Lecture Outline

1. What do we do with NADH + H⁺ and FADH₂ reducing equivalents?
2. Electron Transport – the oxidation phase of Oxidative Phosphorylation
3. ATP synthesis – the Phosphorylation Phase of Oxidative Phosphorylation
4. Why believe the Chemiosmotic hypothesis?
5. Indirect Active Transport into the mitochondrion
6. Comparison of yield from Fatty Acid Oxidation and Monosaccharide Oxidation
7. Life without oxygen - Fermentation

Mitochondrial Functions

**Oxidize compounds to CO₂ + H₂O**

- Fatty acid Oxidation
- Oxidation of Pyruvate
- TCA Kreb’s Cycle

*Produce reduced carriers* NADH & FADH₂

Generate >90% of Typical Cell’s ATP

- Oxidative Phosphorylation
- “electron transport”
- ATP synthesis

*Oxidize reduced carriers to produce ATP or equiv*
Electron transport oxidizes NADH and FADH₂.

Electrons eventually given to oxygen to form water, releasing energy used to make ATP.

Electron transport chain: Oxidize NADH + H⁺ to NAD⁺.
- 2e⁻ and 2H⁺ given to a multiprotein complex (complex I).
- 2e⁻ get passed on.
- 2H⁺ orphaned as electrons transferred.

Energy of oxidation pumps H⁺ to intermembrane space.

Electron transport chain:
- 2H₂ + O₂ = H₂O + BOOM!
Electron Transport Chain

Oxidize NADH + H+ to NAD+
2e− and 2H+ given to multiprotein complex (complex I)
2e− get passed on
2H+ orphaned as electrons transferred
energy of oxidation pumps H+ to intermembrane space

2e− and 2H+ (where from?) get passed to another complex (complex III)
orphaning of 2H+ repeated as 2e− passed on
energy of oxidation pumps H+ to intermembrane space

2e− and 2H+ (where from?) get passed to a third complex (complex IV)
orphaning of 2H+ repeated as 2e− passed on
energy of oxidation pumps H+ to intermembrane space

need to pick up 2H+ (where from?) to form water

Net Result of Electron Transport:
an electrochemical H+ gradient
What happened to complex II?

- Three 2H⁺ pumping steps from NADH + H⁺ oxidation
- Only TWO 2H⁺ pumping steps from FADH₂ oxidation

Electron transport - oxidizes NADH and FADH₂ back to NAD⁺ and FAD

Electron transport - electrons passed in series from one carrier to another eventually give e⁻ and H⁺ to oxygen to form water

BUT PUMP H⁺ in the process across the inner mitochondrial membrane into intermembrane space

FORMS BIG H⁺ gradient

Electron transport - electrons passed in series from one carrier to another eventually give e⁻ and H⁺ to oxygen to form water

BUT PUMP H⁺ in the process across the inner mitochondrial membrane into intermembrane space

FORMS BIG H⁺ gradient

What good is the H⁺ Electrochemical Gradient?

H⁺ gradient forms across the inner mitochondrial membrane between the matrix space and the intermembrane space.

At pH 6 in the matrix space, H⁺ concentration is high.

At pH 8 in the intermembrane space, H⁺ concentration is lower.

H⁺ moves from high concentration to low concentration, creating an electrochemical gradient.

This gradient drives the synthesis of ATP by the ATP synthase enzyme in the inner mitochondrial membrane.
A rotor within the membrane spin... A stator anchored in the membrane... A rod (or "stalk")... Three catalytic sites... Three catalytic sites in the stationary knob join inorganic phosphate to ADP to make ATP.

Mitochondrial H+ ATPase drives conformational change

pH 6

pH 8

pH 6

pH 8

Mitochondrial H+ ATPase

ADP + Pi

ATP

H+ movement

Open

ATP expelled

ATP

ADP + Pi

Open

ATP synthase

H+ movement drives conformational change

Figure 9.14

Mitochondrial matrix
Electron Transport Chain is a Proton Pump

Reducing equivalents power the pump

Gradient powers ATP Synthesis

Uncoupling Oxidation and Phosphorylation

- Must have intact membranes to make ATP
- Agents such as cyanide poison electron transport chain so no H⁺ gradient produced
  - but if supply H⁺ gradient can still make ATP
- Agents that allow H⁺ passage (dissipate gradient) diminish ATP synthesis – dinitrophenol (DNP)

Roughly

3 ATP for each NADH + H⁺
2 ATP for each FADH₂
Can Use H⁺ gradient to power other useful work

How you get pyruvate⁻ (an acid) across the mitochondrion membrane? (not magic!)

