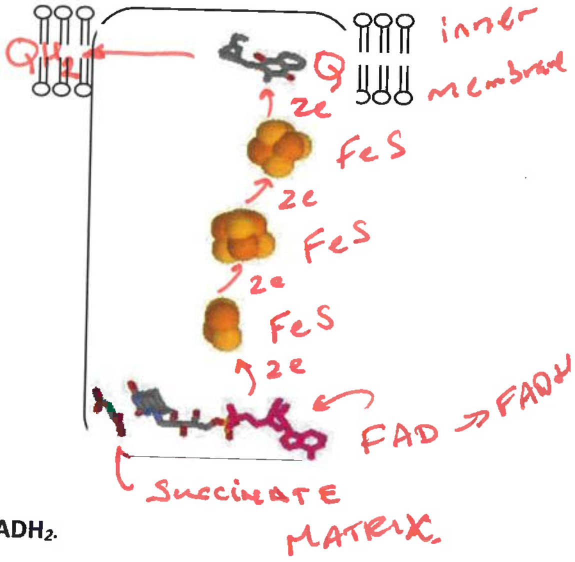
Complex IIA = succinate dehydrogenase (TCA cycle)
Complex IIB = acyl-CoA dehydrogenase (fatty acid oxidation)

Complexes in Electron Transport:

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

- Multi-enzyme complex, contains FAD and Fe-S centers. Electrons are transferred from NADH to FAD, then to FeS, then to Q.
- **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 $FADH_2$ are transferred to CoQ via Fe-S clusters, generating $CoQH_2$.
- **Does not pump any protons.**

***Complex III*: $CoQH_2$-cytochrome c oxidoreductase**

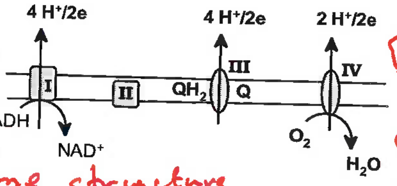
- Transfers electrons from $CoQH_2$ to cytochrome c one electron at a time.
- **Four protons are pumped/NADH or $FADH_2$**

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/O_2.
- Site of oxygen reduction to water.
  - i) Produces 2 water molecules/O_2 molecule.
  - ii) Pumps an additional **two protons/NADH or $FADH_2$.**

Energy Stored in the Proton Gradient



$[H^+]$ high

$[H^+]$ low

The energy 'stored' in a concentration gradient can be considered to consist of two parts: $\Delta G_{TOTAL} = \Delta G_{CONC} + \Delta G_{ELEC}$

+ ΔV −

i) The Gibbs energy due to a concentration difference across a sealed membrane. Defining the reaction direction from inter-membrane space (out) to the matrix (in):

→ 0 Same structure both sides.

$$\Delta G = \Delta G^0 + RT\,ln\frac{[X_{IN}]}{[X_{out}]} = (\cancel{\mu^0_{IN} - \mu^0_{OUT}}) + RT\,ln\frac{[X_{IN}]}{[X_{out}]} = RT ln\frac{[X_{IN}]}{[X_{out}]}$$

The standard chemical potential (μ_0) for the species ([X]) is the same on both the inside and the outside of the membrane, so ΔG^o=0. 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 $\Delta\Psi$(=ΔV), is:

$\boxed{\Delta G_{ELEC} = ZF\Delta\Psi}$ $\Delta\Psi = \Delta V$

- 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
- $\Delta\Psi$ is the voltage difference across the membrane, in volts. This difference is often referred to as the membrane potential: $\Delta\Psi = V_{IN} - V_{OUT}$.

The total Gibbs free energy is the sum of these two terms: $\boxed{\Delta G_{TOTAL} = RT\ln\frac{[H^+]_{IN}}{[H^+]_{OUT}} + ZF\Delta\Psi}$

conc

charged particle thru conc gradient.

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

$[H^+]_{IN}/[H^+]_{out}$ = 0.1 (pH=6.5 outside, 7.5 inside), voltage difference -0.15 V, inside negative.

$$\Delta G = (8.31)(300)\ln(0.1) + (+1)(96{,}000)(-0.150)$$
$$= -5.7kJ/mol - 14.4kJ/mol$$
$$= -20kJ/mol$$

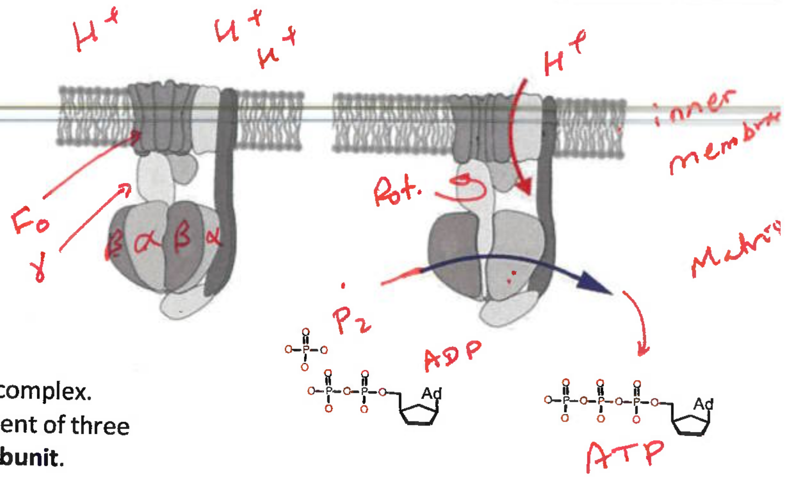
→ ATP synthesis

3 H^+ are used to make one ATP
(due to Mechanism)

ATP Synthesis (Oxidative Phosphorylation):

