Oxidative Phosphorylation, Photosynthesis, and the Calvin Cycle

Benzi Estipona, Sumana Mahata

11/30/16
Oxidative Phosphorylation

What you should know:

-where the process takes place in a cell

-which reactants contribute to the electron transport chain and how the flow of electrons is mediated (reduction potentials)

-which proteins are involved and how they contribute

What you should be able to do:

-understand how this process relates to the other metabolic processes

-track the flow of electrons along the chain

-explain what factors change its effectiveness
The electron-rich NADH and FADH$_2$, which were formed as byproducts of earlier steps of respiration transfer their electrons to carrier proteins along the inner mitochondrial membrane, and the flow of electrons result in protons moving across the membrane. Ultimately, these proton transfers generate a gradient of hydrogen ions that drive ATP production.
The electron transport chain is a series of oxidation-reduction reactions. NADH and FADH$_2$ are good electron donors, while oxygen has a high reduction potential which means it makes a good electron acceptor in the chain. As the chain progresses, the reduction potentials increase until oxygen receives the electrons. The transfer of electrons fuels proton transfers from the matrix to the intermembrane space.
Complex I (NADH-CoQ oxidoreductase)

NADH transfers its electrons to coenzyme Q (Q), which is catalyzed by this complex. This complex has many subunits, but two important ones are a protein that has an iron-sulfur cluster and a flavoprotein with a coenzyme called flavin mononucleotide (FMN). NADH transfers its electrons to FMN, becoming oxidized to NAD+ as FMN is reduced to FMNH$_2$. Next, the flavoprotein is reoxidized and a series of Fe-S clusters are reduced. Lastly, the final reduced Fe-S subunit donates its electrons to Q (aka ubiquinone). 4 protons are pumped into the intermembrane space, and the net effect is Q getting reduced to QH$_2$. 
Complex II (succinate-CoQ oxidoreductase)

As in complex I, complex II transfers electrons to Q; however, complex II is unique in that it receives electrons from succinate (which, when oxidized to fumarate in the citric acid cycle, reduces FAD to FADH$_2$). FADH$_2$ gets reoxidized as it reduces an Fe-S protein, which then gets oxidized as Q is reduced to QH$_2$. No protons are pumped, and the net effect is the reduction of Q to QH$_2$. 
Complex III (CoQH$_2$-cytochrome c oxidoreductase)

This complex facilitates the transfer of electrons from QH$_2$ to cytochrome c. The Q cycle happens in this complex. In the first half of the cycle, QH$_2$ transfers one electron to cytochrome c, which results in 2 protons being pumped into the intermembrane space. The other electron binds to a Q in another binding site. In the second half of the cycle, another QH$_2$ transfers one electron to cytochrome c and one to a Q, resulting in 2 more protons being pumped out and two protons being taken up into the complex, in addition to the formation of QH$_2$. QH$_2$ then can enter the Q pool of the membrane. Thus, the net result is the transfer of two protons into the intermembrane space per QH$_2$. 
Complex IV (cytochrome c oxidase)

This complex is responsible for the transfer of electrons from cytochrome c to oxygen, the last electron acceptor. The complex contains many subunits of cytochrome a, cytochrome a₃, and Cu²⁺ ions. Two molecules of cytochrome c transfer electrons to reduce Fe and Cu. These reduced ions bind O₂⁻ forming a peroxide bridge. Adding in two more electrons from another 2 cytochrome c molecules results in the cleavage of the bridge, and the uptake of two protons results in the formation of water. Overall, 4 cytochrome c electrons are needed to reduce oxygen to water, and four protons are pumped as a result.
Overall, \([H^+]\) increases in the intermembrane space, which decreases pH and increases the voltage difference between the intermembrane and the matrix, creating an electrochemical gradient which is also known as the proton-motive force.
ATP Synthase

Since protons cannot directly pass through the membrane, they go through ATP synthase. ATP synthase comprises two subunits:

$F_0$ subunit: this functions as a proton channel, allowing for protons to travel along the gradient from the intermembrane space through the inner mitochondrial membrane and back into the matrix.

