Chapter 19
Homework Assignment

• We will not be covering sections 19.4 – 19.9, except for a brief overview of photosynthesis
• The following problems will be due once we finish the chapter:

  1, 6, 7, 11, 12, 14

• And these additional problems:
  – Draw a mitochondrion and describe the functions that are associated with various areas
  – Draw and describe the flow of electrons and protons through the respiratory chain (four complexes plus ATP Synthase)
**Cellular Respiration**

**Overview**

**Stage 1:** Acetyl-CoA Production from:
- Glucose (CHP 14)
- Fatty Acids (CHP 17)
- Amino Acids (CHP 18)

**Stage 2:** Acetyl-CoA Oxidation (TCA cycle; CHP 16)

**Stage 3:** Electron Transfer & Oxidative Phosphorylation (NOW!)

---

**Oxidative Phosphorylation**

**Mitochondria: Power Plants of the Cell**

- All fuel oxidation pathways, except for glycolysis, occur in the mitochondrial matrix
- The inner membrane is an essential barrier that organizes transporters for both lateral and transverse translocation of substrates
- What tissues do you think have lots of mitochondria? Few? None?
**Oxidative Phosphorylation**

*Mitochondria: Power Plants of the Cell*

For full oxidation of Glucose:

\[ C_{6}H_{12}O_{6} + 6 O_{2} \rightarrow 6 CO_{2} + 6 H_{2}O \]

2 pyruvates → 2 Acetyl-CoAs
2 Turns of TCA cycle

\[ \frac{1}{2} O_{2} + 2 H^{+} + 2 e^{-} \rightarrow H_{2}O \]

Production of 6 \( H_{2}O \) will require 12 \( e^{-} \) and 12 \( H^{+} \) (or 12 \( H^+ \))

Where do we get these?

**NADH or FADH\(_2\)**

- 2 NADH (Glyc)
- 2 NADH (PDH Complex)
- 6 NADH & 2 FADH\(_2\) (TCA x 2)
The Players: Electron Carrying Molecules

Oxidative Phosphorylation

- NAD(P)^+ and FAD (or FMN) are the electron acceptors associated with Dehydrogenases
- NAD(P)^+–linked DH enzymes remove two hidrogen atoms, one is transferred to NAD(P)^+ as a hydride (H^−), the other is released as H^+
- NAD(P)H is a water soluble electron carrier that diffuse to their binding partner(s) for electron transfer
- FAD (& FMN) are tightly bound to their electron donor proteins in flavoproteins.
- The oxidixed flavin can accept one (yielding the semiquinone) or two electrons (yielding FADH_2 or FMNH_2)
- It cannot diffuse away though so its electrons must be directly transferred

Which enzyme produced reduced FADH_2 during TCA cycle?

Oxidative Phosphorylation

The Players: Electron Carrying Molecules

- The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are membrane bound proteins with prosthetic groups capable of accepting and donating one or two electrons
- The three types of transfer are:
  - Direct transfer of electrons (Fe^{3+} to Fe^{2+})
  - Transfer as a hydrogen atom (H^+ + e^-)
  - Transfer as a hydride atom (H^:\-)
- Ubiquinone (aka. Coenzyme Q) is a lipid soluble benzoquinone and carries electrons in the respiratory chain
- Ubiquinone can accept one electron to become a semiquinone or two electrons to become Ubiquinol
- What about this compound makes it able to move between complexes within the membrane?
Oxidative Phosphorylation
The Players: Electron Carrying Molecules

- **Cytochromes** are proteins with characteristic strong absorption of visible light due to their conjugated porphyrin ring system.

- There are three types of these groups differentiated by their longest wavelength band:
  - Type a – 600 nm
  - Type b – 560 nm
  - Type c – 550 nm

- The heme cofactors of type a and b cytochromes are tightly (but NOT covalently) bound to their associated protein.

- Hemes of c-type cytochromes are covalently bound through a Cys.

- The standard reduction potential of the iron is dependent on the protein environment for each heme, and therefore varies among Cytochromes.

- The Cytochromes of type a and b, and some type c are integral proteins of the inner mitochondrial membrane.

Oxidative Phosphorylation
The Players: Electron Carrying Molecules

- **Iron-Sulfur Proteins** contain an iron in association with either inorganic sulfur or the sulfur atoms of a Cys residue (no heme here!)

