Electron Transport and Oxidative Phosphorylation

The Mitochondrion

Electron Transport

Oxidative Phosphorylation

Control of ATP Production

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$
$$\Delta G^{\circ\prime} = -2823\ kJ{\cdot}mol^{-1}$$

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

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

Electrons are shuttled by NAD^+/NADH and FAD/$FADH_2$ into electron transport chain

Proton gradient that develops drives synthesis of ATP from ADP + P_i (oxidative phosphorylation)

38 ATP maximum potential production

The Mitochondrion
(site of eukaryotic oxidative metabolism)

Outer membrane

porin - nonspecific pore for <10-kDa molecules
nucleoside diphosphate kinase

Intermembrane space

adenylate kinase
nucleoside diphosphate kinase

Inner membrane

electron transport chain proteins
transporters/translocators for ATP, ADP, pyruvate, Ca^{2+}, P_i
permeable to O_2, CO_2, and H_2O

Cristae

invaginations of inner membrane
increases with respiratory activity of tissue

Matrix

citric acid cycle enzymes
pyruvate dehydrogenase
genetic machinery

The Mitochondrion

Mitochondrial transport systems

ADP-ATP translocator - antiport for exchange of ADP^{3-} and ATP^{4-}, driven by membrane potential ($\Delta\psi$)

P_i-H^+ transporter - symport driven by ΔpH

Ca^{2+} transport - driven by $\Delta\psi$ and Ca^{2+}-Na^+ exchanger

NADH "transport":
reducing equivalents are transported, not NADH

Glycerophosphate shuttle

3-phosphoglycerol dehydrogenase
flavoprotein dehydrogenase
NADH $\cong$ 2 ATP

Malate-aspartate shuttle

malate dehydrogenase
malate-α-ketoglutarate carrier
transaminase
glutamate-aspartate carrier
NADH $\cong$ 3 ATP

Electron Transport

Thermodynamics of electron transport

Reoxidation of NADH and $FADH_2$ by O_2 is coupled to ATP synthesis

$$NAD^+ + H^+ + 2e^- \Leftrightarrow NADH \quad \varepsilon^{\circ\prime} = -0.315 \text{ V}$$

$$0.5O_2 + 2H^+ + 2e^- \Leftrightarrow H_2O \qquad \varepsilon^{\circ\prime} = +0.815 \text{ V}$$

$$0.5O_2 + NADH + H^+ \Leftrightarrow H_2O + NAD^+$$

$$\Delta\varepsilon^{\circ\prime} = 0.815 - (-0.315) = 1.130 \text{ V}$$

$$\Delta G^{\circ\prime} = -nF\Delta\varepsilon^{\circ\prime}$$

$$= -2(96{,}494 \text{ C}\cdot\text{mol}^{-1})(1.130 \text{ J}\cdot\text{C}^{-1}) = 218 \text{ kJ}\cdot\text{mol}^{-1}$$

5 to 7 ATP if 100% efficient

Distribute free energy change to electron transport protein complexes, each coupled to ATP synthesis (oxidative phosphorylation)

Electron Transport

The sequence of electron transport

Series of four protein complexes (in order)

Complex I - NADH-coenzyme Q reductase

$$NADH + CoQ_{ox} \rightarrow NAD^{+} + CoQ_{red}$$

$\Delta\varepsilon\,^{\circ\prime} = +0.36$ V $\quad \Delta G^{\circ\prime} = -70\ kJ{\cdot}mol^{-1}$

inhibited by rotenone and amytal

Complex II - succinate-coenzyme Q reductase

$$FADH_2 + CoQ_{ox} \rightarrow FAD + CoQ_{red}$$

$\Delta\varepsilon\,^{\circ\prime} = +0.015$ V $\quad \Delta G^{\circ\prime} = -2.9\ kJ{\cdot}mol^{-1}$

Complex III - coenzyme Q-cytochrome c reductase

$$CoQ_{red} + \text{cytochrome } c_{ox} \rightarrow CoQ_{ox} + \text{cytochrome } c_{red}$$

$\Delta\varepsilon\,^{\circ\prime} = +0.19$ V $\Delta G^{\circ\prime} = -37\ kJ{\cdot}mol^{-1}$

inhibited by antimycin A

Complex IV - cytochrome c oxidase

$$\text{cytochrome } c_{red} + 0.5O_2 \rightarrow \text{cytochrome } c_{ox} + H_2O$$

$\Delta\varepsilon^{\circ\prime} = +0.58$ V $\Delta G^{\circ\prime} = -110\ kJ{\cdot}mol^{-1}$

inhibited by cyanide (CN^-), CO, azide (N_3^-)

