AUTOTROPHIC NUTRITION
(or, Life is a photochemical phenomenon)

I. Overview of photosynthesis

Photosynthesis can be defined as the light-driven synthesis of carbohydrate. Recall the equation for this reaction:

\[
\text{CO}_2 + \text{H}_2\text{O} + \text{light} + \text{chloroplast} \rightarrow (\text{CH}_2\text{O})_n + \text{O}_2
\]

From this simple equation we can make some elegant conclusions:

A. Photosynthesis is a redox reaction.
   - CO₂ is reduced to a carbohydrate [(CH₂O)ₙ]
   - Water is oxidized (to oxygen)
   - Water supplies the electrons for the reduction of carbon dioxide; water is cleaved in the process yielding oxygen as a byproduct.
   - Light provides the energy for the reduction. Light essentially splits water (photolysis) to yield electrons and protons for photosynthesis.

B. Photosynthesis is an energy conversion process.

During photosynthesis, light energy is ultimately converted to chemical energy (carbohydrate). In a broad sense, it is an example of the 1st Law of Thermodynamics (recall that energy cannot be created nor destroyed, but it can be changed from one form to another).

C. Steve’s extra special BLACK BOX model for photosynthesis.

Diagram provided in class. This model shows that there are three major energy conversions during photosynthesis:

Radiant energy (sunlight) → electrical energy (passage of electrons via a series of carrier) → chemical energy (ATP, NADPH; labile =unstable) → chemical energy (carbohydrates; stable).

The first two conversions are part of the light-dependent reactions that occur in the thylakoid membranes of the chloroplast; the last step refers to the light-independent reactions (Calvin cycle) that occur in the stroma.

II. Leaves - the primary photosynthetic organs

Leaves are perfect solar collectors. These organs are broad and flat to allow for efficient light harvest. The leaves are broad to maximize surface area for light harvest and they are thin since light cannot penetrate too deeply into the leaf (the amount of light decreases exponentially with distance). As an aside, although the majority of light is absorbed near the leaf surface, in some situations, plant tissues can act like fiber optic cables that can funnel some light deeply into the plant body. Even within the thin leaf, most chloroplasts are found in the upper layer of cells, the
palisade layer, which is a tissue layer just beneath the upper epidermis of the leaf. This makes "sense" since these cells receive the greatest amount of light of any region in the leaf.

Leaves double as a means to exchange photosynthetic gases (take up carbon dioxide and get rid of oxygen) with the environment. Leaves have pores in the surface (stomata) that regulate the entry/exit of gases and prevent the loss of excessive water.

The spongy layer of the leaf acts like a "lung" increasing the internal surface area and provides for more rapid diffusion within the leaf. Note again that leaves are thin - this avoids the need for lungs or other type of pump to move gases. Since diffusion rates are inversely related to distance, simple diffusion can account for gas movements into/out of a leaf. An added advantage of having large leaves for light harvest is that they provide lots of surface area for absorption of carbon dioxide.

Veins and vascular tissue supply the leaf with water (xylem) and transport the end products to other parts of the plant (phloem).

III. Chloroplasts - specialized organelles that carry out the process of photosynthesis

A. Structure

Remember the cell unit? To jog your memory, reread the cell chapter. Terms that you should know are thylakoid (or lamellae), inter-membrane space = lumen, double membrane, stroma, granum (grana).

B. Chemistry  Chloroplasts contain....

1. Assorted Goodies.
   - DNA - circular loop, similar to a bacterial chromosome though much smaller - only enough nucleotides (ca. 120-160 kilobases) to code for about 120 proteins;
   - RNA;
   - ribosomes;
   - proteins - some are encoded by the genes in the nucleus, others by the genes in the chloroplast DNA. For example, rubisco, an important enzyme, has 2 different subunits, one coded by each source. The nuclear genes are essential for chloroplast function, but the reverse is not true.

2. Pigments.

These make up about 7% of the chloroplast. A pigment is any molecule with a color that absorbs light. These occur in the thylakoids because they are highly hydrophobic (fat-soluble). There are two major groups of pigments in higher plants - chlorophylls and carotenoids/xanthophylls.