- These complexes range from simple (1 Fe – 4 S) to complex (4 Fe – 4 S).

- **Rieske iron-sulfur proteins** are a variation on this theme in which one iron atom is coordinated to two His rather than two Cys.

- All Fe-S clusters participate in one electron transfers in which one iron in the cluster is reduced or oxidized.

- Again, the reduction potential is protein environment dependent.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\Delta \text{G}^{\circ}$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$</td>
<td>-0.414</td>
</tr>
<tr>
<td>$\text{NAD}^+ + \text{H}^+ + \text{e}^- \rightarrow \text{NADH}$</td>
<td>-0.323</td>
</tr>
<tr>
<td>$\text{NADP}^+ + \text{H}^+ + \text{e}^- \rightarrow \text{NADPH}$</td>
<td>-0.324</td>
</tr>
<tr>
<td>$\text{NADP}^+$ (dehydrogenase) + $\text{2H}^+ + 2\text{e}^- \rightarrow \text{NADPH}$ (dehydrogenase)</td>
<td>-0.324</td>
</tr>
<tr>
<td>Lactate $+ 2\text{H}^+ + 2\text{e}^- \rightarrow$ aldehyde + water</td>
<td>-0.157</td>
</tr>
<tr>
<td>Cytochrome $c_1$ (Fe$^{3+}$) $+ \text{e}^- \rightarrow$ cytochrome $c_1$ (Fe$^{2+}$)</td>
<td>0.22</td>
</tr>
<tr>
<td>Cytochrome $b_2$ (Fe$^{3+}$) $+ \text{e}^- \rightarrow$ cytochrome $b_2$ (Fe$^{2+}$)</td>
<td>0.24</td>
</tr>
<tr>
<td>Cytochrome $a$ (Fe$^{3+}$) $+ \text{e}^- \rightarrow$ cytochrome $a$ (Fe$^{2+}$)</td>
<td>0.24</td>
</tr>
<tr>
<td>Cytochrome $a_3$ (Fe$^{3+}$) $+ \text{e}^- \rightarrow$ cytochrome $a_3$ (Fe$^{2+}$)</td>
<td>0.46</td>
</tr>
<tr>
<td>$\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}$</td>
<td>0.81 kJ/mol</td>
</tr>
</tbody>
</table>
So, how much ATP do we really need (and use) each day?

- 50 micrograms
- 50 milligrams
- 50 grams
- 50 kilograms

We need to identify two processes:

- Remember, that \( 2H_2 + O_2 \rightarrow 2H_2O \) is an explosive reaction yielding lots of energy.
- First: We need a way to transfer the electrons harvested from our diet (NADPH and FADH_2) to molecular oxygen
  - ELECTRON TRANSPORT CHAIN
- Second: We need a way to harness the energy from above and use it to synthesize ATP
  - ATP SYNTHASE

Two systems that work in concert!
Oxidative Phosphorylation
The components of the “electron transport chain” are physically separable!

Oxidative Phosphorylation
The sequence of electron carriers should be predictable based on their redox potential (Why?)
Oxidative Phosphorylation
Pathway Sequence Determination

• The carriers have spectral properties that are sensitive to their oxidation state
• When oxygen is suddenly added to fully reduced mitochondria, the rate at which each carrier gets oxidized should reveal its place in the chain

The sequence of electron carriers can also be predicted using inhibitors...

\[
\begin{align*}
\text{NADH} & \rightarrow Q \rightarrow \text{Cyt } b \rightarrow \text{Cyt } c_1 \rightarrow \text{Cyt } c \rightarrow \text{Cyt } (a + a_3) \rightarrow O_2 \\
\text{NADH} & \rightarrow Q \rightarrow \text{Cyt } b \rightarrow \text{Cyt } c_1 \rightarrow \text{Cyt } c \rightarrow \text{Cyt } (a + a_3) \rightarrow O_2 \\
\text{NADH} & \rightarrow Q \rightarrow \text{Cyt } b \rightarrow \text{Cyt } c_1 \rightarrow \text{Cyt } c \rightarrow \text{Cyt } (a + a_3) \rightarrow O_2
\end{align*}
\]
Oxidative Phosphorylation
Taa Daa! The Respiratory Chain

Chapter 17

Embedded in the inner membrane, it employs integral membrane proteins having prosthetic groups that can carry 1 or 2 electrons. The two *mobile* carriers are Coenzyme Q and Cyt c.

