Electron Transport Chain (overview)

- The NADH and $FADH_2$, formed during glycolysis, β-oxidation and the TCA cycle, give up their electrons to reduce molecular O_2 to H_2O.
- Electron transfer occurs through a series of protein electron carriers, the final acceptor being O_2; the pathway is called as the electron transport chain.
- ETC takes place in inner mitochondrial membrane where all of the electron carriers are present.
- The function of ETC is to facilitate the controlled release of free energy that was stored in reduced cofactors during catabolism.

Oxidative Phosphorylation

- Energy is released when electrons are transported from higher energy NADH/$FADH_2$ to lower energy O_2.
- This energy is used to phsophorylate ADP.
- This coupling of ATP synthesis to NADH/$FADH_2$ oxidation is called oxidative phosphorylation.
- Oxidative phosphorylation is responsible for 90 % of total ATP synthesis in the cell.

The Chemiosmotic Theory

- The chemiosmotic theory explains the mechanism of oxidative phosphorylation.
- When electrons are transported along the components of the ETC, the accompanying protons are released.
- Part of the free energy harvested during the ETC is used to pump protons out of the mitochondrial matrix.
- The resulting uneven distribution of protons generates a pH gradient and a charge gradient across the inner mitochondrial membrane.
- The electrochemical potential energy generated by these gradients is called as **Proton Motive Force**.
- The return of protons to the mitochondrial matrix is coupled to ATP synthesis.

Mitochondria are Biochemical Hubs

- The mitochondrial matrix contains enzymes of PDH, TCA cycle, β-oxidation and amino acid oxidation.
- Mitochondrial matrix is enclosed by two membranes.
- Components of the ETC are located on the inner membrane; the folded cristae provide a large surface area.
- The inner membrane is highly impermeable and requires specific transporters.
- Transporters specific for pyruvate, fatty acids, amino acids, ATP/ADP, phosphate and protons are found in the inner membrane.
- The outer membrane is permeable to small molecules and ions because of Porins: transmembrane proteins that form channels in the outer membrane.

Standard Reduction Potentials

- In oxidative phosphorylation, the electron transfer potential of NADH and $FADH_2$ is converted into the phosphoryl transfer potential of ATP.
- The standard reduction potential (E_0) is a quantitative measure of the ease with which a compound can be reduced; or how readily it accepts electrons.
- The more positive the E_0, the more readily the compound accepts electrons. The more negative the E_0, the more readily it gives up electrons.
- The redox potential is measured relative to that of a proton which is assigned as zero. $2H^+ + 2e^- \rightarrow H_2$. $E_o = 0$.
- For biochemical reactions, $[H^+]$ of 10^{-7} is considered standard and we use E_o' instead of E_o.

Relationship between ΔE_o' and ΔG^o'

- The standard free energy change is related to the change in standard reduction potential: $\Delta G^o' = -nF\Delta E_o'$; n is the number of electrons transferred and F is a constant that converts energy from volts to KJ. F = 96.5 kJ/volt.mol.
- Based on this relationship, electrons can be spontaneously transferred from a compound with a lower E_o' to a higher E_o' (ΔE_o' needs to be positive) but not the other way around.
- If NADH is the electron donor and O_2 is the electron acceptor,

  $\Delta G^{0'} = -nF\Delta E_0'$

  $\Delta G^{0'} = -$ (2 electrons)(96.5 kJ/volt.mol)(0.82volt–(–0.32volt))

  $\Delta G^{0'} = -$ 220 kJ/mol
- The great difference in E_o' between NADH/$FADH_2$ and O_2 results in a highly negative ΔG^o' and drives the ETC.