   - Chlorophylls - green; look like a tennis racket. The head of the racket is a ring system based on the structure of heme (a type of porphyrin). Magnesium is inserted in the ring. Chlorophyll also has a long (C-20) hydrocarbon tail. The tail is important for positioning the molecule in the membrane. The interaction of the chlorophyll with the membrane is non-covalent and is
important because it ultimately determines the physical properties of the chlorophyll.

- Carotene/xanthophylls - both are large hydrophobic molecules (C-40). Carotenoids are hydrocarbons; xanthophylls are oxygenated. These pigments are orange or yellow in color.
- Autumn color is due to a change in the pigment composition of the leaf. For more details, check out my essay, "Behind the Green Curtain."


There are three major complexes (groups of proteins, pigments and electron carriers) in the chloroplast membranes (thylakoids). These complexes are physically separate from one another and can be isolated from the chloroplast by biochemical techniques (electrophoresis and ultra centrifugation). These complexes are (don't memorize the individual components - simply appreciate the diversity and complexity):

(a) Photosystem II (PSII) Complex

- six integral proteins - coded by the chloroplast genome
- three peripheral proteins - coded by nuclear genome; they bind Ca\(^{2+}\) and Cl\(^{-}\)
- 4 MN\(^{2+}\)
- LHCII - Light harvesting pigment complex associated with PSII. It is comprised of: (1) about 250 chlorophyll \(a\) & \(b\); in approximately equal amounts; (2) xanthophyll; (3) proteins - each pigment is associated with protein. The protein is coded by the nuclear genome
- P680 reaction center \(\frac{i}{2} a\) a unique chlorophyll \(a\), maximum red light absorption at 680nm; it may actually be two chlorophyll \(a\) molecules; this is the chlorophyll that "looses" electrons

(b) Cytochrome b/f Complex

- cytochrome b
- cytochrome f
- other proteins including a non-heme iron-sulfur protein (2Fe-2 S)

(c) Photosystem I (PSI) Complex

- 11 polypeptides
- 50-100 chl \(\alpha\)
- electron carriers
- LHCI - contains about 100 chlorophylls; 4:1 ratio of chl \(\alpha\): chl \(b\); the protein is encoded by nuclear genome
- P700 reaction center \(\frac{i}{2} a\) unique chlorophyll \(a\) that absorbs light in the red region at 700 nm

4. ATP synthase/Coupling Factor Complex

This is a group of nine polypeptides (some nuclear, some chloroplastic) that is responsible for making ATP. It looks like a "lollipop" inserted into the thylakoid. The stalk of the lollipop (CF\(\text{a}\)) is
embedded in the thylakoid while the head (CF₁) is in the stroma. This complex is essentially the same as the one in the mitochondrion.

**IV. From photons to electrons**

**A. Nature of light**

- part of the electromagnetic spectrum - radiation emitted by sun.
- acts as discrete particles (called photons)
- travels as waves
- wavelength - distance between any two crests (or troughs). Symbolized by lambda (λ);
- frequency - number of waves passing a point in one second (ν). Frequency is inversely related to wavelength $\nu = \frac{c}{\lambda}$ where $c =$ speed of light ($3 \times 10^{10}$ cm sec⁻¹).
- The energy of a photon is a quantum.

**B. Which photons are important in photosynthesis?**

Run an *action spectrum* (graph of a physiological process vs. wavelength).

****refer to action spectrum of photosynthesis****

Conclusion: radiations between 400-700 nm are photosynthetically active (termed PAR). Specifically, red (600-700 nm) and blue (400-500 nm) light is particularly important.

**C. Photons must be absorbed to be used in a photochemical reaction.**

So, which molecules absorb the red and blue light? Run an *absorption spectrum* of potential pigment candidates (plot of light absorption vs. wavelength) and compare it to the action spectrum.

****refer to absorption spectrum of photosynthetic pigments.****

Chlorophyll a & b absorb light in red and blue regions of the visible spectrum. Note that their absorption spectrum matches the action spectrum of photosynthesis perfectly and hence, strongly implicates (though doesn’t prove) them in the process. (Subsequent work has shown the chlorophylls to be the major photosynthetic pigments).