Complex I: NADH to Ubiquinone

- Complex I (aka. NADH Dehydrogenase) is a large complex composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least 6 Fe-S centers
- The L shaped complex catalyzes two coupled processes:
  - First, the exergonic transfer of a hydride ion (from NADH) and a proton (from the Matrix) to Ubiquinone
  - Second, the endergonic transfer of four protons from the Matrix to the Intermembrane Space.
- Ubiquinone’s hydrophobic tail enable it to transverse the membrane interior for interaction with Complex III
- The presence of the Flavoprotein and Ubiquinone provide a conduit between the two-electron donor of NADH and the one-electron acceptors, the cytochromes
Oxidative Phosphorylation
Complex II: Succinate to Ubiquinone

- We have already met Complex II (aka. Succinate Dehydrogenase) in the TCA cycle.
- This enzyme was the only membrane bound enzyme in that cycle. Why do you think it must be membrane bound in this situation?
- It contains five prosthetic groups (FAD, Heme b and Fe-S Clusters) and has four subunits (A, B, C and D)
- Subunits A and B extend into the Matrix and contain the FAD, Fe-S clusters and the succinate binding site.
- Subunits C and D are integral membrane proteins that contain Heme b and the Ubiquinone binding site.
- The electrons from FAD are passed through the Fe-S clusters to Ubiquinone, a distance of ~40 Å

Complex III: Ubiquinone to Cytochrome c

- Complex III couples the transfer of electrons from Ubiquinol (QH₂) to Cyt c with simultaneous transfer of protons from the Matrix to the IMS.
- The complex is a homodimer, with each monomer containing two heme bs, one heme c and a Fe-S cluster.
- The redox reaction for the movement of these electrons is called the Q cycle:

\[
QH_2 + 2 Cyt c_1 (oxid) + 2 H^+ \rightarrow Q + 2 Cyt c_1 (reduced) + 4 H^+ 
\]

- Cyt c is a soluble protein of the IMS that diffuses to Complex IV to donate its electron
Oxidative Phosphorylation

**Complex IV: Cyt c to O₂**

- Complex IV (aka Cytochrome Oxidase) carries electrons from Cyt c to molecular oxygen, reducing it to water.
- Complex IV is a large enzyme (13 subunits; MM 204 kDa)
- Three of these subunits appear to be critical to activity:
  - **Subunit II** contains two copper ions complexed with two enzyme Cys residues in a binuclear center (Cu₆); these accept e⁻ from Cyt c and pass them to heme a
  - **Subunit I** contains two heme groups (a and a₃) and another copper ion (Cu₆); heme a₂ and Cu₆ accept the e⁻ from heme a
  - **Subunit III**, whose role is not well understood
- For every four e⁻ that pass through this complex, four H⁺ are consumed from the matrix
- In addition, the energy produced by this reaction allows for the pumping of 4 other H⁺ across the membrane:

\[
4 \text{Cyt } c_1 \text{(red)} + 8 \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow 4 \text{Cyt } c_1 \text{(oxd)} + 4 \text{H}^+_p + 2 \text{H}_2\text{O}
\]

Chapter 17 21

**Oxidative Phosphorylation**

**So, where does the energy come from?**

- Transfer of electrons through the Respiratory Chain leads to a transmembrane potential

\[
\text{NADH} + 11 \text{H}^+_N + \frac{1}{2} \text{O}_2 \rightarrow \text{NAD}^+ + 10 \text{H}^+_p + \text{H}_2\text{O}
\]

- The electrochemical energy inherent in this difference in proton concentration and separation of charge represents a temporary conservation of much of the energy of electron transfer.
- The energy stored in such a gradient is called **proton-motive force**
Oxidative Phosphorylation

Proton-Motive Force

- The PMF has two components:
  - The chemical potential energy due to the difference in concentration of a chemical species (H⁺)
  - The electrical potential energy that results from the separation of charge when a proton moves across the membrane without a counter ion

By pumping the protons across the membrane, they can now be moved spontaneously down their electrochemical gradient, making energy available for work!

\[ \Delta G_t = 200 \text{ kJ per 10 } H^+ \text{ pumped} \]
\[ \text{For 2 } e^- \text{ from FADH}_2 \text{ only 6 } H^+ \]
\[ \Delta G_t = 120 \text{ kJ} \]

What do you think the mitochondria uses this energy for?