$F_1$ subunit: this is the portion of ATP synthase that utilizes the energy released from the electrochemical gradient to phosphorylate ADP to ATP. Acting like a turbine, an ADP molecule first binds to the subunit, gets phosphorylated, then is released as ATP.
The rotor itself has three states which arise from conformational changes of the alpha and beta subunits:

- the “loose” conformation: ADP and $P_i$ bind reversibly
- the “tight” conformation: formation of ATP
- the “open” conformation: release of ATP

It takes about 3.3 protons to form 1 ATP.
Photosynthesis and the Calvin Cycle
Photosynthesis and the Calvin Cycle

What you should know:

**Photosynthesis:**
Electron transfer in Photosystems I and II; how they are interconnected
Generation of the proton gradient that drives ATP Synthesis
Reaction/Products of the cycle (NADPH)

**Calvin Cycle**
Products and reactants and how each transformation is achieved
Regulation and committed steps
Photosynthesis

**Process:**

Convert electromagnetic radiation into chemical energy. Synthesize fuel, such as glucose, from carbon dioxide and water by using sunlight as an energy source.

**Where does it occur:**

Chloroplasts (similar to mitochondria in oxidative phosphorylation)
Oxidation of Carbon; Source of Energy

![Chemical structures and energy levels](Figure 15-9)

<table>
<thead>
<tr>
<th>Compound</th>
<th>ΔG°_{oxidation} (kJ mol⁻¹)</th>
<th>ΔG°_{oxidation} (kcal mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane</td>
<td>-820</td>
<td>-196</td>
</tr>
<tr>
<td>Methanol</td>
<td>-703</td>
<td>-168</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>-523</td>
<td>-125</td>
</tr>
<tr>
<td>Formic acid</td>
<td>-285</td>
<td>-68</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Figure 15-9: Biochemistry, Sixth Edition*
Photosynthesis Breakdown

**Light Reaction**

Convert light into 1) reducing power (NADPH) and 2) ATP

Photosynthesis I

- Generates NADPH

Photosynthesis II

- Oxidizes water to O₂ and transfers the electrons to generate ATP

**Dark Reaction (Calvin Cycle)**

Convert the products of the light reaction to drive the reduction of CO₂ and subsequently synthesize glucose and other sugars
Parallels with Oxidative Phosphorylation

**OXIDATIVE PHOSPHORYLATION:**
Protons pumped from Matrix to Intermembrane Space
→ ATP Generated when Protons flow back in to Matrix

**PHOTOSYNTHESIS:**
Protons pumped from Thylakoid Space to Stroma
→ ATP, NADPH Generated when Protons flow back in to Stroma
Thylakoid Membrane

Contains the energy-transforming machinery

Like the inner membrane, impermeable to most molecules and ions

Light-induced transfer of $H^+$ into the thylakoid space is accompanied by the transfer of either $Cl^-$ in the same direction or $Mg^{2+}$ in the opposite direction

Influx of $Mg^{2+}$ into the stroma plays a role in the regulation of the Calvin Cycle
Parallels with Oxidative Phosphorylation

In cellular respiration:

We leverage the oxidation of glucose to CO₂ with the reduction of O₂ to water to generate ATP

In photosynthesis, we reverse the process synthesizing glucose by reducing CO₂ and oxidizing H₂O

In Both:

Generation of high-energy electrons that drive an ATP synthase to form ATP
Photosystems I and II

Electron flow is not cyclic

**Photosystem II**

- Catalyzes the light-driven transfer of electrons from water to plastoquinone

\[ 2 \text{Q} + 2\text{H}_2\text{O} \rightarrow \text{O}_2 + 2\text{QH}_2 \]

Photosystem II drives the reaction in a thermodynamically uphill direction by using the free energy of light

Protein structure binds more than 30 chlorophyll molecules to increase light absorption efficiency

