CHAPTER 10
PHOTOSYNTHESIS

OUTLINE

I. Photosynthesis in Nature
   - A. Plants and other autotrophs are the producers of the biosphere
   - B. Chloroplasts are the sites of photosynthesis in plants

II. The Pathways of Photosynthesis
   - A. Evidence that chloroplasts split water molecules enabled researchers to track atoms through photosynthesis: *science as a process*
   - B. The light reactions and the Calvin cycle cooperate in converting light energy to the chemical energy of food: *an overview*
   - C. The light reactions transform solar energy to the chemical energy of ATP and NADPH: *a closer look*
   - D. The Calvin cycle uses ATP and NADPH to convert CO_2 to sugar: *a closer look*
   - E. Alternative mechanisms of carbon fixation have evolved in hot, arid climates
   - F. Photosynthesis is the biosphere's metabolic foundation: *a review*

OBJECTIVES

After reading this chapter and attending lecture, the student should be able to:

1. Distinguish between autotrophic and heterotrophic nutrition.
2. Distinguish between photosynthetic autotrophs and chemosynthetic autotrophs.
3. Describe the location and structure of the chloroplast.
4. Explain how chloroplast structure relates to its function.
5. Write a summary equation for photosynthesis.
6. Explain van Niel's hypothesis and describe how it contributed to our current understanding of photosynthesis.
7. Explain the role of REDOX reactions in photosynthesis.
8. Describe the wavelike and particlelike behaviors of light.
9. Describe the relationship between an action spectrum and an absorption spectrum.
10. Explain why the absorption spectrum for chlorophyll differs from the action spectrum for photosynthesis.
11. List the wavelengths of light that are most effective for photosynthesis.
12. Explain what happens when chlorophyll or accessory pigments absorb photons.
13. List the components of a photosystem and explain their function.
14. Trace electron flow through photosystems II and I.
15. Compare cyclic and noncyclic electron flow and explain the relationship between these components of the light reactions.

16. Summarize the light reactions with an equation and describe where they occur.
17. Describe important differences in chemiosmosis between oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts.
18. Summarize the carbon-fixing reactions of the Calvin cycle and describe changes that occur in the carbon skeleton of the intermediates.
19. Describe the role of ATP and NADPH in the Calvin cycle.
20. Describe what happens to rubisco when the O_2 concentration is much higher than CO_2.
21. Describe the major consequences of photorespiration.
22. Describe two important photosynthetic adaptations that minimize photorespiration.
23. Describe the fate of photosynthetic products.

KEY TERMS

photosynthesis
autotrophs
heterotrophs
chlorophyll
mesophyll
stomata
stroma
light reactions
Calvin cycle
NADP+
photophosphorylation
carbon fixation
wavelength
electromagnetic spectrum
visible light
photons
spectrophotometer
absorption spectrum
chlorophyll *a*
action spectrum
chlorophyll *b*
carotenoids
photo systems
reaction center
primary electron acceptor
photosystem I
photosystem II
noncyclic electron flow
noncyclic photophosphorylation
cyclic electron flow
cyclic photophosphorylation
glyceraldehyde 3-phophate (G3P)
rubisco
C_3 plants
photorespiration
C_4 plants
bundle-sheath cells
mesophyll cells
PEP carboxylase
crassulacean acid metabolism
CAM plants

LECTURE NOTES

I. Photosynthesis in Nature

Photosynthesis transforms solar light energy trapped by chloroplasts into chemical bond energy stored in sugar and other organic molecules. This process:

- Synthesizes energy-rich organic molecules from the energy-poor molecules, CO_2 and H_2O
- Uses CO_2 as a carbon source and light energy as the energy source
- Directly or indirectly supplies energy to most living organisms

A. Plants and other autotrophs are the producers of the biosphere

Organisms acquire organic molecules used for energy and carbon skeletons by one of two nutritional modes: 1) autotrophic nutrition or 2) heterotrophic nutrition.

Autotrophic nutrition = (Auto = self; trophos = feed); nutritional mode of synthesizing organic molecules from inorganic raw materials

- Examples of autotrophic organisms are plants, which require only CO_2, H_2O and minerals as nutrients.
- Because autotrophic organisms produce organic molecules that enter an ecosystem's food store, autotrophs are also known as *producers*.

- Autotrophic organisms require an energy source to synthesize organic molecules. That energy source may be from light (*photoautotrophic*) or from the oxidation of inorganic substances (*chemoautotrophic*).
  - *Photoautotrophs* = Autotrophic organisms that use light as an energy source to synthesize organic molecules. Examples are photosynthetic organisms such as plants, algae, and some prokaryotes.
  - *Chemoautotrophs* = Autotrophic organisms that use the oxidation of inorganic substances, such as sulfur or ammonia, as an energy source to synthesize organic molecules. Unique to some bacteria, this is a rarer form of autotrophic nutrition.

