So far we have discussed the catabolism involving oxidation of 6 carbons of glucose to CO$_2$ via glycolysis and CAC without any oxygen molecule directly involved.

In all the oxidative reactions so far, the electron acceptors (i.e., the oxidizing agents) were NAD$^+$ and a FAD.

The free energy released in these oxidation reactions were stored as reduced compounds (NADH and FADH$^2$) and synthesis of net 4 ATP molecules.

The ATP production during glycolysis is achieved by formation of a high energy compound 1,3-biphosphoglycerate in GAPDH reaction followed by transfer of the one of the inorganic phosphate to ADP yielding ATP. This is referred to as **substrate level phosphorylation**.

Similarly formation of GTP during Succinyl CoA synthetase reaction of CAC is also a **substrate level phosphorylation**.

Major chunk of ATP molecules are generated by a special biochemical machinery in which oxidation of NADH and FADH$_2$ is coupled to ATP synthesis in inner mitochondrial membrane. This process is called **Oxidative phosphorylation**.

Electrons from NADH and FADH$_2$ are transported to the ultimate electron acceptor, O$_2$ via various protein-bound redox centers present in inner mitochondrial membrane. The free energy released is used to pump proton across the membrane, and the energy of proton gradient thus built is used to drive synthesis of ATP by $F_o$, $F_i$ ATPase (or ATP synthase) system.

In the next few lectures we will discuss the followings;
- The mitochondrial structure
- Electron transport from NADH and FADH$_2$ to O$_2$
  - a. Thermodynamics of Electron transport
  - b. Sequence of electron transport
- Oxidative phosphorylation
  - a. Chemiosmotic theory
  - b. ATP synthase
- Physiological implications of aerobic metabolism

---

### Table 19-2

<table>
<thead>
<tr>
<th>Redox reaction (half-reaction)</th>
<th>$E^\circ$ (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2H$^+$ + 2e$^-$ $\rightarrow$ H$_2$</td>
<td>-0.414</td>
</tr>
<tr>
<td>NADH + H$^+$ + 2e$^-$ $\rightarrow$ NAD$^+$</td>
<td>-0.320</td>
</tr>
<tr>
<td>NADPH + H$^+$ + 2e$^-$ $\rightarrow$ NADP$^+$</td>
<td>-1.00</td>
</tr>
<tr>
<td>NADH dehydrogenase (NADH) + 2H$^+$ + 2e$^-$ $\rightarrow$ NADH dehydrogenase (NADH$_2$)</td>
<td>-0.10</td>
</tr>
<tr>
<td>Ubiquinone + 2H$^+$ + 2e$^-$ $\rightarrow$ ubiquinone</td>
<td>0.045</td>
</tr>
<tr>
<td>Cytochrome $b$ (Fe$^{2+}$) + e$^-$ $\rightarrow$ cytochrome $b$ (Fe$^{3+}$)</td>
<td>0.079</td>
</tr>
<tr>
<td>Cytochrome $c$ (Fe$^{2+}$) + e$^-$ $\rightarrow$ cytochrome $c$ (Fe$^{3+}$)</td>
<td>0.22</td>
</tr>
<tr>
<td>Cytochrome $c$ (Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome $c$ (Fe$^{2+}$)</td>
<td>0.254</td>
</tr>
<tr>
<td>Cytochrome $a$ (Fe$^{2+}$) + e$^-$ $\rightarrow$ cytochrome $a$ (Fe$^{3+}$)</td>
<td>0.29</td>
</tr>
<tr>
<td>Cytochrome $a$ (Fe$^{3+}$) + e$^-$ $\rightarrow$ cytochrome $a$ (Fe$^{2+}$)</td>
<td>0.55</td>
</tr>
<tr>
<td>$FADH_2$ + 2H$^+$ + 2e$^-$ $\rightarrow$ FAD$^+$</td>
<td>0.838</td>
</tr>
</tbody>
</table>

**Thermodynamics of Electron transport:**

Virtually all the complexes of electron transport chain and oxidative phosphorylation are located in the inner mitochondrial membrane.

Electrons from NADH and FADH$_2$ are transported to the ultimate electron acceptor, O$_2$ via four protein complexes containing various redox centers, called complex I, II, III and IV.

---

### Mitochondria

In most aerobic organisms, mitochondria is the major site for the reactions of PDC, citric acid cycle, and ATP generation through electron transport chain and oxidative phosphorylation. Hence called powerhouse of cells.

- It is an intracellular organelle with a size as big as a bacterium.
- It has two membranes outer and inner mitochondrial membranes.
- Mitochondria has its own DNA encoding some of the genes required in ETC and other functions.
- Mitochondria also have their own transcription and translation machinery. The mitochondrial ribosomes similar to that of bacteria.
Electrons are transferred through different electron-carriers sequentially in mitochondrial membrane. Most of electron-carriers (except coenzyme Q) are proteins with prosthetic groups capable of accepting or donating electrons.