Quantitation of ATP synthesis

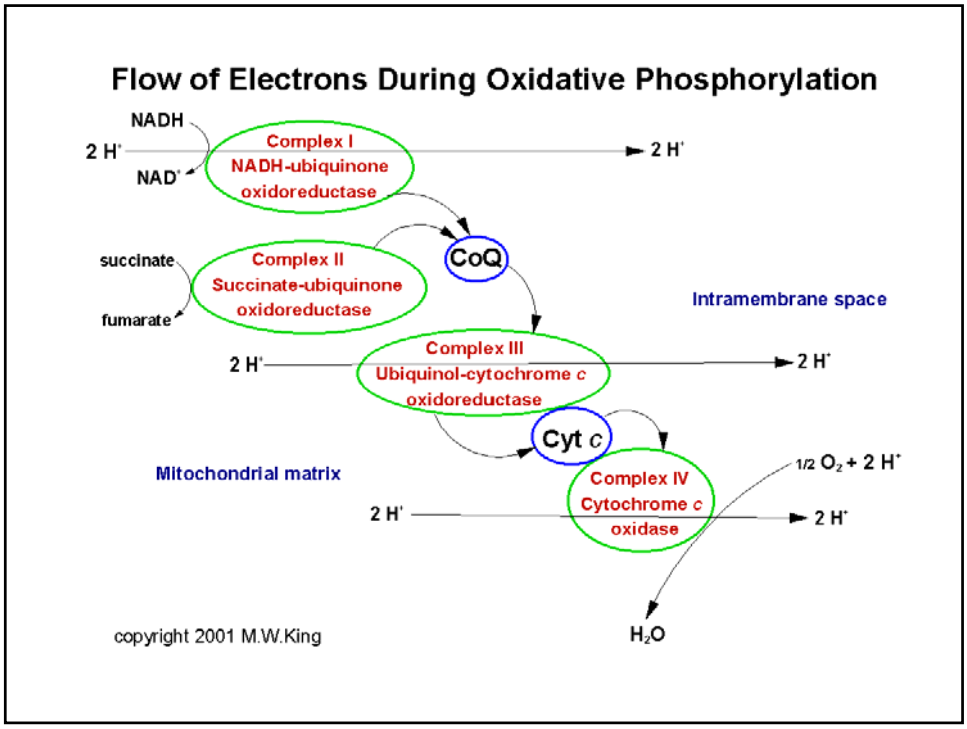
- $\Delta G^{o'}$ for transfer of 2 electrons from NADH to O_2 is –220 kJ/mol. This is sufficient to synthesize 7 molecules of ATP ($\Delta G^{o'}$ for ATP synthesis is 31 kJ/mol).
- However, a significant amount of energy is used up to pump H^+ out of the mitochondria. Only a third is used for ATP synthesis.
- Actually, by the process of oxidative phosphorylation:

  oxidation of each mole of NADH = 2.5 moles of ATP

  oxidation of each mole of $FADH_2$ = 1.5 moles of ATP

Components of the Electron Transport Chain

- In the ETC, the electron carriers are arranged such that the flow of electrons is spontaneous. Each acceptor has sequentially greater electron affinity (greater $\Delta E_0'$) than the electron donor.
- The series of oxidation-reduction reactions requires four membrane-bound multi-protein complexes called complexes I, II, III and IV.
- Each complex consist of multiple proteins and Fe-S, heme or copper prosthetic groups.
- Complexes I, III and IV are also proton pumps
- Complex II consists of succinate dehydrogenase, the enzyme of the TCA cycle.



Complex I

Complex I: NADH-CoQ oxidoreductase

*Entry site for NADH + H^+

*Contains:

Fe-S cluster (non-heme protein)

flavin mononucleotide phosphate (FMN)

Coenzyme Q (free in membrane)