Heterotrophic nutrition = (Heteros = other; trophos = feed); nutritional mode of acquiring organic molecules from compounds produced by other organisms. Heterotrophs are unable to synthesize organic molecules from inorganic raw materials.

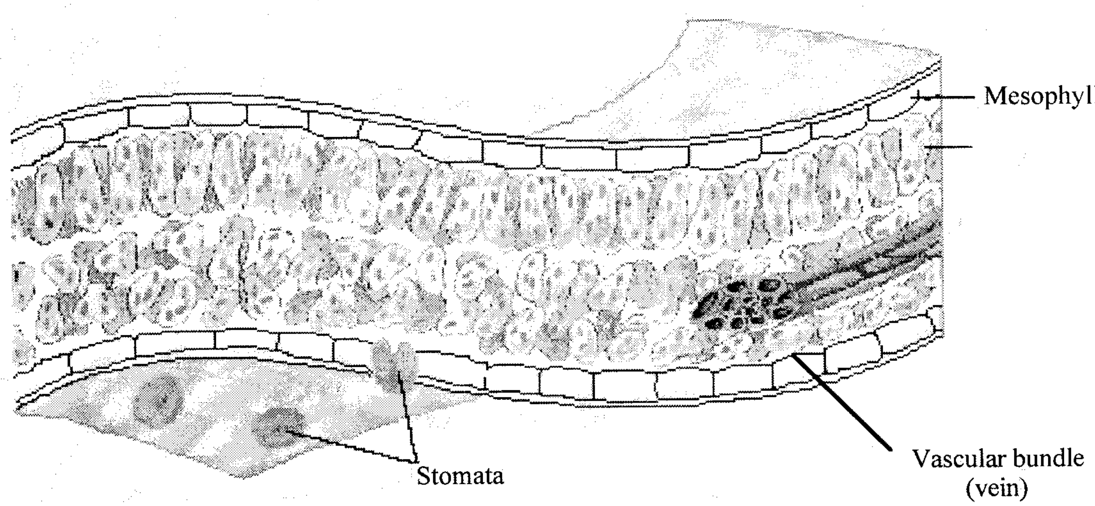
- Heterotrophs are also known as *consumers*.
- Examples are animals that eat plants or other animals.
- Examples also include *decomposers*, heterotrophs that decompose and feed on organic litter. Most fungi and many bacteria are decomposers.
- Most heterotrophs depend on photoautotrophs for food and oxygen (a by-product of photosynthesis).

B. Chloroplasts are the sites of photosynthesis in plants

Although all green plant parts have chloroplasts, leaves are the major sites of photosynthesis in most plants (see Campbell, Figure 10.2).

- *Chlorophyll* is the green pigment in chloroplasts that gives a leaf its color and that absorbs the light energy used to drive photosynthesis.

Leaf cross-section:



- Chloroplasts are primarily in cells of *mesophyll*, green tissue in the leaf's interior.
- CO_2 enters and O_2 exits the leaf through microscopic pores called *stomata*.
- Water absorbed by the roots is transported to leaves through veins or *vascular bundles* which also export sugar from leaves to nonphotosynthetic parts of the plant.

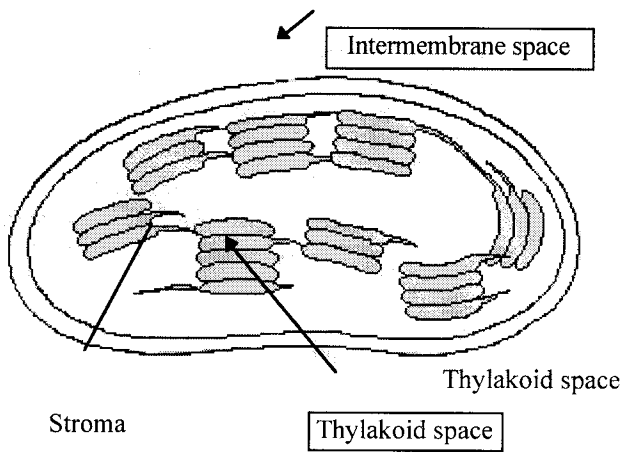
Chloroplasts are lens-shaped organelles measuring about 2 – 4 µm by 4 – 7 µm. These organelles are divided into three functional compartments by a system of membranes:

1. **Intermembrane space**

   The chloroplast is bound by a double membrane which partitions its contents from the cytosol. A narrow *intermembrane space* separates the two membranes.

2. **Thylakoid space**

   Thylakoids form another membranous system within the chloroplast. The thylakoid membrane segregates the interior of the chloroplast into two compartments: *thylakoid space* and *stroma*.