**D. Duh!**

In reality it wasn’t necessary to prepare action and absorption spectra to learn that red and blue light was important for photosynthesis. Since leaves are green, it means that they don’t absorb the green wavelengths of light in the visible spectrum but absorb the remainder (blue and red). Thus, these are the ones available for photosynthesis. As an example, if you look through a pair of red sunglasses the world looks rosy. This is because the pigment in the lens absorbs all wavelengths of light except red which can then be transmitted thru the lens to your eye.
E. Quantity vs. Quality

1. Light quality. Refers to the wavelengths of light that are important. Photosynthetically active radiations (PAR) range from 400 - 700 nm with peaks in the red and blue.

2. Light quantity. Refers to the amount of light (PAR) received; units of mol m\(^{-2}\) s\(^{-1}\), called the photon fluence rate; or units of energy, J m\(^{-2}\) s\(^{-1}\).

F. What happens when chlorophyll absorbs light?

The chlorophyll molecule becomes excited (this takes only 10\(^{-15}\) sec = femptosec) and an electron moves to an outer energy level. CHL (ground state) \(\rightarrow\) CHL\(^*\) (excited state)

Blue light excites an electron to a higher energy level than red light. Imagine the "bell ringer at a carnival." Electrons don't stay excited long (10\(^{-9}\) sec) - they can:

1. return to the ground and release energy as heat (thermal deactivation);
2. return to ground state and release energy as light (fluorescence);
3. the energy may be transferred to another molecule, kind of like hitting pool balls (resonance transfer). Example:
   \[ \text{CHL}_1^- + \text{CHL}_2^- \rightarrow \text{CHL}_1^- + \text{CHL}_2^* \]
4. be used in a photochemical reaction (passed on to an electron carrier). Example:
   \[ \text{CHL}^* + \text{electron carrier}_{(ox)} \rightarrow \text{CHL}^+ + \text{electron carrier}_{(red)} \]

G. Why excite electrons?

The ultimate purpose of exciting electrons from chlorophyll is to provide the energy needed to transfer electrons from water to NADP\(^+\). This is necessary because water has a lower energy state than NADP\(^+\) - thus, the only way get electrons from water to NADP\(^+\) is to first excite them to a high energy state so they can flow downhill to NADP\(^+\).

IV. The Z-Scheme (Or, the Light-Dependent Reactions; Or, Non-cyclic photophosphorylation)

A. Overview.

During the light-dependent reactions of photosynthesis, electrons are transferred from water to NADP\(^+\). This reaction is depicted as follows: H\(_2\)O \(\rightarrow\) NADP\(^+\)

In the thylakoid, three of the complexes mentioned above (III.B.3&4) are responsible for transferring the electrons from water to NADP\(^+\). These are Photosystem II (PSII), the cytochrome b/f complex (cyt b/f), and Photosystem I (PSI). After electrons are removed from water, they are sequentially shuttled from PSII to the cyto b/f complex to PSI and then finally to NADP\(^+\). Thus:

\[ \text{H}_2\text{O} \rightarrow \text{PSII} \rightarrow \text{Cytb/f} \rightarrow \text{PSI} \rightarrow \text{NADP}^+ \]

Since the three complexes are physically separated from one another in the thylakoid membrane, there must be a means to transfer electrons between the complexes. A mobile form of plastoquinone (PQ) transfers electrons from PSII to cyt b/f. A copper-containing protein, plastocyanin (PC), transfers electrons from the cytochrome b-f complex to PSI. Thus, the reaction sequence is modified as follows:
H₂O → PSII → PQ → Cytb/f → PC → PSI → NADP⁺

The transfer of electrons from PSI to NADP⁺ requires a soluble carrier found in the stroma, ferredoxin (Fd). Thus our revised equation:

H₂O → PSII → PQ → Cytb/f → PC → PSI → Fd → NADP⁺

The transfer of electrons from water to PSII involves an "oxygen evolving complex" (OEC) which is a part of PSII and rich in chloride and manganese ions. Thus,

H₂O → OEC → PSII → PQ → Cytb/f → PC → PSI → Fd → NADP⁺

B. Origin of the name, Z Scheme

Derived from the arrangement of components with regard to energy potential. But, why don’t we call it the N-scheme?