1. Electrons can be transferred directly: e.g. reduction of Fe+++ to Fe++ or by

2. Transfer of H atom: H+ + e⁻ or by

3. Transfer of hydride ion :H⁻ as in NADH oxidation to NAD⁺

Membrane-Bound Electron Carriers

Ubiquinone or Coenzyme Q

It is a highly hydrophobic, lipid soluble benzoquinone with a long isoprenoid side chain.

Ubiquinone can accept one electron to become semiubiquinone radical or it can accept two electrons to become ubiquinol.

Due to its small size and lipid solubility, it can freely diffuse through lipid bilayer, thus it is capable of shuttling the reducing equivalents (electron) among other less mobile electron carriers.

Since it carries both electron and protons, it plays central role in coupling electron flow to proton movement.

Flavoproteins

Flavoproteins: Proteins tightly (or covalently) bound to Flavin nucleotides FMN or FAD.

The reduction potential of flavin nucleotides depends on the protein it is bound to. Local interaction of functional groups of amino acids distorts the electron orbitals of flavin ring, thus changing the stability of oxidized or reduced forms.

Flavoproteins are capable of accepting or donating one or two electrons.

Iron sulfur proteins

These are proteins containing Fe atom co-ordinated to sulfur atoms of either Cys residues of the proteins or also with inorganic sulfur atoms. The Fe atom is oxidized or reduced.

The reduction potential of Fe-S proteins varies from 0.65 to +0.45V depending on the microenvironment around Fe atom in the protein.

There are 8 different Fe-S proteins that function in mitochondrial electron transfer.

Cytochromes

Cytochromes are proteins with a Fe-containing heme prosthetic group.

They absorb light in visible range due to the heme gp (chrome=colour)

There are three classes of cytochromes depending of the type of heme group they have.

Heme of cyt-a and b are tightly bound to protein (non-covalently) whereas that in c type cytochrome is covalently bound.

Cyt-a and b are membrane proteins whereas cyt-c in mitochondria is a soluble protein associated with the outer surface of membrane by electrostatic interaction.
Absorption spectra of cytochrome c in oxidized and reduced form.

Isolation of various components of ETC and oxidative phosphorylation

---

**Table 19.4**

Some Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

<table>
<thead>
<tr>
<th>Type of Inhibitor</th>
<th>Component</th>
<th>Target of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of electron transfer</td>
<td>Dioxygen</td>
<td>inactivate cytochrome oxidase</td>
</tr>
<tr>
<td>Inhibition of ATP synthesis</td>
<td>ATP synthase</td>
<td>inhibit ADP synthase</td>
</tr>
<tr>
<td>Uncoupling of phosphorylation from electron transfer</td>
<td>UCP</td>
<td>inhibit ADP synthase</td>
</tr>
<tr>
<td>Inhibition of ATPADP exchange</td>
<td>Atpase</td>
<td>inhibit ADP synthase</td>
</tr>
</tbody>
</table>

---

**Table 19.5**

ATP Yield from Complete Oxidation of Glucose

<table>
<thead>
<tr>
<th>Process</th>
<th>Direct product</th>
<th>Final ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td>2 NADH (cytosolic)</td>
<td>3 or 5*</td>
</tr>
<tr>
<td>Pyruvate oxidation (two per glucose)</td>
<td>2 ATP</td>
<td>2</td>
</tr>
<tr>
<td>Acetyl-CoA oxidation in citric acid cycle (two per glucose)</td>
<td>6 NADH (mitochondrial matrix)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>2 FADH$_2$</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2 ATP or 2 GTP</td>
<td>2</td>
</tr>
<tr>
<td>Total yield per glucose</td>
<td></td>
<td>30 or 32</td>
</tr>
</tbody>
</table>

*The number depends on which shuttle system transfers reducing equivalents into mitochondria.
Complex I: NADH-Coenzyme Q oxidoreductase:

It is the largest protein complex in mitochondrial membrane containing 43 polypeptides and total approximate molecular weight 850kDa.

It contains 6-7 iron sulfur clusters and 1 FMN as prosthetic group. It has binding site for Coenzyme Q which is a substrate for this complex.

Complex II: Succinate dehydrogenase;

Contain four different proteins

One protein has a covalently bound FAD prosthetic gp and an Fe-S centre with four Fe atoms. Another protein is also an iron-sulfur protein.

Electrons pass from succinate to FAD and then through Fe-S centres to Ubiquinone.

Other mitochondrial dehydrogenases can pass electrons from different substrates to ubiquinone in the respiratory chain.

During beta oxidation of fatty acids, acyl-CoA dehydrogenase catalyses the transfer of electron to FAD then to electron transferring flavoprotein (ETF) then to ETF:ubiquinone oxidoreductase. This enzyme passes electron to ubiquinone.

Similarly, glycerol 3-phosphate dehydrogenase is a flavoprotein on the outer surface of inner mitochondrial membrane and transfer electron to Ubiquinone via FAD.