   Thylakoids = Flattened membranous sacs inside the chloroplast

   - Chlorophyll is found in the thylakoid membranes.
   - Thylakoids function in the steps of photosynthesis that initially convert light energy to chemical energy.

   Thylakoid space = Space inside the thylakoid

   Grana = (Singular, granum); stacks of thylakoids in a chloroplast

3. **Stroma**

   Reactions that use chemical energy to convert carbon dioxide to sugar occur in the *stroma*, viscous fluid outside the thylakoids.

   Photosynthetic prokaryotes lack chloroplasts, but have chlorophyll built into the plasma membrane or membranes of numerous vesicles within the cell.

   - These membranes function in a manner similar to the thylakoid membranes of chloroplasts.
   - Photosynthetic membranes of cyanobacteria are usually arranged in parallel stacks of flattened sacs similar to the thylakoids of chloroplasts.

II. The Pathways of Photosynthesis

A. Evidence that chloroplasts split water molecules enabled researchers to track atoms through photosynthesis: *science as a process*

Some steps in photosynthesis are not yet understood, but the following summary equation has been known since the early 1800s:

$$6\,CO_2 + 12\,H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6\,O_2 + 6\,H_2O$$

- Glucose ($C_6H_{12}O_6$) is shown in the summary equation, though the main products of photosynthesis are other carbohydrates.
- Water is on both sides of the equation because photosynthesis consumes 12 molecules and forms 6.

Indicating the net consumption of water simplifies the equation:

$$6\,CO_2 + 6\,H_2O + \text{light energy} \longrightarrow C_6H_{12}O_6 + 6\,O_2$$

- In this form, the summary equation for photosynthesis is the reverse of that for cellular respiration.
- Photosynthesis and cellular respiration both occur in plant cells, but plants do not simply reverse the steps of respiration to make food.

The simplest form of the equation is: $CO_2 + H_2O \longrightarrow CH_2O + O_2$

- CH_2O symbolizes the general formula for a carbohydrate.
- In this form, the summary equation emphasizes the production of a sugar molecule, one carbon at a time. Six repetitions produces a glucose molecule.

1. The splitting of water

The discovery that O_2 released by plants is derived from H_2O and not from CO_2, was one of the earliest clues to the mechanism of photosynthesis.

- In the 1930s, C.B. van Niel from Stanford University challenged an early model that predicted that:
  a. O_2 released during photosynthesis came from CO_2.
     $CO_2 \longrightarrow C + O_2$
  b. CO_2 was split and water was added to carbon.
     $C + H_2O \longrightarrow CH_2O$
- Van Niel studied bacteria that use hydrogen sulfide (H_2S) rather than H_2O for photosynthesis and produce yellow sulfur globules as a by-product.
  $CO_2 + 2\,H_2S \longrightarrow CH_2O + H_2O + 2\,S$
- Van Niel deduced that these bacteria split H_2S and used H to make sugar. He generalized that all photosynthetic organisms required hydrogen, but that the source varied:
  general: $CO_2 + 2\,H_2X \longrightarrow CH_2O + H_2O + 2\,X$
  sulfur bacteria: $CO_2 + 2\,H_2S \longrightarrow CH_2O + H_2O + 2\,S$
  plants: $CO_2 + 2\,H_2O \longrightarrow CH_2O + H_2O + O_2$
- Van Niel thus hypothesized that plants split water as a source of hydrogen and release oxygen as a by-product.

Scientists later confirmed van Niel's hypothesis by using a heavy isotope of oxygen (^{18}O) as a tracer to follow oxygen's fate during photosynthesis.

- If water was labeled with tracer, released oxygen was ^{18}O:
  Experiment 1: $CO_2 + 2\,H_2O^* \longrightarrow CH_2O + H_2O + O_2$
- If the ^{18}O was introduced to the plant as CO_2, the tracer did not appear in the released oxygen:
  Experiment 2: $CO_2^* + 2H_2O \longrightarrow CH_2O^* + H_2O^* + O_2$

An important result of photosynthesis is the extraction of hydrogen from water and its incorporation into sugar.

- Electrons associated with hydrogen have more potential energy in organic molecules than they do in water, where the electrons are closer to electronegative oxygen.
- Energy is stored in sugar and other food molecules in the form of these high-energy electrons.

2. Photosynthesis as a redox process

Respiration is an exergonic redox process; energy is *released* from the oxidation of sugar.

- Electrons associated with sugar's hydrogens lose potential energy as carriers transport them to oxygen, forming water.
- Electronegative oxygen pulls electrons down the electron transport chain, and the potential energy released is used by the mitochondrion to produce ATP.

Photosynthesis is an endergonic redox process; energy is *required* to reduce carbon dioxide.

- Light is the energy source that boosts potential energy of electrons as they are moved from water to sugar.
- When water is split, electrons are transferred from the water to carbon dioxide, reducing it to sugar.