Oxidative Phosphorylation

How is Proton Pumping Accomplished?

- Bacteriorhodopsin provides a model for proton pumping.
  - The following amino acids provide a “proton wire” through the protein: ASP-85, ARG-82, GLU-194, GLU-204, and water molecules.

- Conformational changes induce changes in pKₐ’s and provide the driving force for proton translocation.
Experimental evidence demonstrates obligatory coupling between electron transport and ATP synthesis.

Why would succinate start $O_2$ consumption (Black Line) and ATP synthesis (Red Line)?

Why would Cyanide (CN⁻) have any affect?

Experimental evidence demonstrates obligatory coupling between electron transport and ATP synthesis in the other direction too!

ATP synthesis is needed to start oxygen consumption, unless the mitochondria are uncoupled by a compound like dinitrophenol (DNP).
In the case where succinate is oxidized to fumarate...

- Oxygen is consumed
- ATP is made
- Inhibiting either process blocks the other
- **Except** with uncouplers, where respiration continues, but ATP is not made
- Mitchell knew that electron transfer caused protons to be pumped out
- And proposed that ATP synthesis results from protons flowing back in!

---

**Oxidative Phosphorylation**

**Uncouplers are Very Toxic**

- DNP (dinitrophenol) and FCCP both have dissociable protons and are very hydrophobic
  - Both can carry protons through the inner membrane, thereby dissipating the proton gradient-preventing ATP synthesis but allowing continued electron transport
- **Ionophores** (such as vanomycin) also serves as an uncoupler by moving ions across the membrane

*Why would this decouple?*
The P/O Ratio (phosphorylated nucleotide / Oxygen consumed) is the number of moles of ATP made divided by (½) the number of moles of oxygen consumed.

These are experimentally measurable values.
- When NADH is substrate: P/O = 2.5
- When succinate is used: P/O = 1.2

Why are they different?

Oxidative Phosphorylation

You should now be familiar with:
- The identities and the order of the six electron carriers in the electron transport chain
- The 3 techniques used to establish the order of the carriers
- The ultimate source(s) of electrons
- The tight coupling between electron transport and ATP synthesis
- P/O ratios for NADH and succinate (FADH₂) and why they are different
- The definition of proton motive force
ATP Synthesis
How is a concentration gradient of H⁺ transformed into ATP?

• First, how much energy do we need to convert ADP and Pᵢ into ATP? Anyone remember the ∆G for that reaction?
• With that in mind, does the energy produced during oxidative phosphorylation meet that value?
• So, approximately how many ATP should we produce if we start with:
  – NADH?
  – Succinate (FADH₂)?

Mitchell’s Chemiosmotic Model

• The Chemiosmotic model proposed that the difference in [H⁺] and the separation in charge across the membrane drives the synthesis of ATP as protons flow passively back into the matrix via a proton pore associated with ATP Synthase
• The trick was to figure out how proton motive force could be used to drive ATP synthesis…
**ATP Synthesis**

**Can an Artificial Gradient Drive ATP Synthesis?**

- **Top**: mitochondria are equilibrated with pH 9 buffer and 0.1 M KCl, then shifted to…..
- **Bottom**: pH 7 buffer, no KCl, with valinomycin
- Now, $\Delta$pH = 2, and K$^+$ flows out, providing a charge imbalance, and
- **And yes…ATP is synthesized too!**

---

**Chapter 17**

---

**ATP Synthesis**

**ATP Synthase**

- **ATP Synthase** is an F-type ATPase (aka. Complex V)
- This enzyme is located on the inner mitochondrial membrane and catalyzes the formation of ATP from ADP and P$_i$, accompanied by the flow of electrons from the P side to the N side of the membrane.
- ATP Synthase has two distinct components:
  - Catalytic F$_1$, a peripheral membrane component
  - Proton-Conducting component F$_o$, which is integral to the membrane
ATP Synthesis

ATP Synthase – $F_0$ Component

- Proton translocation takes place in the $F_0$ portion, composed of 6-10 copies of subunit $c$.
- A proton enters from the intermembrane space into the cytosolic half-channel to neutralize the charge on an aspartate residue in a $c$ subunit.
- With this charge neutralized, the $c$ ring can rotate clockwise into the membrane by one $c$ subunit, moving an aspartic acid residue out of the membrane into the matrix half-channel.
- This proton can move into the matrix, resetting the system to its initial state.