One molecule of \( \text{O}_2 \) generated from the four electrons transferred
Photosystem II
Link Between Photosystem I and II

Cytochrome $bf$ links photosystem II to photosystem I

Catalyzes the transfer of electrons from plastoquinol to plastocyanin

$QH_2 + 2Pc(Cu^{2+}) \rightarrow Q + 2 \text{Pc}(Cu^{2+}) + 2H^+_{\text{thylakoid lumen}}$

(synonymous with Complex III in oxidative phosphorylation) through two $b$-type hemes

Complex catalyzes the reaction by proceeding through the Q cycle, one electron at a time. The electrons from plastoquinol flow through the Fe-S protein to convert oxidized plastocyanin into its reduced form

In the second half of the Q cycle, cytochrome $bf$ reduces a molecule of plastoquinone from Q pool to plastoquinol, taking up two protons from one side of the membrane and then reoxidizes plastoquinol to release the protons on the other side
Photosystem I
Overall Photosynthetic Pathway
Ferredoxin-NADP$^+$ reductase converts NADP$^+$ into NADPH

Ferredoxin, while an important reductant for nitrogen fixation, is not useful for many reactions since it carries only one available electron.

The enzyme ferredoxin-NADP$^+$ reductase, a flavoprotein with a FAD prosthetic group, accepts two electrons and two protons from two molecules of reduced ferredoxin to form FADH$_2$. The enzyme then transfers a hydride ion to NADP$^+$ to form NADPH on the stromal side of the membrane.

Uptake of a proton in the reduction of NADP$^+$ contributes to generate the proton gradient.
Photosynthesis and Calvin Cycle
Three Stages of the Calvin Cycle
Calvin Cycle in its Entirety
Useful Enzymes
Net Reaction of the Calvin Cycle

$$6 \text{CO}_2 + 18 \text{ATP} + 12 \text{NADPH} + 12 \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 18 \text{ADP} + 18 \text{P}_i + 12 \text{NADP}^+ + 6\text{H}^+$$

- three molecules of ATP and two molecules of NADPH are consumed to incorporate a single CO$_2$ molecule into a hexose

The rest of the monophosphate pool stored as either starch or sucrose

- Starch is synthesized and stored in chloroplasts
- Sucrose synthesized in the cytoplasm
Practice Questions
Which complexes are associated with...

Pumping a proton into the intermembrane space? I, III, IV

Acquiring electrons from NADH? I

Acquiring electrons from FADH$_2$? II

Having the highest reduction potential? IV

Involve cytochrome c? III, IV
What are the 3 conformations of the ATP synthase \( F_1 \) complex? What happens at each one?

1. the “loose” conformation: ADP and Pi bind
2. the “tight” conformation: formation of ATP
3. the “open” conformation: release of ATP
Supplementary Section
On Electron Transfer...

What prevents charge recombination?

1) Distance
   a) The next electron acceptor is less than 10 Å from BPh⁻

2) Rapid electron transfer from the reduced cytochrome
   a) The hemes of the cytochrome subunit is less than 10 Å from the special pair

The cytochrome subunit takes two electrons from reduced QH₂

- QH₂ first enters the Q pool in the membrane where it is reoxidized to Q by complex bc₁ (homologous to complex II in respiratory electron transport)
- Bc₁ transfers the electrons to cytochrome c₂ in the periplasm and pumps protons into the periplasmic space
Stoichiometry for the Light Reactions

Absorption of four photons by PSII generates one molecule of $O_2$ and releases four protons into the thylakoid lumen

Two molecules of plastoquinol are oxidized by the Q cycle of cytochrome $b_f$ complex to release eight protons into the lumen

The electrons from four molecules of reduced plastocyanin are driven to ferredoxin by the absorption of four additional photons. The four molecules of reduced ferredoxin generate two molecules of NADPH

$$2 \text{H}_2\text{O} + 2 \text{NADP}^+ + 10 \text{H}^+_{\text{stroma}} \rightarrow \text{O}_2 + 2 \text{NADPH} + 12 \text{H}^+_{\text{lumen}}$$