B. The light reactions and the Calvin cycle cooperate in transforming light to the chemical energy of food: *an overview*

Photosynthesis occurs in two stages: the *light reactions* and the *Calvin cycle.*

Light reactions = In photosynthesis, the reactions that convert light energy to chemical bond energy in ATP and NADPH. These reactions:

- Occur in the thylakoid membranes of chloroplasts
- Reduce $NADP^+$ to NADPH
  - Light absorbed by chlorophyll provides the energy to reduce $NADP^+$ to NADPH, which temporarily stores the energized electrons transferred from water.
  - $NADP^+$ (nicotinamide adenine dinucleotide phosphate), a coenzyme similar to NAD^+ in respiration, is reduced by adding a pair of electrons along with a hydrogen nucleus, or H^+.
- Give off O_2 as a by-product from the splitting of water
- Generate ATP. The light reactions power the addition of a phosphate group to ADP in a process called *photophosphorylation.*

Calvin cycle = In photosynthesis, the carbon-fixation reactions that assimilate atmospheric CO_2 and then reduce it to a carbohydrate; named for Melvin Calvin. These reactions:

- Occur in the stroma of the chloroplast
- First incorporate atmospheric CO_2 into existing organic molecules by a process called *carbon fixation*, and then reduce fixed carbon to carbohydrate

Carbon fixation = The process of incorporating CO_2 into organic molecules.

The Calvin cycle reactions do not require light directly, but reduction of CO_2 to sugar requires the *products* of the light reactions:

- NADPH provides the reducing power.
- ATP provides the chemical energy.

Chloroplasts thus use light energy to make sugar by coordinating the two stages of photosynthesis (see Campbell, Figure 10.4).

- Light reactions occur in the thylakoids of chloroplasts.
- Calvin cycle reactions occur in the stroma.
- As $NADP^+$ and ADP contact thylakoid membranes, they pick up electrons and phosphate respectively, and then transfer their high-energy cargo to the Calvin cycle.

C. The light reactions transform solar energy to the chemical energy of ATP and NADPH: *a closer look*

To understand how the thylakoids of chloroplasts transform light energy into the chemical energy of ATP and NADPH, it is necessary to know some important properties of light.

1. The nature of sunlight

Sunlight is *electromagnetic energy.* The quantum mechanical model of electromagnetic radiation describes light as having a behavior that is both wavelike and particlelike.

a. Wavelike properties of light

- *Electromagnetic energy* is a form of energy that travels in rhythmic waves which are disturbances of electric and magnetic fields.
- A *wavelength* is the distance between the crests of electromagnetic waves.
- The electromagnetic spectrum ranges from wavelengths that are less than a nanometer (gamma rays) to those that are more than a kilometer (radio waves) (see Campbell, Figure 10.5).
- *Visible light*, which is detectable by the human eye, is only a small portion of the electromagnetic spectrum and ranges from about 380 to 750 nm. The wavelengths most important for photosynthesis are within this range of visible light.

b. Particlelike properties of light

- Light also behaves as if it consists of discrete particles or quanta called *photons*.
- Each photon has a fixed quantity of energy which is *inversely* proportional to the wavelength of light. For example, a photon of violet light has nearly twice as much energy as a photon of red light.

The sun radiates the full spectrum of electromagnetic energy.

- The atmosphere acts as a selective window that allows visible light to pass through while screening out a substantial fraction of other radiation.
- The visible range of light is the radiation that drives photosynthesis.
- Blue and red, the two wavelengths most effectively absorbed by chlorophyll, are the colors most useful as energy for the light reactions.

2. Photosynthetic pigments: the light receptors

Light may be reflected, transmitted, or absorbed when it contacts matter (see Campbell, Figure 10.6).

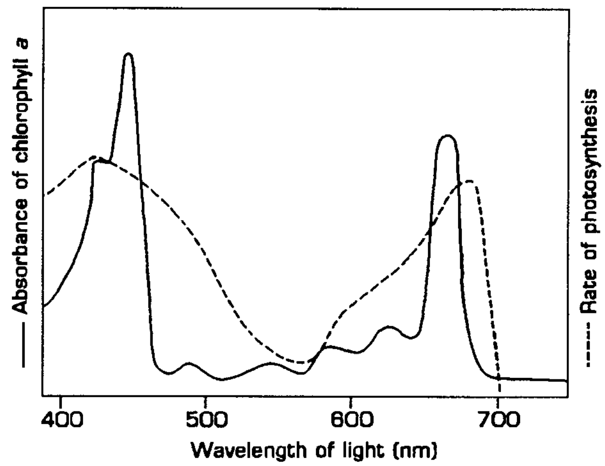
Pigments = Substances which absorb visible light

- Different pigments absorb different wavelengths of light.
- Wavelengths that are absorbed disappear, so a pigment that absorbs all wavelengths appears black.
- When white light, which contains all the wavelengths of visible light, illuminates a pigment, the color you see is the color most reflected or transmitted by the pigment. For example, a leaf appears green because chlorophyll absorbs red and blue light but transmits and reflects green light.