VI. Photophosphorylation

- Literal translation - "the light driven synthesis of ATP"
- Occurs by the same mechanism, "Chemiosmotic Hypothesis", that occurs during ATP synthesis in the mitochondria
- the passage of electrons through the carrier complexes results in the movement of electrons from the stroma into the intermembrane space.
- pH gradient is generated across the membrane, the stoma pH is ca. 8 while the lumen is ca. 5.
- This happens because the thylakoid, like the inner membrane of the mitochondrion, is impermeable to protons
- the pH gradient provides the energy for the synthesis of ATP
- ATP is synthesized by an ATPase associated with the CF complex; and
- protons escaping through the channel drives ATP synthesis something like water turning a mill.

Evidence: lots!

1. a pH gradient exists in the chloroplast. Discharging the gradient with buffers prevents ATP synthesis;
2. uncouplers like DNP "poke holes" in the thylakoid making it "leaky" and discharging the gradient preventing ATP synthesis. Note that this doesn’t stop electron flow - in fact, it usually increases the rate.
VII. The final frontier - Calvin-Benson Cycle or Photosynthetic Carbon Reduction

A. General:
- called "dark reactions" or "light independent reactions" because the reactions don't require light - However, note that these reactions can (and do) occur in the light. In one sense they can be considered "light dependent" since they require the ATP and NADPH generated during the Z scheme.
- called the Calvin cycle - after the fellow and his colleagues who worked out most of the reactions. If you had done it, you too, would own a Nobel Prize
- occurs in the stroma
- there are four major steps: fixation → reduction → rearrangement → charging (note this is slightly different than the text)

B. Carbon dioxide fixation - carbon dioxide is fixed (trapped, bound) to form an organic compound (phosphoglyceric acid, PGA)
- carbon dioxide binds to RuBP (ribulose bisphosphate; C5) to form 2 molecules of PGA (C3)
- first product of carbon fixation is PGA (Calvin's experiments)
- catalyzed by the enzyme ribulose bisphosphate carboxylase (rubisco)
- rubisco is the most abundant protein on earth; it makes up 50% of leaf protein

C. Reduction - step in which the "temporary" chemical (ATP) and reducing (NADPH) energy that were generated in the light-dependent reactions are used to reduce the PGA from an acid to a carbonyl (glyceraldehyde 3-phosphate; abbreviated G3P or GAP)
- PGA is reduced to G3P
- this is a two-step reaction sequence
- first, PGA is phosphorylated with ATP to 1,3-bisphosphoglycerate which is subsequently reduced to G3P (note a phosphate is lost during this reaction). NADPH provides the electrons for the reduction
- energy requirements - at this point in the cycle, for each carbon dioxide fixed, two ATP and two NADPH are required (one for each of the two PGAs)

D. Rearrangement - complex series of reactions that result in the net removal of a C3 carbohydrate from the cycle and the production of the precursor to the starting material:
- see overhead and diagram in text for details
- the cycle must turn 3 times for the production of one net three carbons sugar
- the end product of the cycle is ribulose-5-P (RuP)

D. Charging - the original starting material (RuBP) is produced using the chemical potential (ATP) generated during the light-dependent reactions.
- ATP converts ribulose-5-P to RuBP
- ATP comes from the Z scheme
E. Summary: the fixation of 1 carbon dioxide requires: 3 ATP and 2 NADPH.

VIII. C3 Plants

Plants that exhibit the type of photosynthetic carbon reduction that we described above are termed C3 plants. In other words, the first product of carbon dioxide fixation is a 3-carbon compound (PGA). Thus, when radioactively labeled carbon dioxide is fed to a plant, the first place that it shows up is PGA.

IX. Photorespiration - Light stimulated production of carbon dioxide in the presence of oxygen

- not associated with mitochondrial respiration
- requires light
- not accompanied by ATP synthesis
- wastes energy (i.e., ATP, NADPH)

A. The problem - Rubisco.

Unlike most enzymes, rubisco is not substrate specific - it also has an oxygenase function. In addition to its normal substrate (carbon dioxide) rubisco also binds oxygen to RuBP. Although rubisco has a higher affinity for binding carbon dioxide (Km = 9 μM), if enough oxygen is present, it will act as a competitive inhibitor (the Km for oxygen is 535 μM).