Assume there are 12 subunit III components of CF$_0$. We expect 12 protons must pass through CF$_0$ to complete one full rotation of CF$_1$
ATP synthase regulation in chloroplasts

Dependent on a disulfide bond in the $\gamma$ subunit

- maximal activity when it reduced to two cysteines

Thioredoxin which is formed from ferredoxin generated in PSI by ferredoxin thioredoxin reductase

2 Reduced ferredoxin + thioredoxin disulfide $\Leftrightarrow$ 2 oxidized ferredoxin + reduced thioredoxin + 2H$^+$

Conformational changes in $\varepsilon$ subunit between one conformation that inhibits ATP hydrolysis while the other in response to an increase in proton-motive force allows ATP synthesis and facilitates the reduction of the disulfide bond in the $\gamma$ subunit
Cyclic electron flow through photosystem I and the production of ATP instead of NADPH

When NADPH:NADP$^+$ is high NADP$^+$ may be unavailable to accept electrons from reduced ferredoxin

- Cyclic electron flow that powers ATP synthesis
  - Electrons from P700 generate reduced ferredoxin, the electron is transferred to cytochrome $bf$ complex rather than NADP$^+$ The electron then flows back through the cytochrome $bf$ complex to reduce plastocyanin, which can be reoxidized by P700$^+$

Net outcome: pumping of protons by cytochrome $bf$ which drives the synthesis of ATP

- cyclic photophosphorylation (no NADPH formed)

  Photosystem II does not participate (What does this mean about O$_2$ formed?)
Cyclic Phosphorylation

Thus, eight photons are required to yield three molecules of ATP (2.7 photons/ATP)

In cyclic photophosphorylation, the absorption of four photons by photosystem I leads to the release of eight protons into the lumen by the cytochrome $bf$ system. Two absorbed photons yield one molecule of ATP (but no NADPH)

Pigments absorb light and funnel the energy to the reaction center for conversion

Resonance energy transfer allows energy to move from site of initial absorbance to reaction center
Calvin Cycle Regulation

- Altering the stromal environment by the light reactions
  - lead to increase in stromal pH
  - an increase in stromal concentrations of Mg$^{2+}$, NADPH, and reduced ferredoxin]
- Activity of rubisco increases because light facilitates the carbamate formation
  - pH increase leads to an increase in Mg$^{2+}$ resulting from proton pumping
  - Carbamate is favored at alkaline pH
- Thioredoxin
  - Reduced ferredoxin and NADPH are good signals that conditions are right for biosynthesis
  - Reduced form of thioredoxin activates many biosynthetic enzymes including ATP synthase by reducing disulfide bridges
  - Inhibits several degradative enzymes
  - Oxidized thioredoxin is reduced by ferredoxin
  - Activities of the light and dark reactions are coordinated through electron transfer from reduced ferredoxin to thioredoxin
Rubisco

In the absence of CO$_2$, rubisco binds ribulose 1,5-bisphosphate. Uses ATP to release the bound substrate, coordinates rubisco activity with the light reactions

Also catalyzes photorespiration which produces phosphoglycolate and 3-phosphoglycerate

- requires the carbamate, which forms only in the presence of CO$_2$ thus preventing rubisco from catalyzing the oxygenase reaction exclusively when CO$_2$ is absent

Phosphoglycolate is salvaged by converting it into glycolate which enters peroxisomes to be oxidized to glyoxylate by glycolate oxidase. The H$_2$O$_2$ produced is cleaved by catalase to H$_2$O and O$_2$. Transamination yields glycine. Two glycine can form serine with the release of CO$_2$ and ammonium. The ammonium ion is salvaged by glutamine synthetase reaction.
Activity of the Calvin Cycle

- Reduced thioredoxin from the light-driven reactions activates enzymes of the Calvin cycle by reducing disulfide bridges
- Light-induced increase in pH and Mg\(^{2+}\) of the stroma stimulates the carboxylation of ribulose 1,5-bisphosphate