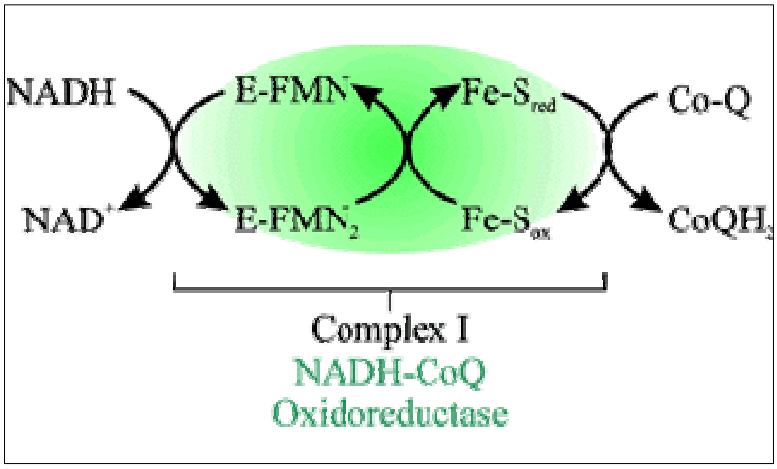
***Net reaction: NADH + H^+ + CoQ ---> NAD^+ + $CoQH_2$**

*$\Delta G^{\circ\prime}$ = -81.0 kJ/mol

* complex I pumps protons outside the mitochondria

* ATP is produced

Complex I



From: http://www.geocities.com/CapeCanaveral/Lab/2041/

Complex II

Complex II: Succinate-CoQ reductase

*Entry site for $FADH_2$

*Contains:

Fe-S cluster (non-heme protein)

Coenzyme Q (free in membrane)

***Net reaction: Succinate + CoQ --> Fumarate + $CoQH_2$**

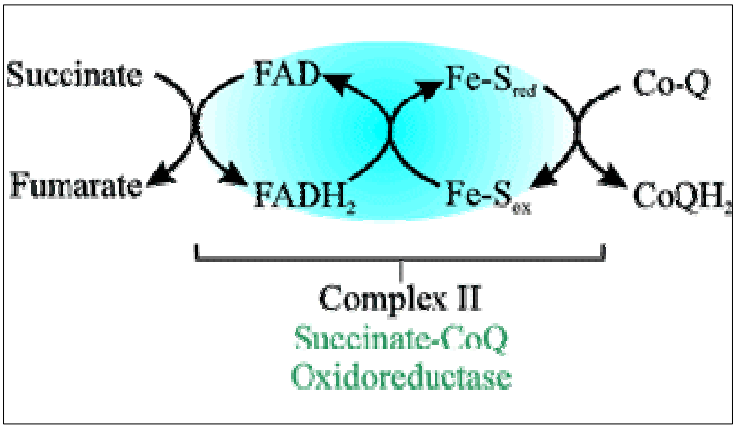
*$\Delta G^{\circ\prime}$ = -13.5 kJ/mol

* Conversion of succinate to fumarate is reaction of TCA cycle and is catalyzed by succinate dehydrogenase

* Not a proton pump

* No ATP produced

Complex II



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Complex III

Complex III: CoQH2-cytochrome c oxidoreductase

*Contains:

cytochrome c (free in membrane)

cytochrome b

cytochrome c1

Several Fe-S cluster (non-heme protein)

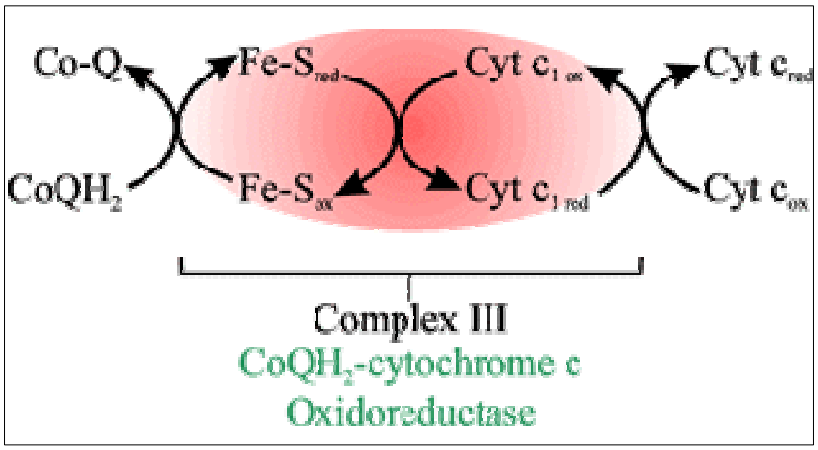
***Net reaction: $CoQH_2$ + 2 cyt c [Fe (III)] ---> CoQ + 2 cyt c [Fe (II)] + 2 H^+**

