Chapter 20

Electron Transport and Oxidative Phosphorylation

Biochemistry
by
Reginald Garrett and Charles Grisham

Essential Question

- How do cells oxidize NADH and [FADH₂]
- How do cells convert their reducing potential into the chemical energy of ATP?

Outline of chapter 20

1. Where in the Cell Are Electron Transport and Oxidative Phosphorylation Carried Out?
2. What Are Reduction Potentials, and How Are They Used to Account for Free Energy Changes in Redox Reactions?
3. How Is the Electron-Transport Chain Organized?
4. What Are the Thermodynamic Implications of Chemiosmotic Coupling?
5. How Does a Proton Gradient Drive the Synthesis of ATP?

• Electron Transport:
  - Electrons carried by reduced coenzymes, NADH or FADH₂, are passed through a chain of proteins and coenzymes, finally reaching O₂, the terminal electron acceptor
  - and to drive the generation of a proton gradient across the inner mitochondrial membrane
• Oxidative Phosphorylation:
  - The proton gradient runs downhill to drive the synthesis of ATP
20.1 - Where in the Cell Are Electron Transport and Oxidative Phosphorylation Carried Out?

- The processes of electron transport and oxidative phosphorylation are membrane associated
  - in bacteria, is carried out at the plasma membrane
  - In eukaryotic cells, happens in or at the inner mitochondrial membrane
- The mitochondria is about 0.5 ± 0.3 micron in diameter and from 0.5 to several micron long

Mitochondrial functions are localized in specific compartments

1. Outer membrane
   - Fatty acid elongation
   - Fatty acid desaturation
   - Phospholipid synthesis
   - Monoamine oxidase

2. Inner membrane
   - Electron transport
   - Oxidative phosphorylation
   - Transport system
   - Fatty acid transport

3. Intermembrane space
   - Creatine kinase
   - Adenylate kinase

4. Martix

Mitochondrial functions are localized in specific compartments

1. Outer membrane
2. Inner membrane
3. Intermembrane space
4. Martix
   - Pyruvate dehydrogenase complex
   - TCA cycle
   - Glutathione dehydrogenase
   - Fatty acid oxidation
   - Urea cycle
   - DNA replication
   - Transcription
   - Translation
20.2 – What Are Reduction Potentials, and How Are They Used to Account for Free Energy Changes in Redox Reactions?

**Reduction potential:**
- The tendency of an electron donor to reduce its conjugate acceptor
- The standard reduction potential, \( E_0 \) (25°C, 1 M), is the tendency of a reductant to lose an electron
- The higher the standard reduction potential \( E_0 \), the higher the tendency of the oxidized membrane of a redox couple to attract electrons.

*High \( E_0 \) indicates a strong tendency to be reduced*
- Crucial equation: \( \Delta G^o' = -nF \Delta E_0' \)
  \( \Delta E_0' = E_0'(acceptor) - E_0'(donor) \)
  \( F \): Faraday’s constant (96.5 kJ/mol \cdot V)
- Electrons are donated by the half reaction with the more negative reduction potential and are accepted by the reaction with the more positive reduction potential: \( \Delta E_0' \) positive, \( \Delta G^o' \) negative
- If a given reaction is written so the reverse is true, then the \( \Delta E_0' \) will be a negative number and \( \Delta G^o' \) will be positive
20.3 – How Is the Electron Transport Chain Organized?

NADH (reductant) + H+ + O2 (oxidant) → NAD+ + H2O

Half-reaction:
NAD+ + 2 H+ + 2 e- → NADH + H+

\( E_o' = -0.32 \text{ V} \)

\( \frac{1}{2} \text{O}_2 + 2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{H}_2\text{O} \)

\( E_o' = +0.816 \text{ V} \)

\[ \Delta E_o' = 0.816 - (-0.32) = 1.136 \text{ V} \]

\[ \Delta G_o' = -219 \text{ kJ/mol} \]

Figure 20.3

Eo' and E values for the components of the mitochondrial electron-transport chain. Values indicated are consensus values for animal mitochondria. Black bars represent Eo'; red bars, E.
The Electron Transport Chain

The electron-transport chain involves several different molecular species:

1. Flavoproteins: FAD and FMN
2. A lipid soluble coenzyme Q (UQ, CoQ)
3. A water soluble protein (cytochrome c)
4. A number of iron-sulfur proteins: Fe$^{2+}$ and Fe$^{3+}$
5. Protein-bound copper: Cu$^+$ and Cu$^{2+}$

All these intermediates except for cytochrome c are membrane associated.