B. The reaction catalyzed by ribulose bisphosphate carboxylase/oxygenase.

When rubisco binds oxygen to RuBP, the RuBP is essentially split in half to a 3 carbon piece and a 2 carbon fragment according to the following reaction: RuBP + oxygen → PGA (C3) + phosphoglycolate (C2)

Compare this to the normal reaction: RuBP + oxygen → 2 PGA (C3)

Thus, rubisco has oxygenase activity as well as a carboxylase.

C. What determines which process will occur?

Oxygenase activity occurs during periods of active photosynthesis when:

1. carbon dioxide levels are low; and
2. oxygen levels are high - due to the activity of PSII; high light intensity.

The ratio of [carbon dioxide]/[oxygen] ultimately determines the product of the rubisco reaction.

- if [carbon dioxide/oxygen] = high; then it favors normal Calvin cycle
- if [carbon dioxide/oxygen] = low; then it favors oxygenase activity
X. Photosynthetic carbon oxidation (PCO), or, Glycolate Cycle.

The purpose of this pathway is to metabolize and reclaim the carbon in phosphoglycolate

A. Overview of the major steps:

1. The products of rubisco oxygenase activity are phosphoglycolate (C2) and PGA (C3);
2. PGA enters the Calvin cycle as normal;
3. Phosphoglycolate is further metabolized in the peroxisome and mitochondria and some (75%) of the carbon in PGA is eventually reclaimed. The other carbons atoms are released as carbon dioxide from the mitochondria (not associated with cellular respiration) and hence the reason this is called a "respiration".
4. Note that the cycle is oxidative (redox reactions occur) and takes place in three organelles;

B. Why do plant photorespire?

From a Darwinian perspective, would expect that this process would have been selected against. However, the fact that so many plants do it, suggests that it may have an unappreciated function. Possibilities include: (a) salvage the carbon lost during rubisco oxygenase action; (b) mechanism to help prevent destruction by excess light.

XI. C4 Photosynthesis, or, How maize avoids photorespiration

Plants that avoid photorespiration have a unique modification of photosynthesis. They are called C4 plants because the first product of carbon dioxide fixation is a 4-carbon compound, not PGA as it is in C3 plants.

Examples: There are many plants that have this specialized modification. Found in many different and unrelated groups of plants which indicates that it apparently evolved independently several times. Even within a genus, some members can be C4 others C3.

C4 photosynthesis is common in grasses like maize, sorghum, crabgrass. But, not all grasses are C4; for example, Kentucky blue grass (*Poa pratensis*; common lawn grass) is C3.

A. How do C4 plants avoid photorespiration?

The answer is ingenious - C4 plants separate the site of oxygen production (PSII) from rubisco (Calvin cycle). But how? PSII and rubisco are placed in different:

1. Cells.
   In typical C3 plants the chloroplasts are dispersed throughout the mesophyll. Usually there is a well-defined palisade and spongy layer. In contrast, C4’s have a more or less uniform mesophyll layer with a well-developed bundle sheath around each vein. This is called Kranz anatomy, because the bundle sheaths appears like a wreath surrounding the vein. In C4 plants, the Calvin cycle activity occurs primarily in the bundle sheath cells, whereas PSII activity occurs in the mesophyll cells.
2. Chloroplasts.
The chloroplasts of C4 are of two forms. Bundle sheath cell (BSC) chloroplasts are agranal and have little PSII activity; but, they do have hi PSI activity. The mesophyll cell (MC) chloroplasts have typical granal stacking, but low rubisco activity. Thus, most carbon fixation (carbohydrate production) occurs in the BSC. Smart, eh?

B. Carbon Shuttle - Since C4 plants have separated the Calvin cycle PSII, there must be a mechanism to get carbon dioxide into the BSC since:

1. there is relatively slow diffusion to deep, interior regions of the leaf, especially considering;
2. the ambient level of carbon dioxide is low.