Each pigment has a characteristic *absorption spectrum* or pattern of wavelengths that it absorbs. It is expressed as a graph of absorption versus wavelength.

- The absorption spectrum for a pigment in solution can be determined by using a *spectrophotometer*, an instrument used to measure what proportion of a specific wavelength of light is absorbed or transmitted by the pigment (see Campbell Methods Box).
- Since chlorophyll *a* is the light-absorbing pigment that participates directly in the light reactions, the absorption spectrum of chlorophyll *a* provides clues as to which wavelengths of visible light are most effective for photosynthesis (see Campbell, Figure 10.7a).

A graph of wavelength versus rate of photosynthesis is called an *action spectrum* and profiles the relative effectiveness of different wavelengths of visible light for driving photosynthesis (see Campbell, Figure 10.7b).



- The action spectrum of photosynthesis can be determined by illuminating chloroplasts with different wavelengths of light and measuring some indicator of photosynthetic rate, such as oxygen release or carbon dioxide consumption (see Campbell, Figure 10.7c).
- It is apparent from the action spectrum of photosynthesis that blue and red light are the most effective wavelengths for photosynthesis and green light is the least effective.

The *action spectrum* for photosynthesis does not exactly match the *absorption spectrum* for chlorophyll *a*.

- Since chlorophyll *a* is not the only pigment in chloroplasts that absorb light, the absorption spectrum for chlorophyll *a* underestimates the effectiveness of some wavelengths.
- Even though only special chlorophyll *a* molecules can participate directly in the light reactions, other pigments, called *accessory pigments*, can absorb light and transfer the energy to chlorophyll *a*.

The *accessory pigments* expand the range of wavelengths available for photosynthesis. These pigments include:

- *Chlorophyll b*, a yellow-green pigment with a structure similar to chlorophyll *a*. This minor structural difference gives the pigments slightly different absorption spectra (see Campbell, Figure 10.8).
- *Carotenoids*, yellow and orange hydrocarbons that are built into the thylakoid membrane with the two types of chlorophyll (see Campbell, Figure 10.7a).

3. **Photoexcitation of chlorophyll**

What happens when chlorophyll or accessory pigments absorb photons (see Campbell, Figure 10.9)?

- Colors of absorbed wavelengths disappear from the spectrum of transmitted and reflected light.
- The absorbed photon boosts one of the pigment molecule's electrons in its lowest-energy state (*ground state*) to an orbital of higher potential energy (*excited state*).

The only photons absorbed by a molecule are those with an energy state equal to the difference in energy between the ground state and excited state.

- This energy difference varies from one molecule to another. Pigments have unique absorption spectra because pigments only absorb photons corresponding to specific wavelengths.
- The photon energy absorbed is converted to potential energy of an electron elevated to the excited state.

The excited state is unstable, so excited electrons quickly fall back to the ground state orbital, releasing excess energy in the process. This released energy may be:

- Dissipated as heat
- Reradiated as a photon of lower energy and longer wavelength than the original light that excited the pigment. This afterglow is called *fluorescence*.

Pigment molecules do not fluoresce when in the thylakoid membranes, because nearby *primary electron acceptor* molecules trap excited state electrons that have absorbed photons.

- In this redox reaction, chlorophyll is photo-oxidized by the absorption of light energy and the electron acceptor is reduced.
- Because no primary electron acceptor is present, *isolated* chlorophyll fluoresces in the red part of the spectrum and dissipates heat.

4. Photosystems: light-harvesting complexes of the thylakoid membrane

Chlorophyll *a*, chlorophyll *b* and the carotenoids are assembled into *photosystems* located within the thylakoid membrane. Each photosystem is composed of:

a. Antenna complex

- Several hundred chlorophyll *a*, chlorophyll *b* and carotenoid molecules are light-gathering antennae that absorb photons and pass the energy from molecule to molecule (see Campbell, Figure 10.10). This process of resonance energy transfer is called *inductive resonance*.
- Different pigments within the antennal complex have slightly different absorption spectra, so collectively they can absorb photons from a wider range of the light spectrum than would be possible with only one type of pigment molecule.

b. Reaction-center chlorophyll

Only one of the many chlorophyll *a* molecules in each complex can actually *transfer* an excited electron to initiate the light reactions. This specialized chlorophyll *a* is located in the *reaction center*.

c. Primary electron acceptor

- Located near the reaction center, a primary electron acceptor molecule traps excited state electrons released from the reaction center chlorophyll.
- The transfer of excited state electrons from chlorophyll to primary electron acceptor molecules is the first step of the light reactions. The energy stored in the trapped electrons powers the synthesis of ATP and NADPH in subsequent steps.