ATP Synthesis

ATP Synthase – $F_1$ Component

- ATP synthesis takes place in $F_1$ in the three non-equivalent $\alpha$-$\beta$ pairs of the “lollipop”.
- At any given time:
  - One $\beta$ subunit is in the “Loose” (L) confirmation with ADP bound
  - One $\beta$ subunit is in the “Tight” (T) confirmation with ATP bound
  - One $\beta$ subunit is in the “Open” (O) confirmation with ATP released
- The rotation of the $\gamma$ subunit interconverts the three $\beta$ subunits
- The Proton-motive force provides the energy to run this rotation (3 protons per turn)
The movement of both components is an example of **rotational catalysis**.

The effect of protons flowing “downhill” through the ATP synthase complex is to produce rotation of the $F_o$ and $F_1$ domains, thereby inducing conformational changes and catalyzing ATP formation.

- Because *charge* favors the export of negative charge, and *concentration* favors the import of protons,
- The antiport ANT can exchange ATP (made inside) with ADP (outside), driven by the net transfer of $-1$ charge to the P-side of the membrane, and
- The symport phosphate translocase can import $H^+$ (to higher pH) together with $H_2PO_4^-$
- Together, these two translocases provide substrate for ATP synthase, and remove product!
ATP Synthesis

ATP Balance Sheet

- It is generally accepted that it requires translocation of 4 H+ to synthesize one ATP
  - 1 H+ to transport P, to F1, and the other 3 H+ to rotate Fo

With that in mind, let’s determine the following:

- How many H+ are pumped per NADH?
- How many ATP does that equal?
- How many H+ are pumped per FADH2 (succinate)?
- How many ATP does that equal?

Now, let’s do some real math with a single glucose molecule….

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>NADH</th>
<th>FADH2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDC / TCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ATP</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chapter 17 39

ATP Synthesis

30 versus 32 ATP Synthesized

- Glycolysis produces 2 NADH per glucose
- The problem is that these NADH are in the cytosol and cannot cross the inner mitochondrial membrane to pass their electrons, so bring on the shuttle(s)!

- **Malate-aspartate shuttle (the FREE shuttle):**
  - In the cytosol, OAA receives electrons from NADH, reducing it to malate, which can be carried in by the malate/α-ketoglutarate transporter
  - Once inside, Malate is reoxidized to regenerate OAA & NADH
  - NADH now has access to Complex I and 2.5 ATP’s will result
  - The OAA is transaminated, and carried out by the glutamate/aspartate transporter to the cytosol…

So 32 ATP per Glucose!
ATP Synthesis
The Malate – Aspartate Shuttle

- In liver, kidney & heart MT, this shuttle is used to move reducing equivalents of NADH from cytosol into the matrix.
- No cost so you still generate 2.5 ATP per NADH.
- Note that the electro-neutral glutamate-aspartate transporter (bottom) can also benefit the urea cycle.

ATP Synthesis
The $1 Shuttle

- In skeletal muscle and brain, an alternative shuttle, the Glycerol-3-phosphate shuttle, to deliver cytosolic reducing equivalents of NADH.
- These equivalents are delivered directly to Coenzyme Q and thus to Complex III obviating the need for any membrane transport systems.
- This shortcut results in fewer protons being pumped and less ATP per electron pair will result.

So 30 ATP per Glucose!
Regulation of Oxidative Phosphorylation

Cellular Energy Needs

• Because of the coupling between electron transport and ATP synthesis, the availability of ADP limits respiration ("acceptor control" - think through how this works!)

• Moreover, the [ATP]/[ADP][P_i] mass action ratio is important, and is usually very high

• If ADP levels increase (reflecting energy consumption), respiration increases accordingly to restore high ATP levels

• Once achieved, respiration slows again: overall, ATP is only made as fast as it is being used…

• But sometimes, cells uncouple respiration from ATP synthesis – why?