In order to solve this problem, plants required a mechanism to:

1. fix carbon dioxide in regions of the leaf where it occurs in high concentration (i.e., MC). The enzyme that catalyzes this reaction is phosphoenolpyruvate carboxylase (PEPcase). This enzyme binds carbon dioxide (actually bicarbonate) to PEP to form oxaloacetate (reaction diagram). This reaction occurs in the cytoplasm. Note that OAA is a C4 compound. Hence these plants are called C4 - because the first product of carbon fixation is a four carbon compound.
2. transport the fixed carbon dioxide (which is in the form of a C4 compound like malate or aspartate) from the MC to the BSC. The C4 molecule is converted to another C4 compound that, in turn, migrates to the BSC where it is decarboxylated and used in the Calvin cycle. The "leftover" C3 shuttles back to the MC to pick up another carbon dioxide and repeat the process.

C. General scheme - on overhead, covered in class

D. Advantages of C4 metabolism.

Plants that exhibit this type of photosynthesis are characteristic of hot, tropical environments that have a high light fluence. The advantage of C4 in these circumstances is that C4 metabolism:

1. avoids the photorespiratory loss of carbon
2. improves the water use efficiency of the plants
3. results in higher rates of photosynthesis at high temperatures
4. improves the efficiency of nitrogen utilization (because C3 require lots of rubisco)

XII. Crassulacean Acid Metabolism - CAM plants

A. Origin of the name.

Crassulacean refers to the Stonecrop family (Crassulaceae) and related succulents in which this process is common. To date, plants in more than 18 different families including Cactaceae (Cactus family) and Bromeliaceae (Pineapple family) have been shown to carry out CAM
metabolism. Acid is derived from the observation that these plants accumulate large amounts of organic acids in the dark.

Plants with CAM metabolism typically evolved in dry, hot, high light environments. This is largely a mechanism to conserve water. Recall the photosynthesis/transpiration compromise (paradox)? Plants in dry environments can't afford to compromise - they loose too much water opening their stomates during the day. CAM plants solved this problem by opening up the stomates at night to obtain carbon dioxide. This strategy is just the reverse of "normal" plants. But, this presents another problem - ATP & NADPH, which are products of the light dependent reactions, are not available when the carbon dioxide is fixed. The solution to this problem was to store the carbon dioxide during the night until ATP and NADPH were available the following day. Thus, there is a temporal separation of initial carbon fixation via PEPcase and the Calvin cycle. (C4 plants have a spatial separation).

B. PEPcase.

This is the initial enzyme that fixes carbon dioxide. The product is ultimately malate, an organic acid, which accumulates in the vacuole during the night (hence the "acid" term).

C. Sequence of events.

Night → stomates open → nocturnal transpiration (lower than diurnal) and carbon fixation by PEPcase → OAA produced → reduced with NADPH to malate → shuttled into vacuole → acid content of vacuole increases → starch depleted to provide PEP for carboxylation → day → stomates close → transpiration decreased → acid content decreases → malate decarboxylated to provide carbon dioxide for Calvin cycle → starch content increases
### XIII. Comparison of C3, C4 and CAM photosynthesis

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<th></th>
<th>C3</th>
<th>C4</th>
<th>CAM</th>
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<tbody>
<tr>
<td><strong>Leaf anatomy</strong></td>
<td>no distinct bundle sheath</td>
<td>Kranz anatomy</td>
<td>Usually no palisade cells, large vacuoles</td>
</tr>
<tr>
<td><strong>Initial carboxylating enzyme</strong></td>
<td>rubisco</td>
<td>PEPcase</td>
<td>PEPcase</td>
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<tr>
<td><strong>chloroplasts</strong></td>
<td>one type</td>
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| **transpiration ratio**  
(H$_2$O/dry wt)            | 450-950                   | 250-350                   | 18-125                    |
| **carbon dioxide compensation point** | 30-70                     | 0-10                      | 0-5 (in dark)             |
| **photosynthesis inhibited by oxygen?** | Yes                       | No                        | Yes                       |
| **photorespiration detectable?** | yes                       | only in bundle sheath     | late afternoon            |
| **optimum temperature for photosynthesis** | 15-25                     | 30-47                     | 35                        |
| **dry matter production (tons ha$^{-1}$ yr$^{-1}$)** | 22                        | 39                        | low, variable             |