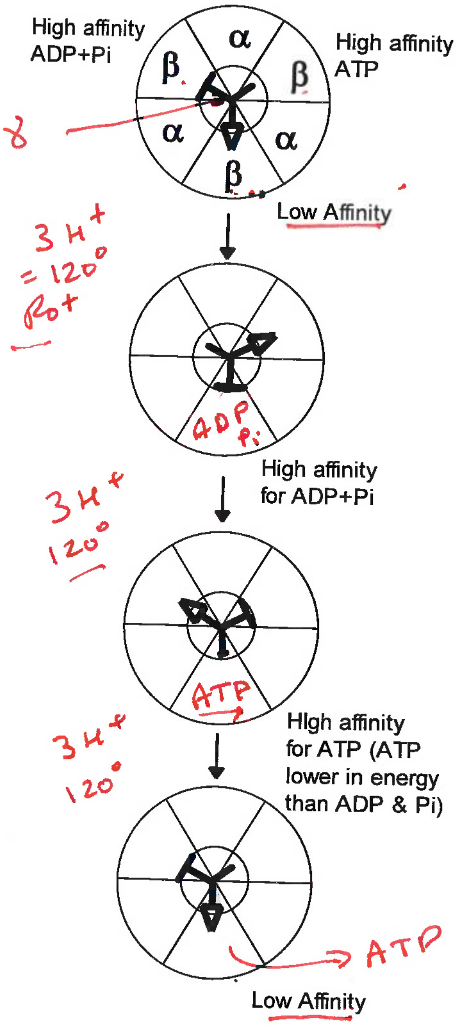
*ATP synthesis is attained by coupling the free energy of a proton gradient to the chemical synthesis of ATP. The enzyme that accomplishes this coupling is called **ATP-synthase** (also known as F_oF_1 ATPase)*

9 H^+ transported = 3 ATP synthesized



Structural Features:

1. The F_o Complex
   - Membrane-spanning, multi-protein complex.
   - Responsible for coupling the movement of three protons to 120° rotations of the **γ-subunit**.
2. The F_1 Complex
   - Attached to F_o, it protrudes into the mitochondrial matrix.
   - Composed of five different subunits: $\alpha_3\beta_3\gamma\delta\varepsilon$
   - The γ subunit is the shaft at the center of the $\alpha_3\beta_3$ disk. **γ rotates 120° 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 P_i.**
     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 + P_i becomes higher than the energy of ATP, thus ATP forms spontaneously from the bound ADP and P_i.
- The newly-formed ATP is released with the transport of three additional protons.
- The actual synthesis, or formation of the bond between ADP and P_i, 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 (on average).

NADH	~10 protons pumped	~ 3 ATP
$FADH_2$	~6 protons pumped	~2 ATP

9H+ : 3 subunits
⇒ 3 ATP

Anaerobic Metabolism and Inter-tissue Cooperation:

NAD^+ is required as the electron acceptor in glycolysis.

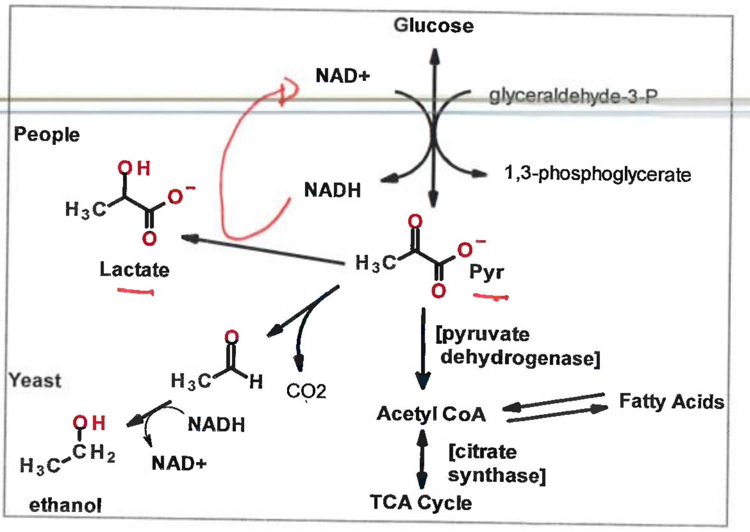
How is NAD^+ regenerated when oxygen is present?

ETC (IV)

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

STOPS

Alternative electron acceptors can be used to regenerate NAD+
   a) pyruvate → lactate
   b) pyruvate → ethanol.



Anaerobic metabolism produces 2 ATP and 2 lactate/glucose molecule, much less than when oxygen is used as an electron acceptor.

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

a) During intense exercise muscle tissue cannot get sufficient O_2 for electron transport, it can only do glycolysis.

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

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