Table 20.2

<table>
<thead>
<tr>
<th>Complex</th>
<th>Mass (kD)</th>
<th>Subunits</th>
<th>Prosthetic Group</th>
<th>Binding Site For:</th>
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<tbody>
<tr>
<td>NADH-UQ reductase</td>
<td>850</td>
<td>&gt;30</td>
<td>FMN</td>
<td>NADH (matrix side)</td>
</tr>
<tr>
<td>Succinate-UQ reductase</td>
<td>140</td>
<td>4</td>
<td>FAD</td>
<td>UQ (lipid core)</td>
</tr>
<tr>
<td>UQ-Cyt $e$ reductase</td>
<td>250</td>
<td>9-10</td>
<td>Heme $b$</td>
<td>Cyt $e$ (intermembrane space side)</td>
</tr>
<tr>
<td>Heme $b_{5}$</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme $b_{6}$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cyt $e$</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cyt $c$</td>
<td>15</td>
<td>1</td>
<td>Heme $c$</td>
<td>Cyt $c$, Cyt $a$</td>
</tr>
<tr>
<td>Cytochrome $c$</td>
<td>162</td>
<td>&gt;10</td>
<td>Heme $a$</td>
<td>Cyt $c$ (intermembrane space side)</td>
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<td>Heme $a_{5}$</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cyt $a$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu$^{+}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td></td>
<td></td>
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Complex I Oxidizes NADH and Reduces Coenzyme Q

**NADH-CoQ Reductase or NADH dehydrogenase**

- Electron transfer from NADH to CoQ
- More than 30 protein subunits - mass of 850 kD
- Path: NADH $\rightarrow$ FMN $\rightarrow$ Fe-S $\rightarrow$ CoQ
- Four H$^+$ transported out per 2 e$^-$
**Complex II Oxidizes Succinate and Reduces Coenzyme Q**

**Succinate-CoQ Reductase**

- Also called succinate dehydrogenase or flavoprotein 2 (FP₂) - FAD covalently bound
- four subunits, including 2 Fe-S proteins
- Three types of Fe-S cluster: 4Fe-4S, 3Fe-4S, 2Fe-2S
- Path: Succinate → FADH₂ → 2Fe²⁺ → CoQH₂
- Net reaction:
  
  \[
  \text{succinate} + \text{CoQ} \rightarrow \text{fumarate} + \text{CoQH₂}
  \]

  \[\Delta E^{o} = 0.029 \text{ V}\]
Figure 20.7
The fatty acyl-CoA dehydrogenase reaction, emphasizing that the reaction involves reduction of enzyme-bound FAD (indicated by brackets).

Complex III Mediates Electron Transport from Coenzyme Q to Cytochrome c

**CoQ-Cytochrome c Reductase**

- CoQ passes electrons to cyt c (and pumps H⁺) in a unique redox cycle known as the Q cycle
- The principal transmembrane protein in complex III is the \( b \) cytochrome - with hemes \( b_L \) and \( b_H \)
- Cytochromes, like Fe in Fe-S clusters, are one-electron transfer agents
- The Q cycle
- CoQH₂ is a lipid-soluble electron carrier
- cyt c is a water-soluble mobile electron carrier
Complex IV Transfers Electrons from Cytochrome c to Reduce Oxygen on the Matrix Side

Cytochrome c Oxidase

- Electrons from cyt c are used in a four-electron reduction of $\text{O}_2$ to produce $2\text{H}_2\text{O}$
  
  $$4\text{cyt c (Fe}^{2+}\text{)} + 4\text{H}^+ + \text{O}_2 \rightarrow 4\text{cyt c (Fe}^{3+}\text{)} + 2\text{H}_2\text{O}$$

- Oxygen is thus the terminal acceptor of electrons in the electron transport pathway

- Cytochrome c oxidase utilizes 2 hemes ($a$ and $a_3$) and 2 copper sites (CuA and CuB)

- Complex IV also transports $2\text{H}^+$

Figure 20.9  Typical visible absorption spectra of cytochromes.

Figure 20.10  The structures of iron protoporphyrin IX, heme c, and heme a.

Figure 20.11  Molecular graphic image of subunits I, II, and III of cytochrome c oxidase.

Figure 20.12  The subunit structure of mitochondrial cytochrome c oxidase.

Figure 20.13  The structure of mitochondrial cytochrome c. The heme is shown at the center of the structure, covalently linked to the protein via its two sulfur atoms (yellow). A third sulfur from a methionine residue coordinates the iron.
Figure 20.16
Molecular graphic image of cytochrome c oxidase. Seven of the 10 nuclear DNA-derived subunits (IV, Vla, Vlc, VIIa, VIIb, VIIc, and VIII) possess transmembrane segments. Three (Va, Vb, and Vlb) do not. Subunits IV and Vlc are transmembrane and dumbbell-shaped. Subunit Vla is globular and bound to the matrix side of the complex, whereas Vlb is a globular subunit on the cytosolic side of the membrane complex. Vb is globular and matrix-side associated as well, but it has an N-terminal extended domain. Vla has a transmembrane helix and a small globular domain. Subunit Vlla consists of a tilted transmembrane helix, with another short helical segment on the matrix side of the membrane. Subunits VIIa, VIIb, and VIII consist of transmembrane segments with short extended regions outside the membrane.

Figure 20.17
The electron transfer pathway for cytochrome oxidase. Cytochrome c binds on the cytosolic side, transferring electrons through the copper and heme centers to reduce \( \text{O}_2 \) on the matrix side of the membrane.

Figure 20.18
(a) The CuA site of cytochrome oxidase. Copper ligands include two histidine imidazole groups and two cysteine side chains from the protein. (b) The coordination of histidine imidazole ligands to the iron atom in the heme a center of cytochrome oxidase.