Electron Transport

Phosphorylation and oxidation are rigidly coupled

P/O ratios:

moles of ADP phosphorylated/
moles of NADH ($FADH_2$) oxidized

Traditional approach

complex I - 9/3 = 3

complex II - 6/3 = 2

complex IV - 3/3 = 1

38 total ATP from complete oxidation of glucose

Modern approach

H^+ translocated/$4H^+$ per ATP synthesis and transport

complex I - 10/4 = 2.5

complex II - 6/4 = 1.5

complex IV - 2/4 = 0.5

29.5 to 31 total ATP from complete oxidation of glucose

Electron Transport

Components of electron transport chain

Laterally mobile
Non-equimolar ratios
No apparent higher-order structures

Complex I (NADH-coenzyme Q reductase)

passes electrons from NADH (2 e^- donor/acceptor) to CoQ

850-kDa protein

1 flavin mononucleotide (FMN) - accept/donate 1 or 2 e^-

6 to 7 iron-sulfur clusters - accept/donate 1 or 2 e^-

coenzyme Q - accept/donate 1 or 2 e^-, hydrophobic tail, 10 isoprenoid units (Q_{10})

Electron Transport

Components of the electron-transport chain

Complex II (succinate-coenzyme Q reductase)

passes electrons from succinate to CoQ

Succinate dehydrogenase

covalent bound FAD

1 [4Fe-4S] cluster

2 [2Fe-2S] cluster

1 cytochrome b_{560}

$\Delta\varepsilon^{\circ\prime}$ is insufficient to provide ΔG for ATP synthesis

Electron Transport

Components of the electron-transport chain

Complex III (coenzyme Q-cytochrome *c* reductase)

passes electrons from CoQ to cytochrome *c*

2 cytochrome *b* (cyt $b_{562 \text{ or } H}$ and cyt $b_{566 \text{ or } L}$)

1 cytochrome c_1

1 [2Fe-2S] cluster (Rieske iron-sulfur protein)

cytochromes have characteristic absorption bands and chemical structures

Electron Transport

Components of the electron-transport chain

Cytochrome *c*

passes electrons from cyt c_1 of Complex III to cytochrome oxidase (Complex IV)

peripheral membrane protein

protein portion of cytochromes directs path of electron transfer

charged amino acids on surface facilitate protein-protein interactions

Electron Transport

Components of the electron-transport chain

Complex IV (cytochrome oxidase)

$$4\text{Cyt } c^{2+} + 4H^{+} + O_2 \rightarrow 4\text{cyt } c^{3+} + 2H_2O$$

mammalian complex ~200-kDa transmembrane dimeric protein

6 to 13 subunits

subunits I and II contain redox-active centers

2 heme *a* (heme *a* and heme a_3)

2 Cu centers (CuA or *a* and CuB or a_3)

charged Asp/Glu residues interact with Lys residues on surface of cyt *c*

reaction cycle proceeds through binuclear (Cu-Fe) complex and ferryl (Fe^{4+}) intermediate

Oxidative Phosphorylation

Energy coupling (energy transduction) - free energy from electron transport chain utilized by proton-translocating ATP-synthase (Complex V)

Energy coupling hypotheses:

1. The chemical coupling hypothesis - reactive intermediates drove oxidative phosphorylation
2. The conformational-coupling hypothesis - electron transport causes proteins to assume activated conformations, whose relaxation back to deactivated states drove ATP synthesis
3. The chemiosmotic hypothesis - Most consistent model, ΔG of electron transport is conserved by pumping H^+ from mitochondrial matrix to intermembrane space, creating electrochemical H^+ gradient across inner membrane, which drive ATP synthesis

Oxidative Phosphorylation

Proton gradient generation

Electron transport causes Complexes I, III, and IV to transport H^+ from the matrix (region of low $[H^+]$ or high pH and negative electrical potential) across the inner membrane to the intermembrane space (region of high $[H^+]$ or low pH and positive electrical potential)

ΔG of the resulting electrochemical gradient = proton motive force (pmf) (recall: discussion of nonconjugate flow)

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

Z is charge on proton (+1), *F* is faraday constant, $\Delta\Psi$ is membrane potential ($\Delta\Psi$ is positive when ion transported from negative to positive)

It takes energy to transport H^+ from matrix to intermembrane space

$$1\ H^+ \rightarrow \Delta G \sim 21.5\ kJ{\cdot}mol^{-1}$$

$\sim 3\ H^+$ to synthesize 1 ATP

Oxidative Phosphorylation

Proton gradient generation

Proton transport mechanisms

1. The redox loop mechanism - reduction of redox center involves accepting e^-s and H^+ from matrix and that 1st redox carrier contains more H atoms in its reduced state than in its oxidized state and that the 2nd redox carrier have no difference in its hydrogen atom content between states. Addition of Q cycle to explain missing ($H + e^-$) carrier, but cannot participate in Complex IV

$$CoQH_2 + \text{cyt } c_1(Fe^{3+}) \rightarrow CoQ^{\cdot -} + \text{cyt } c_1\ (Fe^{2+}) + 2H^+\ (\textit{cytosolic})$$

$$CoQH_2 + CoQ^{\cdot -} + \text{cyt } c_1\ (Fe^{3+}) + 2H^+(\textit{mitochondrial}) \rightarrow CoQ + CoQH_2 + \text{cyt } c_1\ (Fe^{2+}) + 2H^+\ (\textit{cytosolic})$$

$$CoQH_2 + 2\text{cyt } c_1\ (Fe^{3+}) + 2H^+\ (\textit{mitochondrial}) \rightarrow CoQ + 2\text{cyt } c_1\ (Fe^{2+}) + 4H^+\ (\textit{cytosolic})$$

2. The proton pump mechanism - electron transfer results in conformational changes that influence pKs of amino acid residues (similar to Bohr effect in hemoglobin)

More experimental work is required!