*$\Delta G^{\circ\prime}$ = -34.2 kJ/mol

* Complex III pumps protons outside the mitochondria

* ATP produced

Complex III



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Complex IV

Complex IV: cytochrome oxidase

*Contains:

cytochrome a

cytochrome a3

Copper

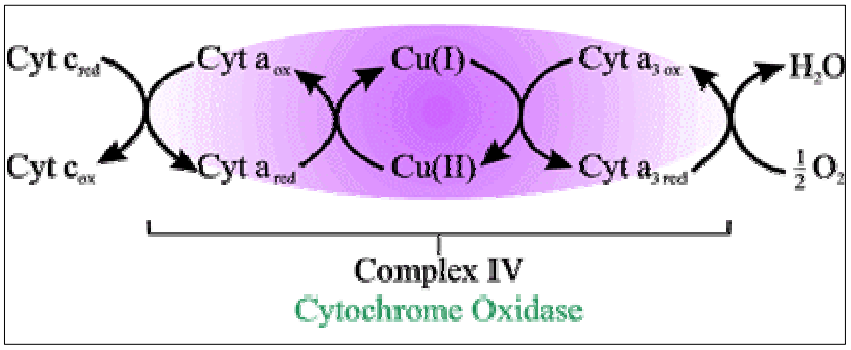
***Net reaction: 2 cyt c [Fe (II)] + 1/2 O_2 + 2 H^+ ---> 2 cyt c [Fe (III)] + H_2O**

*$\Delta G^{\circ\prime}$ = -110.0 kJ/mol

* Complex IV pumps protons outside the mitochondria

* ATP produced

Complex IV



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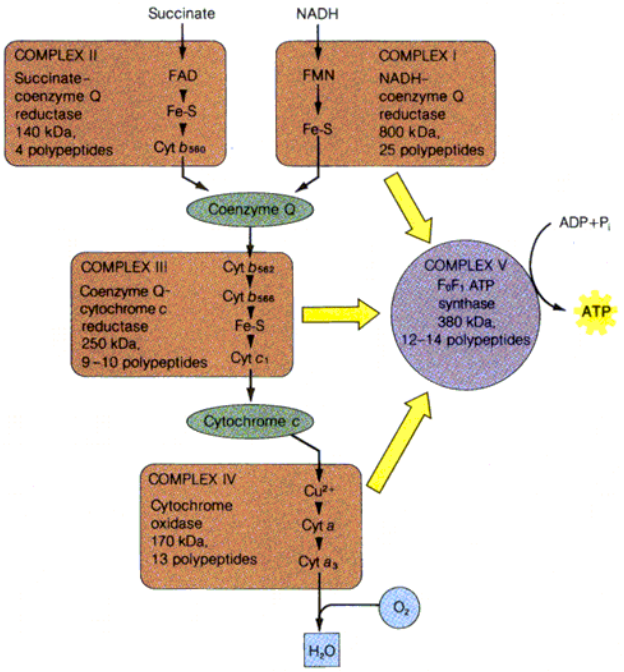
Overall Electron Transport Chain