Two types of photosystems are located in the thylakoid membranes, *photosystem I* and *photosystem II*.

- The reaction center of photosystem I has a specialized chlorophyll *a* molecule known as *P700*, which absorbs best at 700 nm (the far red portion of the spectrum).
- The reaction center of photosystem II has a specialized chlorophyll *a* molecule known as *P680*, which absorbs best at a wavelength of 680 nm.

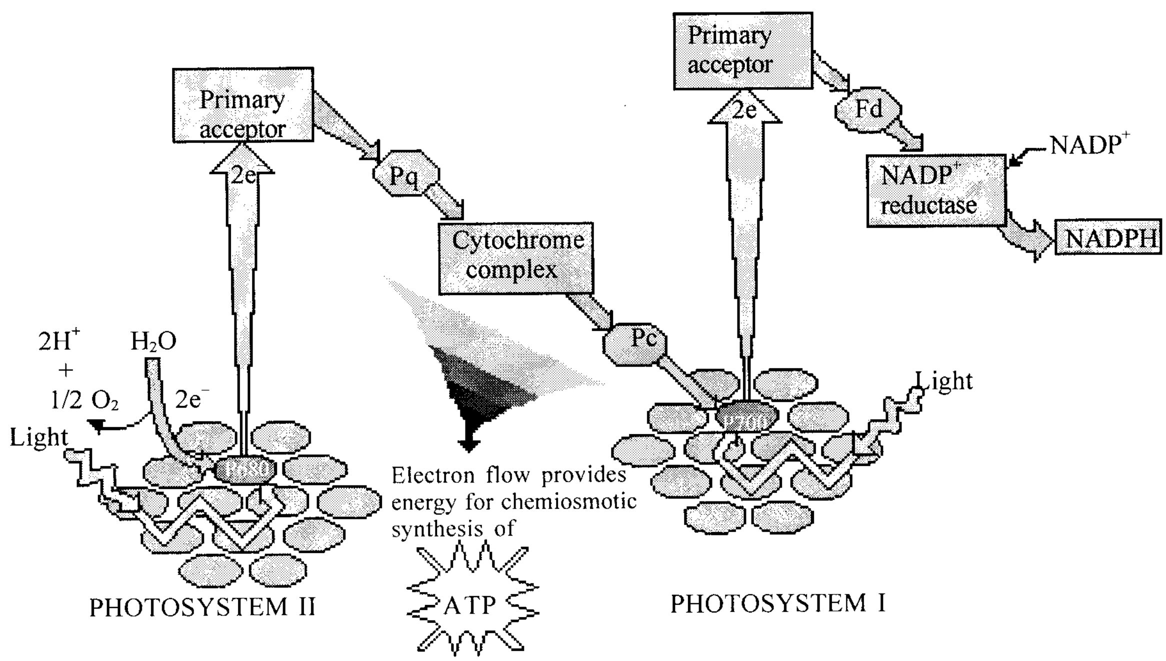
- P700 and P680 are identical chlorophyll *a* molecules, but each is associated with a different protein. This affects their electron distribution and results in slightly different absorption spectra.

5. Noncyclic electron flow

There are two possible routes for electron flow during the light reactions: *noncyclic flow* and *cyclic flow*.

Both photosystem I and photosystem II function and cooperate in noncyclic electron flow, which transforms light energy to chemical energy stored in the bonds of NADPH and ATP (see Campbell, Figure 10.11). This process:

- Occurs in the thylakoid membrane
- Passes electrons continuously from water to $NADP^+$
- Produces ATP by *noncyclic photophosphorylation*
- Produces NADPH.
- Produces O_2



Light excites electrons from P700, the reaction center chlorophyll in photosystem I. These excited state electrons do not return to the reaction center chlorophyll, but are ultimately stored in NADPH, which will later be the electron donor in the Calvin cycle.

- Initially, the excited state electrons are transferred from P700 to the primary electron acceptor for photosystem I.
- The primary electron acceptor passes these excited state electrons to *ferredoxin* (Fd), an iron-containing protein.
- *$NADP^+$ reductase* catalyzes the redox reaction that transfers these electrons from ferredoxin to $NADP^+$, producing reduced coenzyme – NADPH.
- The oxidized P700 chlorophyll becomes an oxidizing agent as its electron "holes" must be filled; photosystem II supplies the electrons to fill these holes.

When the antenna assembly of photosystem II absorbs light, the energy is transferred to the P680 reaction center .