Regulation of Oxidative Phosphorylation

Prevention of ATP Hydrolysis

• When a cell is deprived of oxygen, such as during a heart attack or stroke, electron transfer to oxygen and proton pumping ceases
  – This results in a breakdown in the PMF

• Under these conditions, ATP Synthase runs in the reverse direction, becoming an ATP Hydrolase and pumping protons out of the mitochondria

• A small protein (IF_1) binds simultaneously to two ATP Synthase molecule, inhibiting their ATP Hydrolase activity

• This protein is only inhibitory in its dimeric form, which is favored at low pH (< 6.5)

Why would a cell starved for oxygen experience a decrease in pH?
Regulation of Oxidative Phosphorylation

Uncoupled Mitochondria Generate Heat

- Respiration does not always slow when ATP is abundant.
- “Brown fat” mitochondria use respiration to generate heat, not ATP
- This adipose tissue uses a specialized protein, **thermogenin**, to form a proton pore to dissipate the membrane potential
- Electron transport (and proton pumping) continues vigorously, vainly attempting to maintain membrane potential
- This action generates heat!
- Which is good for newborn mammals, and hibernating bears...

Why do you think we call it “Brown Fat”? 

Regulation of Oxidative Phosphorylation

Coordination of Regulation
Regulation of Oxidative Phosphorylation
Other Interfering Agents

TABLE 19-4  Agents That Interfere with Oxidative Phosphorylation or Photophosphorylation

<table>
<thead>
<tr>
<th>Type of interference</th>
<th>Compound*</th>
<th>Target/mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition of electron transfer</td>
<td>Cyanide</td>
<td>Inhibit cytochrome oxidase</td>
</tr>
<tr>
<td>Antimycin A</td>
<td>Blocks electron transfer from cytochrome b to cytochrome c&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Veratridine</td>
<td>Prevent electron transfer from Fe-S center to ubiquinone</td>
<td></td>
</tr>
<tr>
<td>Amytal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavohemoglobin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCMU</td>
<td>Competes with O&lt;sub&gt;2&lt;/sub&gt; for binding site in PSII</td>
<td></td>
</tr>
<tr>
<td>Inhibition of ATP synthase</td>
<td>Aurovertin</td>
<td>Inhibits F&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td>Oligomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venturicidale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncoupling of phosphorylation from electron transfer</td>
<td>DCCO&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Blocks proton flow through F&lt;sub&gt;0&lt;/sub&gt; and O&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>FCCP</td>
<td>Hydrophobic proton carriers</td>
<td></td>
</tr>
<tr>
<td>Cerr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valinomycin</td>
<td>K&lt;sup&gt;+&lt;/sup&gt; ionophore</td>
<td></td>
</tr>
<tr>
<td>Thermogenin</td>
<td>In brown fat, forms proton-conducting pores in inner mitochondrial membrane</td>
<td></td>
</tr>
<tr>
<td>Inhibition of ATP-ADP exchange</td>
<td>Attractylsaponide</td>
<td>Inhibits adenine nucleotide translocase</td>
</tr>
</tbody>
</table>

*DCMU: 3,3' ,4,4'-tetrachlorophenol; FCCP, 1,1'-diocyl-4,4'-biphenyl; Cerr, cyclohexylbenzeneisothiocyanate; PCCP, cyanide-mercuriooxophosphorylation inhibitor; SWR, 2,4,6-trichlorophenol.

Chapter 17

Regulation of Oxidative Phosphorylation
Some Interesting Questions

- Why should blocking cytochrome oxidase stop ATP synthesis?
- Why should inhibition of ATP synthesis prevent respiration from continuing?
- Why should membrane fragments be able to continue respiration, but not synthesize ATP?
- What are “uncouplers” and how do they work?
- What is the real role of the proton gradient, and how could you prove its involvement?
Mitochondrial Genes
The Mitochondrial Genome

- MT have a DNA genome separate from the nucleus
- It is a circular piece of DNA (mtDNA)
- Inheritance is maternal, non-Mendelian
- 13 proteins of the electron transport chain are encoded
- 900 mitochondrial proteins are encoded by the nuclear DNA and imported
- Mutations in mtDNA frequently seen as muscle or neurological problems (especially visual)
- Reactive oxygen species produced by e-transport chain are mutagenic