From: Biochemistry by Matthews

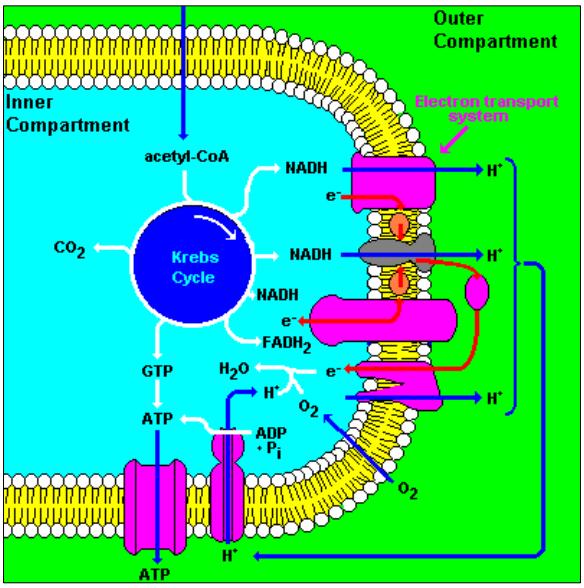
ATP synthase (also called complex V)

- The electrochemical potential energy generated by the proton and pH gradients across the mitochondrial inner membrane is called as Proton Motive Force and is used to drive ATP synthesis.
- Protons return to the mitochondrial matrix through an integral membrane protein (of the mitochondrial inner membrane) known as ATP synthase (sometimes called as Complex V of the ETC).
- ATP synthase is a multiple subunit complex that binds ADP and inorganic phosphate and converts them to ATP
- Proton transport is coupled to ATP synthesis. This is called as the **chemiosmotic theory** of oxidative phosphorylation. ATP is not synthesized unless there is a simultaneous transport of H^+ across the inner mitochondrial membrane.

ATP-ADP translocase

- ATP and ADP do not diffuse freely across the inner mitochondrial membrane
- A specific transport protein ADP-ATP translocase (also called adenine nucleotide translocase, ANT) is a antiporter that exchanges each ATP from the matrix for an ADP from the cytosol.
- Exchange occurs by the mechanism of translocase eversion
- ANT has a single nucleotide binding site and ADP and ATP have the same binding affinity
- When there is a positive membrane potential (higher + charge outside), when ATP is bound to the matrix side, the ANT undergoes rapid eversion (since ATP has one extra negative charge)
- A fourth of the energy harvested by ETC is used by ANT

Overall Scheme of Electron Transport and Oxidative Phosphorylation



From: http://www.people.virginia.edu/~rjh9u/eltrans.html

Inhibitors of Oxidative Phosphorylation

- Complex I: Rotenone
- Complex II: Carboxin
- Complex III: Antimycin A
- Complex IV: Cyanide, Azide, Carbon monoxide
- ATP synthase: Oligomycin
- ATP-ADP translocase: Atractyloside (a plant glycoside)

Uncouplers

- Uncouplers inhibit oxidative phosphorylation.
- They 'uncouple' the ETC from oxidative phosphorylation.
- The ETC remains intact and electrons are transferred to O_2 to generate H_2O. However, uncouplers carry protons across the mitochondrial membrane making it 'leaky' for H^+. The pH and electrical gradient is not generated and ATP is not synthesized.
- In the presence of an uncoupling agent, energy released via the ETC is converted into heat.
- This mechanism is used by hibernating animals to stay warm in the winter, since they don't need ATP for anabolic processes while they are resting.
- Examples of uncouplers: Natural: Thermogenin or uncoupling protein (UCP). Synthetic: 2,4,-dinitrophenol.

Transport of NADH into mitochondria

- Glycolytic pathway results in the reduction of NAD^+ to NADH in the cytosol
- NADH is oxidized to NAD^+ by the ETC in the mitochondria
- The mitochondrial membrane is impermeable to NADH, thus, a transport system would be required to allow entry to NADH into the mitochondrial matrix
- Instead of NADH molecule directly entering the mitochondria, there are electron shuttle systems that accept electrons from cytosolic NADH, enter mitochondria, and give up the electrons to electron acceptors in the mitochondrial matrix

