Mitochondrial Electron Transport

A series of metalloproteins bound to the inner membrane of the mitochondria act, in essence, as a molecular wire, carrying electrons from the strongly reducing products of the citric acid cycle (NADH, succinate) to highly oxidizing dioxygen. The electrons are passed through a series of metal clusters and cofactors embedded in large membrane-bound proteins, and the electron transfer is coupled to the transport of H⁺ across the membrane, from the interior matrix space of the mitochondria to the intermembrane space. The energy released by the electron flow is used to create a proton concentration gradient across the membrane. The potential energy inherent in that gradient is used to drive the synthesis of ATP when protons flow back across the membrane through another enzyme complex, ATP synthase.

There are four large proteins associated with mitochondrial electron transport, called Complexes I through IV. All but Complex II act to "pump" protons across the membrane. Electron transfer between these complexes is accomplished by the mobile coenzymes ubiquinone (in the lipid membrane, from Complexes I and II to Complex III) and cytochrome c (in the intermembrane space, from Complex III to Complex IV).

<table>
<thead>
<tr>
<th>Complex</th>
<th>subunits</th>
<th>MW</th>
<th>redox centres</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: NADH-ubiquinone oxidoreductase</td>
<td>~44</td>
<td>~980 kDa</td>
<td>FMN 8 FeS clusters ([2Fe2S] and [4Fe4S])</td>
</tr>
<tr>
<td>II: succinate dehydrogenase</td>
<td>4</td>
<td>120 kDa</td>
<td>FAD [2Fe2S] [4Fe4S] [3Fe4S] cyt b₅₆₀</td>
</tr>
<tr>
<td>III: cytochrome b₅₆₀ complex</td>
<td>2 × 11</td>
<td>480 kDa</td>
<td>cyt b₅₆₂ cyt b₅₆₆ Rieske FeS cyt c₁</td>
</tr>
<tr>
<td>IV: cytochrome c oxidase</td>
<td>2 × 13</td>
<td>420 kDa</td>
<td>Cuₐ cyta Cu₉/cyt a₃</td>
</tr>
</tbody>
</table>

Complex I – NADH-ubiquinone oxidoreductase

- ~44 protein chains, ~980kDa, FMN and 8 FeS clusters and four H⁺ channels
- hydrophilic arm contains redox cofactors, hydrophobic arm contains membrane-spanning helices that form four proton pumping channels
- e⁻ transfer from NADH to FMN, then e⁻ pass through a chain of FeS clusters: 1×[2Fe2S] and 6×[4Fe4S], with another [2Fe2S] likely acting as temporary reservoir for first NADH e⁻
- last [4Fe4S] lies near proposed binding site for Q, held within hydrophobic reaction chamber
energy of redox reaction used to induce long-range conformational changes within membrane domain – edges of protein subunits anchored by β-hairpin turns and a long helix (HL) running parallel to the membrane surface, while a centre band of proteins shifts back and forth to open proton channels in turn to each side.
Complex III – cytochrome bc₁ complex
(ubiquinol-cytochrome c oxidoreductase)

- dimer of 2×11 chains, ~480kDa
- overall reaction:
  \[2\ \text{QH}_2 + \text{Q} + 2\text{H}^+(\text{"in"}) + 2\ \text{cyt c}^\text{III} \rightarrow 2\ \text{Q} + 4\text{H}^+(\text{"out"}) + \text{QH}_2 + 2\ \text{cyt c}^\text{II} \ E^\circ \sim +210\ \text{mV}\]

\(\text{QH}_2\) is oxidized at \(Q_o\) site, near IMS ("out").
2\(\text{H}^+\) are released to IMS, and 2\(\text{e}^-\) are split up and transferred via two pathways.

**Path 1:** \(\text{QH}_2\) to cyt c in IMS
- Rieske [2Fe2S] cluster: 2 cysS 2 hisN
- cytochrome \(c_1\): metS hisN

**Path 2:** \(\text{QH}_2\) to Q at \(Q_i\) near matrix ("in")
- cytochrome \(b_{562}\) (or \(b_L\))
- cytochrome \(b_{566}\) (or \(b_H\)), both 2×hisN

Reduction of Q to \(\text{QH}_2\) at \(Q_i\) removes 2\(\text{H}^+\) from matrix. In effect, \(\text{H}^+\) are transferred across membrane, by consuming/producing \(\text{H}^+\) on different sides.

Complex IV – cytochrome c oxidase

- dimer of 2×13 chains, ~420kDa
- overall reaction:
  \[4\ \text{cyt c}^\text{II} + 4\text{H}^+ + \text{O}_2 \rightarrow 4\ \text{cyt c}^\text{III} + 2\ \text{H}_2\text{O} \ E^\circ \sim +540\ \text{mV}\]
  4\(\text{H}^+\) moved across membrane per 4\(\text{e}^-\) transferred

Electrons are moved from cyt c through four redox centres to \(\text{O}_2\)
(1 for each \(\text{e}^-\) required for \(\text{O}_2\) reduction). The centres are:
- \(\text{CuA}\): an unusual bimetallic Cu cluster
- \(\text{cytochrome a}\): heme bound by 2 hisN groups
- \(\text{CuB / cytochrome a}_3\): \(\text{O}_2\) binds between Cu and Fe

The \(\text{CuB}\) centre has one hisN group linked covalently through the \(\varepsilon\)-N to the aromatic ring of a neighbouring tyrosine (Y) residue, which directs its hydroxyl group into the active site cavity. This -OH group is thought to be involved in both the \(\text{O}_2\) cleavage and \(\text{H}^+\) pumping mechanisms.
The exact mechanism of O₂ activation and proton transfer is not wholly understood. The current best ideas as to the catalytic cycle of O₂ reduction involves five intermediates, called O R A P and F. Various steps are coupled to the motion of H⁺ through the body of the enzyme, forcing them across the membrane. This is thought to involve channels through the body of the enzyme lined with acidic protein residues, starting with a hisN group bound to CuB.

**Mitochondrial Electron Transport Chain – Overall Energetics**

Neglecting Complex II, the overall reaction of the mitochondrial chain, per two e⁻ transferred, can be written as:

\[
\text{NADH} + \frac{1}{2} \text{O}_2 + 11 \text{H}^+ (\text{“in”}) \rightarrow \text{NAD}^+ + \text{H}_2\text{O} + 10 \text{H}^+ (\text{“out”})
\]

Each two e⁻ through the chain results in the net transfer of 10 H⁺, which provides enough energy to synthesize about 2.5 molecules of ATP (one ATP per 4 H⁺), at ~35% efficiency.

\[\Delta G^\circ \text{ for } \text{NADH} + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NAD}^+ + \text{H}_2\text{O} = -nF\epsilon = -(2(96484 \text{C/mol})(+1.135 \text{J/C}) = -219 \text{kJ/mol} \]

\[\Delta G^\circ \text{ for } 2.5 \text{ ADP} + 2.5 \text{ P}_1 \rightarrow 2.5 \text{ ATP} + 2.5 \text{ H}_2\text{O} = +76 \text{kJ/mol} \]

Efficiency = 76 / 219 = 35%