How about ADP⁻⁻ and Pi⁻⁻ into mitochondrion?

How about ATP⁻⁻ out of mitochondrion?

Electrochemical Gradient

Can also power Indirect ACTIVE TRANSPORT of IONS into MATRIX instead of making ATP
So how many ATP from Glucose oxidation?

~36 ATP per 6 carbon sugar

\[ 7.3 \text{ kcal/mole} \times 36 = 263 \text{ kcal/mole} \]

\[ \Delta G = -686 \text{ kcal/mole} \]

\[ \text{39\% efficient} \]

\[ \approx 39\% \text{ efficient} \]

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\[ \Delta \text{G} = -686 \text{ kcal/mole} \]

\[ \approx 39\% \text{ efficient} \]

\[ \approx 36 \text{ ATP per 6 carbon sugar} \]

Compare to Fatty Acid Oxidation

18 carbon fatty acid (9 two carbon units)

Each 2 carbon unit

\[ 1 \times \text{NADH + H}^+ \]

\[ 1 \times \text{FADH}_2 \]

Each acetyl CoA gives in TCA

\[ 3 \text{ NADH + H}^+ \]

\[ 1 \text{ FADH}_2 \]

\[ 1 \text{ GTP} \]

\[ 9 \text{ ATP} \]

\[ 2 \text{ ATP} \]

\[ 1 \text{ ATP} \]

6 ATP per glucose carbon

8.5 ATP per FA carbon

Oxidation of 18 carbon fatty acid yields 198 ATP

In Metabolism:

Highly reduced \[ \text{fully oxidized} \]

\[ \text{Fatty acid} \quad \text{(captured)} \]

\[ \text{carbohydrate} \]

\[ + \text{O}_2 \rightarrow \text{H}_2\text{O} + \text{CO}_2 + \text{energy} \]

\[ \text{(captured)} \]

Partially reduced \[ \text{fully oxidized} \]

\[ + \text{O}_2 \rightarrow \text{H}_2\text{O} + \text{CO}_2 + \text{energy} \]

\[ \text{(captured)} \]
CH₄
R-CH₂-CH₃
R-CH=CH₂
R-CH₂-CH₂-OH
R-CH₂-C=O
R-CH₂-C=O
O=C=O

Organic Oxidation Series

Fatty Acids
Monosaccharides

Life Without Oxygen

Electrons carried via NADH
Electrons transported and pumping of protons (H+)
Electron transport and pumping of protons (H+), which create an ATP gradient across the membrane
Oxidative phosphorylation

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Oxidative phosphorylation
The Regeneration Energy Carriers

Energy carriers (ATP, NAD^+, FAD) present in only minute amounts

Captured in catabolism

NADH + H^+

Energy for cellular work (endergonic, energy-consuming processes)

Energy from catabolism (exergonic, energy-yielding processes)

Cashed in

Run out of NAD^+

Energy carriers (ATP, NAD^+, FAD) present in only minute amounts

Life Without Oxygen - FERMENTATION

No O_2 present

Fermentation

Ethanol or lactate

Mitochondrion

Citric acid cycle

Figure 9.18

Life Without Oxygen - FERMENTATION

Yeast

Wine/beer

Muscles

Method

To Recycle NADH + H^+

No O_2 present

Fermentation

Mitochondrion

Figure 9.18

Running Muscles

2e^- 2H^+ Recycles NADH

Keeps the Motor running

Two pyruvate

Run in reverse

When oxygen returns

Figure 9.17
Fermentation and cellular respiration
- Differ in their final electron acceptor
  - oxygen
  - lactate
  - or ethanol

Cellular aerobic respiration
- Produces tons more ATP
  - 2 ATP in fermentation
  - 36 ATP in aerobic oxidation of glucose

Fermentation allows organisms to eek out a living in low or no O₂

Ancient system – pre oxygen

Where does oxygen come from?
Next time:
photosynthesis
Summary:
1. Oxidative Phosphorylation is comprised of two distinct processes
   - Electron Transport (oxidative) makes H+ gradient
   - ATP synthesis (phosphorylation) uses H+ gradient coupled by H+ gradient
2. Processes are separable:
   - cyanide (poisons electron transport)
   - uncouplers (DNP) (dissipate H+ gradient)
3. Electrochemical gradient powers
   - mitochondrial H+ ATPase
   - indirect active transport
4. Oxidation of fatty acids yields more ATP than oxidation of glucose
5. Can ferment sugars in absence of oxygen, still produce ATP by substrate level phosphorylation but no reducing equivalents captured

The catabolism of various molecules from food

Figure 9.19