Mitochondrial Genes & Their Origin
Mitochondrial Mutations Can Cause Human Diseases

- A growing number of human diseases can be attributed to mutations in the mitochondrial genes
- Many of these diseases are known as mitochondrial encephalomyopathies and affect primarily the brain and skeletal muscle

Leber’s Hereditary Optic Neuropathy (LHON)

- Affects central nervous system (particularly the optic nerve) causing blindness in early adulthood
- A single base change in the ND4 gene results in a Arg to His mutation in a polypeptide of Complex I.
- This mutated form is defective in electron transfer from NADH to Ubiquinone
- These MT can still produce some ATP (via succinate) but not enough to support the activity of the nerves
Mitochondrial Mutations Can Cause Human Diseases

Myoclonic Epilepsy and Ragged-Red Fiber Disease (MERRF)

- Caused by a mutation in the gene encoding a transfer RNA specific for Lysine (Lysyl-tRNA)
- This defect results in defective production of several proteins whose synthesis requires the tRNA.
- Skeletal muscle fibers of individuals with MERRF have abnormally shaped MT that sometimes contain paracrystalline structures.

Mitochondrial Genes & Their Origin

Derived from Bacterial Symbionts?
Mitochondrial Genes & Their Origin
It Sure Looks Reasonable…

- The *E. coli* membrane complexes look like rudimentary electron-transporting proton pumps
- These create a significant pH differential and charge imbalance
- Which can drive ATP synthase (oriented to the cytosolic side), and
- Perform other mechanical work…

Mitochondrial Genes & Their Origin
Big Wheel Keeps On Turnin’…

- Such as the rotation of bacterial flagella, which is driven by Proton Motive Force,
- As protons “fall” back inside through a complex molecular turbine (perhaps not unlike ATP synthase)
**Mitochondrial Genes & Their Origin**

**Similar Cases Where Proton Movement Drives ATP Synthesis**

- Photosynthetic organisms capture light energy and convert it into chemical energy.
- During this conversion they produce ATP and NADPH.
- These energy molecules are used to convert CO$_2$ and H$_2$O into carbohydrates and O$_2$.
- Aerobic Heterotrophs (like us!) eat the carbohydrates and breathe the O$_2$ then produce CO$_2$ and release it back into the atmosphere.

\[
\text{CO}_2 + \text{H}_2\text{O} \xrightarrow{\text{light}} \text{O}_2 + (\text{CH}_2\text{O})
\]
Photosynthesis
Reactions of Photophosphorylation

• Photosynthesis comprises two reaction types:
  – Light-dependent reactions
  – Carbon-fixation reactions

• In the light-dependent reactions, the organisms absorb light energy and conserve it as ATP and NADPH

• This energy is then used to drive the carbon-fixation reactions through the reduction of CO₂ to form triose phosphates, starch and sucrose

Chapter 17

Photosynthesis
The Chloroplast

• These processes take place in the chloroplasts of plants

• These are membrane bound intracellular organelles that can vary in shape and are generally a few micrometers in diameter

• Inside, membranes (Thylakoids) are stacked in Grana and contain the membrane bound machinery that converts the light into ATP and NADPH

• In the aqueous phase surrounding this membrane (the stroma) are all of the enzymes needed for carbon-fixation

Chapter 17
Photosynthesis

Capture of Light

- Light is absorbed by the chloroplasts via pigments including chlorophyll and the carotenoids.
- These pigments are arranged in functional arrays called photosystems.
- Some pigments within these systems are light-harvesting (aka. Antenna molecules) and others are photochemical reaction centers.
- In general, when light strikes a photosystem, the antenna molecules transmit that energy rapidly to the reaction center for conversion to usable energy.

Chapter 17

59

Photosynthesis

Light-Driven e⁻ Flow: Photosystems I and II

- To elevate the energy of the electrons derived from H₂O to the level needed to reduce NADP⁺ each electron must be “lifted” twice by the photons harvested from sunlight.
- One photon is required per electron per photosystem.
- After excitation, the electron flows “downhill” through the carrier chains.
- Movement through Photosystem II results in proton pumping (ATP synthesis!)
- Movement through Photosystem I results in reduction of NADP⁺.

Chapter 17

60
Photosynthesis
ATP and NADPH Production

[Diagram showing the photosynthetic process with labels for ATP and NADPH production.]