Figure 20.19
The binuclear center of cytochrome oxidase. A ligand, L (probably a cysteine S), is shown bridging the CuB and Fe of heme a metal sites.

Figure 20.20
A model for the mechanism of \( \text{O}_2 \) reduction by cytochrome oxidase.
The complexes are independent

Each is a multiprotein aggregate maintained by numerous strong association between peptides of the complex

The four complexes are independently mobile in the membrane

20.4 – What Are the Thermodynamic Implications of Chemiosmotic Coupling?

- Peter Mitchell proposed a novel idea - a proton gradient across the inner membrane could be used to drive ATP synthesis (Nobel prize in 1978)

\[ \Delta G = RT \ln \frac{[C_2]}{[C_1]} + ZF \Delta \psi \]
\[ \Delta G = RT \ln \frac{[H^+_{\text{out}}]}{[H^+_{\text{in}}]} + ZF \Delta \psi \]
\[ \Delta G = RT \ln \Delta pH + ZF \Delta \psi \]

\( Z \): the charge on a proton
\( \Delta \psi \): the potential difference across the membrane
20.5 – How Does a Proton Gradient Drive the Synthesis of ATP?

Proton diffusion through the ATP synthase drives ATP synthesis

• Also called F₁F₀-ATPase
• Consists of two complexes: F₁ and F₀
  – F₁ catalyzes ATP synthesis
  – F₀ An integral membrane protein attached to F₁
• See Figure 20.25 and Table 20.3 for details

Table 20.3

<table>
<thead>
<tr>
<th>Complex</th>
<th>Protein Subunit</th>
<th>Mass (kD)</th>
<th>Stoichiometry</th>
</tr>
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<tbody>
<tr>
<td>F₀</td>
<td>a</td>
<td>55</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>58</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>35k</td>
<td>1</td>
</tr>
<tr>
<td>F₁</td>
<td>a</td>
<td>20k</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>17k</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>8k</td>
<td>9–12</td>
</tr>
</tbody>
</table>

20.5 – How Does a Proton Gradient Drive the Synthesis of ATP?

• The catalytic sites are in the β-subunits
• A ring of c-subunits could form a rotor that turns with respect to the α-subunit, a stator
• γ is anchored to the c-subunit rotor, then the c rotor-γ complex can rotate together relative to the (αβ) complex
Inhibitors of Oxidative Phosphorylation
Reveal Insights About the Mechanism

- Rotenone inhibits Complex I - and helps natives of the Amazon rain forest catch fish
- Cyanide (CN\(^{-}\)), azide (N\(_3\)^{-}) and CO inhibit Complex IV, binding tightly to the ferric form (Fe\(^{3+}\)) of \(a_3\)
- Oligomycin and DCCD are ATP synthase inhibitors
Uncouplers Disrupt the Coupling of Electron Transport and ATP Synthase

Uncoupling e- transport and oxidative phosphorylation

- Uncouplers disrupt the tight coupling between electron transport and oxidative phosphorylation by dissipating the proton gradient
- Uncouplers are hydrophobic molecules with a dissociable proton
- They shuttle back and forth across the membrane, carrying protons to dissipate the gradient
**ATP-ADP Translocase Mediates the Movement of ATP and ADP Across the Mitochondrial Membrane**

**ATP must be transported out of the mitochondria**
- ATP out, ADP in - through a "translocase"
- ATP movement out is favored because the cytosol is "+" relative to the "-" matrix
- But ATP out and ADP in is net movement of a negative charge out - equivalent to a H⁺ going in
- So every ATP transported out costs one H⁺
- One ATP synthesis costs about 3 H⁺
- Thus, making and exporting 1 ATP = 4H⁺

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**20.6 - What Is the P/O Ratio for Mitochondrial Electron Transport and Oxidative Phosphorylation?**

How many ATP made per electron pair through the chain?
- e⁻ transport chain yields 10 H⁺ pumped out per electron pair from NADH to oxygen
- 4 H⁺ flow back into matrix per ATP to cytosol
- 10/4 = 2.5 for electrons entering as NADH
- For electrons entering as succinate (FADH₂), about 6 H⁺ pumped per electron pair to oxygen
- 6/4 = 1.5 for electrons entering as succinate

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**20.7 – How Are the Electrons of Cytosolic NADH Fed into Electron Transport?**

Most NADH used in electron transport is cytosolic and NADH doesn't cross the inner mitochondrial membrane
- "Shuttle systems" effect electron movement without actually carrying NADH
- Glycerophosphate shuttle stores electrons in glycerol-3-P, which transfers electrons to FAD
- Malate-aspartate shuttle uses malate to carry electrons across the membrane
The Net Yield of ATP from Glucose Oxidation Depends on the Shuttle Used

- 30 ATP per glucose if glycerol-3-P shuttle used
- 32 ATP per glucose if malate-Asp shuttle used
- In bacteria - no mitochondria - no extra H⁺ used to export ATP to cytosol, so:
  - $-\frac{10}{3} = -3\text{ATP/NADH}$
  - $-\frac{6}{3} = -2\text{ATP/FADH}_2$