Complex III: Cytochrome bc1 complex or ubiquinone:cytochrome c oxidoreductase complex.

This complex is a dimer of two identical complexes: each containing 11 different subunits.

Three subunits that form the functional core are cytochrome b with two hemes, Rieske iron sulfur centre and cytochrome c1. Electrons from QH2 are transferred via Fe-S centre to cyt.c1 and then to cyt c.

The Q cycle

In complex III, electrons from two electron carrier QH2 are transferred to one electron carriers cytochromes, b, c1 and c and at the same time 4 H+ are translocated to out side via Q cycle.

One electron from QH2 → Fe–S → Cyt c1 → Cyt c

The other electron is transferred to Cyt bl to bh the to oxidized Q leading to the formation of an unstable Q-

The Q- ion is converted to QH2 by taking two H+ from the matrix side. The QH2 is recycled back.
Complex IV: Cytochrome c oxidase: It catalyses the transfer of electron from cyt. C to oxygen leading to the reduction of oxygen to H₂O.

This complex has 13 subunits, total mol, mass 204 kDa. Three proteins critical for electron flow are: Subunit I, II and III.

Subunit I contains two heme groups and a Cu ion (Cu₁). Subunit II contains two Cu atoms bonded by two sulfur atoms of cysteine. This binuclear centre and cyt c binding sites are located on the outside of the membrane.

Subunit III: Its role is not understood well, but it is essential for complex IV function.

Electron flow takes place as indicated in the figure.


b. Experiments to show the uncoupling of ETC to ATP synthesis.

Adding succinate alone does not resume Oxygen consumption, unless ADP and Pi are added. This indicates the coupling of the two reactions as above. Above. Similarly when ATP synthase is inhibited by Oligomycin, both ATP synthesis and O₂ consumption are inhibited. But if a reagent like DNP which breaks the H⁺ gradient is added, electron transport resumes, so is the O₂ consumption but ATP is not synthesized. Thus the DNP uncouples the electron transport and ATP synthesis.
8. THE PROTON PUMP MECHANISM:

- **DURING OXIDATION-REDUCTION REACTION IN ET CHAIN COMPONENTS**
  - REDUCTION CAUSES CONFORMATIONAL CHANGE → DECREASES THE pK of the ET CHAIN AND EXPOSES THEM TO OUTSIDE
  - H+ IS DISSOCIATED
  - RE-OXIDATION RESTORES ORIGINAL CONFORMATION

**SUPPORT FROM PROTON PUMP IN**

[Diagram of bacterial respiration]

EXPLANATION:
- The system uses the generation of a proton gradient to drive proton translocation. At each 2H+ translocation, a proton is transported across a membrane, creating a proton gradient. This gradient is then used to drive the proton pump, which transfers protons across the membrane, maintaining the proton gradient. The process continues, with the proton pump utilizing the proton gradient to drive protons across the membrane and maintain the gradient.
The binding change mechanism for ATP synthesis

- Translocation of h+ carried out by F2.
- Formation of phosphoenolpyruvate bond catalyzed by F2.

Panel Before: 1. Three interacting proteins (AB)
   a. State: binds ADP & P loosely
   b. State: binds AMP & P tightly
   c. State: cycling does not and
   d. State: catalyzes ATP

1. ADP and P bind to 'L' site
2. Free energy-driven conformational change converts 'L' site to 'T' site
   - This step also involves conversion of ATP containing 'T' site to 'L' site
   2. ATP site → 'L' site

3. ATP synthesized on 'T' site on one side
   - While ATP dissociates from 'O' site on other
   - Due unit free energy provided by """"gradient""""

Binding changes are driven by the rotation of """"gradient""""

Supported by direct observation in experiment

The pH drop on ATP synthesis is the consequence of

\[ \text{NADH} \rightarrow 10^5 \text{ units} \text{ out} \]
\[ 2 \text{ ATP molecules synthesized} \]
\[ \text{FADH}_2 \rightarrow \text{O}_{2} \rightarrow 2 \text{ H+} \text{ out} \]
\[ \text{L} \rightarrow \text{ATP} \text{ out} \]
\[ \text{ADP} + \text{Pi} \rightarrow \text{ATP} + \text{H}^+ \]

Due to H+ leak I can only use

\[ \text{Actual pH} \text{ out} \text{ for ATP} \]
Transport of various metabolites in and out of mitochondria uses electrochemical gradient energy.

Aspartate-Malate shuttle: Liver Kidney and Heart use this system.

Skeletal Muscle and Brain use Glycerol 3 Phosphate Shuttle.

http://biosolutions.blogspot.com/2007/05/atp-synthase.html
Heat production in Skunk Cabbage

Control of ATP producing pathway

IF1: Inhibitor of F1, a peptide of 84 amino acid residues, binds to two F1 subunits and blocks ATPase activity during ischemia or hypoxia
Mitochondrial production and disposal of superoxide.