Oxidative Phosphorylation

Mechanisms of ATP synthesis

Proton-translocating ATP synthase (proton pumping ATPase and F_1F_0-ATPase)

multisubunit transmembrane protein

F_0

water insoluble transmembrane protein
10 to 12 subunit types
channel for proton translocation
Oligomycin inhibits ATP synthesis by binding and interfering with H^+ transport

F_1

water soluble peripheral membrane protein
$\alpha_3\beta_3\gamma\delta\varepsilon$ subunit composition (ATP synthesis on β subunit)
isolated form is ATPase

Stalk

contains at least 2 proteins - oligomycin-sensitivity-conferring protein (OSCP) and couping factor 6 (F_6)

Much structural work in recent literature!

Oxidative Phsophorylation

Mechanism of ATP synthesis

Three phases:

1. Translocation of H^+ carried out by F_0
2. Catalysis of formation of phosphoanhydride bond of ATP carried out by F_1
3. Coupling of dissipation of proton gradient with ATP synthesis, requires interactions of F_0 and F_1

Mechanism resembles conformation-coupling hypothesis presented previously

Three steps:

1. Binding of ADP + P_i to "loose" (L) binding site
2. Free energy-driven conformational change, L to "tight" (T)-binding site that catalyzes ATP synthesis, T to "open" (O) site, and O to L site
3. ATP synthesized at T site on one subunit, while ATP is released from O site on another subunit. Free energy of H^+ flow drives ATP release (i.e., T $\rightarrow$ O transition)

Binding driven by rotation of the catalytic assembly, $\alpha_3\beta_3$, with respect to other portions of the assembly (recent studies have demonstrated this motion!)

Oxidative Phosphorylation

Uncoupling of oxidative phosphorylation

Tight coupling of electron transport to ATP synthesis in mitochondrion depends on the impermeability of the inner mitochondrial membrane

An agent that interacts with the inner mitochondrial membrane and increases its permeability to H^+ would allow dissipation of H^+ gradient, thus uncoupling oxidative phosphorylation from electron transport

Production of heat

Agents:

2,4-dinitrophenol (DNP)
carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP)

In brown adipose tissue:

Uncoupling protein (UCP, thermogenin) - 32-kDa dimer forms channel that controls H^+ permeability of inner membrane

Flow through channel is activated by free fatty acids through norepinephrine stimulated pathway

Control of ATP Production

Adult woman requires 6300 to 7500 kJ of metabolic energy per day → hydrolysis of 200 mol of ATP

amount of ATP in body at any time is ~0.1 mol

Pathways that produce ATP are strictly controlled so that ATP is never produced more rapidly than necessary

Control of oxidative phosphorylation

Cytochrome oxidase - irreversible step, controlled by availability of substrate (reduced cytochrome *c*)

$0.5NADH + \text{cyt } c^{3+} + ADP + P_i \Leftrightarrow$

$0.5NAD^+ + \text{cyt } c^{2+} + ATP$

$$\frac{[c^{2+}]}{[c^{3+}]} = \left(\frac{[NADH]}{[NAD^+]}\right)^{1/2} \frac{[ADP][P_i]}{[ATP]} K_{eq}$$

Acceptor control - rate of oxidative phosphorylation increases with [ADP], phosphoryl group acceptor

Control of ATP Production

Coordinated control of ATP production

$$\frac{[c^{2+}]}{[c^{3+}]} = \left(\frac{[\mathrm{NADH}]}{[\mathrm{NAD^+}]}\right)^{1/2} \frac{[\mathrm{ADP}][\mathrm{P_i}]}{[\mathrm{ATP}]} K_{\mathrm{eq}}$$

Glycolysis and citric acid cycle provide input to [NADH]/[NAD^+] ratio

Citrate inhibits glycolysis by inhibiting PFK-1

Physiological implications of aerobic versus anaerobic metabolism

Pasteur effect - decreased glucose consumption under aerobic conditions, more efficient production of ATP

$$C_6H_{12}O_6 + 2ADP + 2P_i \rightarrow 2lactate + 2H^+ + 2H_2O + 2ATP$$

$$C_6H_{12}O_6 + 38ADP + 38P_i + 6O_2 \rightarrow 6CO_2 + 44H_2O + 38ATP$$

Activity of PFK-1 (regulated by citrate and adenine nucleotide) decreases manyfold on switching from anaerobic to aerobic metabolism

PFK-1 inhibited by acid production arising from lactic acid production