Glycerol-3-Phosphate Shuttle

- Functions in the skeletal muscle and brain.
- NADH on the cytoplasmic side is oxidized to NAD^+ with coupled reduction of DHAP to glycerol-3-phosphate. Enzyme: cytoplasmic glycerol-3-phosphate dehydrogenase.
- The oxidation of glycerol 3-phosphate back to DHAP is catalyzed by a mitochondrial membrane bound isoenzyme of glycerol-3-phosphate dehydrogenase.
- The oxidation is coupled to reduction of a FAD prosthetic group of the mitochondrial enzyme to $FADH_2$.
- Reduced $FADH_2$ transfers it electrons to CoQ via the ETC.
- Thus, in muscle and brain, even though 2 NADH are produced by glycolysis, actually, 2 $FADH_2$ are available for entry into the ETC.

The Malate-Aspartate Shuttle

- Functions in the heart and liver
- In the cytosol, electrons are transferred from NADH to oxaloacetate forming NAD^+ and malate. The enzyme is malate dehydrogenase. (NAD^+ is reduced back to NADH by glycolysis). Malate can easily enter mitochondria.
- In the mitochondrial matrix, malate is oxidized to oxaloacetate by the enzyme of the TCA cycle, malate DH. This is coupled to reduction of NAD^+ to NADH
- Oxaloacetate is converted to aspartate by accepting an amino group from glutamate in a reaction catalyzed by an aminotransferase.
- Aspartate readily crosses the mitochondrial inner membrane to enter the cytosol. In the cytosol, aspartate donates its amino group to form oxaloacetate.
- Thus, in heart and liver, electron transfer from cytosol to mitochondria does not involve net expense of energy

Complete Oxidation of glucose

- Complete oxidation of glucose involves the following pathways and net reactions:
- Glycolysis: glucose + 2ADP + 2Pi + $2NAD^+$ → 2pyruvate + 2 ATP + 2NADH + 2 H^+ + $2H_2O$
- PDH complex: 2 pyruvate + 2CoA + 2 NAD^+ → 2acetylCoA + $2CO_2$ + 2 NADH
- TCA cycle: 2acetylCoA + 6 NAD^+ + 2FAD + 2GDP + 2Pi + $4H_2O$ → $4CO_2$ + 6 NADH + $4H^+$ + $2FADH_2$ + 2GTP + 2CoA
- Overall oxidation: glucose + 2ADP + 2GDP + 4 Pi + $8NAD^+$ + 2FAD + $2H_2O$ → $6CO_2$ + 2ATP + 2GTP + 8NADH + $6H^+$ + $2FADH_2$

Total yield of ATP from Glucose

					TOTAL
Pathway		**ATP**	**NADH**	**FAD**	**ATP**
Glycolysis		-2	2	0	
		4			
PDH		0	2	0	
TCA		2	6	2	
Glycerol-3-P shuttle		0	-2	2	
		4	8	4	
ATP Harvested		4	20	6	**30**

Total oxidation of Palmitate

- Activation: Palmitate + CoA + ATP + H2O → Palmitoyl CoA + AMP + PPi + 2H+
- β-oxidation: Palmitoyl CoA + 7CoA + 7FAD + 7NAD+ + 7H2O → 8 acetylCoA + 7FADH2 + 7NADH + 7H+
- TCA cycle: 8acetylCoA + 24 NAD^+ + 8FAD + 8GDP + 8Pi + 16H_2O → 16CO_2 + 24 NADH + 16H^+ + 8$FADH_2$ + 8GTP + 8CoA
- Overall oxidation: Palmitate + ATP + 15FAD + 31NAD^+ + 8GDP + 8Pi + 24H_2O → 16 CO_2 + AMP + PPi + 15$FADH_2$ + 31NADH + 25H^+ + 8GTP

Total yield of ATP from Palmitate

Pathway		ATP	NADH	FAD	TOTAL ATP
activation		-2	0	0	
b-oxidation		0	7	7	
TCA		8	24	8	
		6	31	15	
ATP Harvested		6	77.5	22.5	**106**