- Electrons ejected from P680 are trapped by the photosystem II primary electron acceptor.
- The electrons are then transferred from this primary electron acceptor to an electron transport chain embedded in the thylakoid membrane. The first carrier in the chain, *plastoquinone* (Pq) receives the electrons from the primary electron acceptor. In a cascade of redox reactions, the electrons travel from Pq to a complex of two cytochromes to plastocyanin (Pc) to P700 of photosystem I.
- As these electrons pass down the electron transport chain, they lose potential energy until they reach the ground state of P700.
- These electrons then fill the electron vacancies left in photosystem I when $NADP^+$ was reduced.

Electrons from P680 flow to P700 during noncyclic electron flow, restoring the missing electrons in P700. This, however, leaves the P680 reaction center of photosystem II with missing electrons; the oxidized P680 chlorophyll thus becomes a strong oxidizing agent.

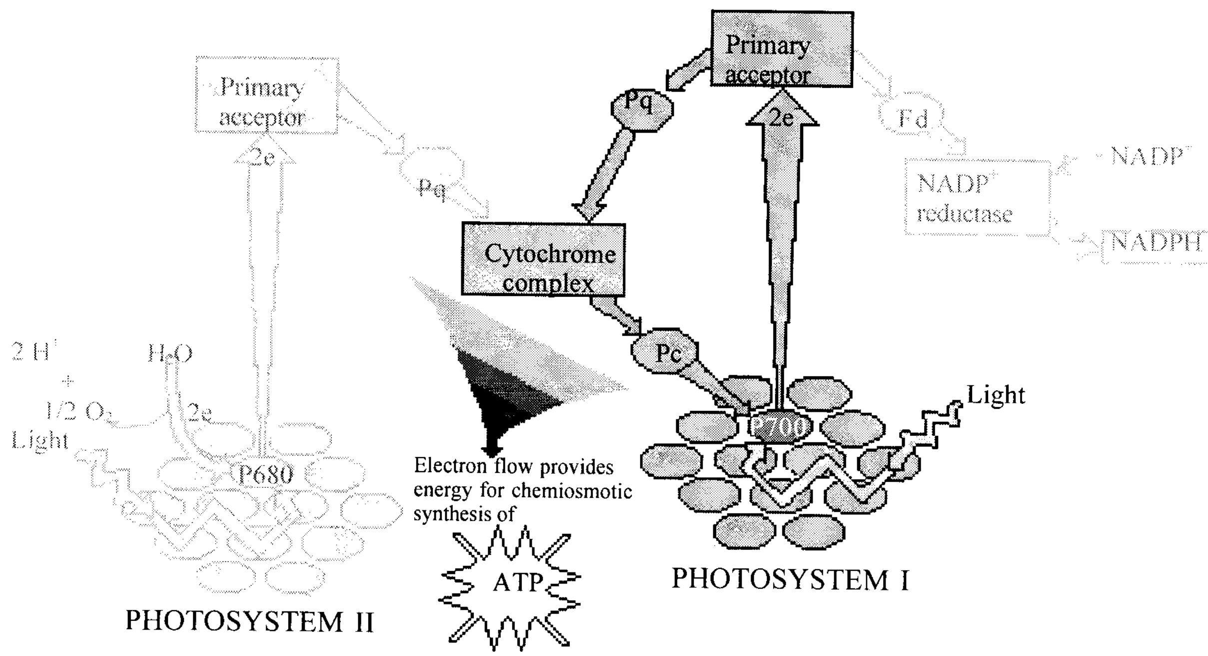
- A water-splitting enzyme extracts electrons from water and passes them to oxidized P680, which has a high affinity for electrons.
- As water is oxidized, the removal of electrons splits water into two hydrogen ions and an oxygen atom.
- The oxygen atom immediately combines with a second oxygen atom to form O_2. It is this water-splitting step of photosynthesis that releases O_2.

As excited electrons give up energy along the transport chain to P700, the thylakoid membrane couples the exergonic flow of electrons to the endergonic reactions that phosphorylate ADP to ATP.

- This coupling mechanism is *chemiosmosis*.
- Some electron carriers can only transport electrons in the company of protons.
- The protons are picked up on one side of the thylakoid membrane and deposited on the opposite side as the electrons move to the next member of the transport chain.
- The electron flow thus stores energy in the form of a proton gradient across the thylakoid membrane – a *proton-motive force*.
- An ATP synthase enzyme in the thylakoid membrane uses the proton-motive force to make ATP. This process is called *photophosphorylation* because the energy required is light.
- This form of ATP production is called *noncyclic photophosphorylation*.

6. Cyclic electron flow

Cyclic electron flow is the simplest pathway, but involves only photosystem I and generates ATP without producing NADPH or evolving oxygen.



- It is cyclic because excited electrons that leave from chlorophyll *a* at the reaction center return to the reaction center.
- As photons are absorbed by Photosystem I, the P700 reaction center chlorophyll releases excited-state electrons to the primary electron acceptor; which, in turn, passes them to ferredoxin. From there the electrons take an alternate path that sends them tumbling down the electron transport chain to P700. This is the same electron transport chain used in noncyclic electron flow.
- With each redox reaction along the electron transport chain, electrons lose potential energy until they return to their ground-state orbital in the P700 reaction center.
- The exergonic flow of electrons is coupled to ATP production by the process of chemiosmosis. This process of ATP production is called *cyclic photophosphorylation.*
- Absorption of another two photons of light by the pigments send a second pair of electrons through the cyclic pathway.

The function of the cyclic pathway is to produce additional ATP.

- It does so *without* the production of NADPH or O_2.
- Cyclic photophosphorylation supplements the ATP supply required for the Calvin cycle and other metabolic pathways. The noncyclic pathway produces approximately equal amounts of ATP and NADPH, which is not enough ATP to meet demand.
- NADPH concentration might influence whether electrons flow through cyclic or noncyclic pathways.

7. A comparison of chemiosmosis in chloroplasts and mitochondria

Chemiosmosis = The coupling of exergonic electron flow down an electron transport chain to endergonic ATP production by the creation of an electrochemical proton gradient across a membrane. The proton gradient drives ATP synthesis as protons diffuse back across the membrane.

Chemiosmosis in chloroplasts and chemiosmosis in mitochondria are similar in several ways:

- An electron transport chain assembled in a membrane translocates protons across the membrane as electrons pass through a series of carriers that are progressively more electronegative.
- An ATP synthase complex built into the same membrane, couples the diffusion of hydrogen ions down their gradient to the phosphorylation of ADP.
- The ATP synthase complexes and some electron carriers (including quinones and cytochromes) are very similar in both chloroplasts and mitochondria.

Oxidative phosphorylation in mitochondria and photophosphorylation in chloroplasts differ in the following ways:

a. Electron transport chain

- Mitochondria transfer chemical energy from food molecules to ATP. The high-energy electrons that pass down the transport chain are extracted by the oxidation of food molecules.
- Chloroplasts transform light energy into chemical energy. Photosystems capture light energy and use it to drive electrons to the top of the transport chain.

b. Spatial organization

- The inner mitochondrial membrane pumps protons from the matrix out to the intermembrane space, which is a reservoir of protons that power ATP synthase.
- The chloroplast's thylakoid membrane pumps protons from the stroma into the thylakoid compartment, which functions as a proton reservoir. ATP is produced as protons diffuse from the thylakoid compartment back to the stroma through ATP synthase complexes that have catalytic heads on the membrane's stroma side. Thus, ATP forms in the stroma where it drives sugar synthesis during the Calvin cycle (see Campbell, Figure 10.14).

There is a large proton or pH gradient across the thylakoid membrane.

- When chloroplasts are illuminated, there is a thousand-fold difference in H^+ concentration. The pH in the thylakoid compartment is reduced to about 5 while the pH in the stroma increases to about 8.
- When chloroplasts are in the dark, the pH gradient disappears, but can be reestablished if chloroplasts are illuminated.
- Andre Jagendorf (1960s) produced compelling evidence for chemiosmosis when he induced chloroplasts to produce ATP in the dark by using artificial means to create a pH gradient. His experiments demonstrated that during photophosphorylation, the function of the photosystems and the electron transport chain is to create a proton-motive force that drives ATP synthesis.

A tentative model for the organization of the thylakoid membrane includes the following:

- Proton pumping by the thylakoid membrane depends on an asymmetric placement of electron carriers that accept and release protons (H^+).
- There are three steps in the light reactions that contribute to the proton gradient across the thylakoid membrane:
    1. Water is split by Photosystem II on the thylakoid side, releasing protons in the process.
    2. As plastoquinone (Pq), a mobile carrier, transfers electrons to the cytochrome complex, it translocates protons from the stroma to the thylakoid space.
    3. Protons in the stroma are removed from solution as $NADP^+$ is reduced to NADPH.
- NADPH and ATP are produced on the side of the membrane facing the stroma where sugar is synthesized by the Calvin cycle.

> Students must be able to visualize the spatial arrangement of electron carriers in the membrane, since this arrangement is a crucial component of the chemiosmosis model. Figure 10.15 nicely illustrates this spatial arrangement.

8. Summary of light reactions

During *noncyclic electron flow*, the photosystems of the thylakoid membrane transform light energy to the chemical energy stored in NADPH and ATP. This process:

- Pushes low energy-state electrons from water to NADPH, where they are stored at a higher state of potential energy. NADPH, in turn, is the electron donor used to reduce carbon dioxide to sugar (Calvin cycle).
- Produces ATP from this light driven electron current
- Produces oxygen as a by-product

During *cyclic electron flow*, electrons ejected from P700 reach ferredoxin and flow *back* to P700. This process:

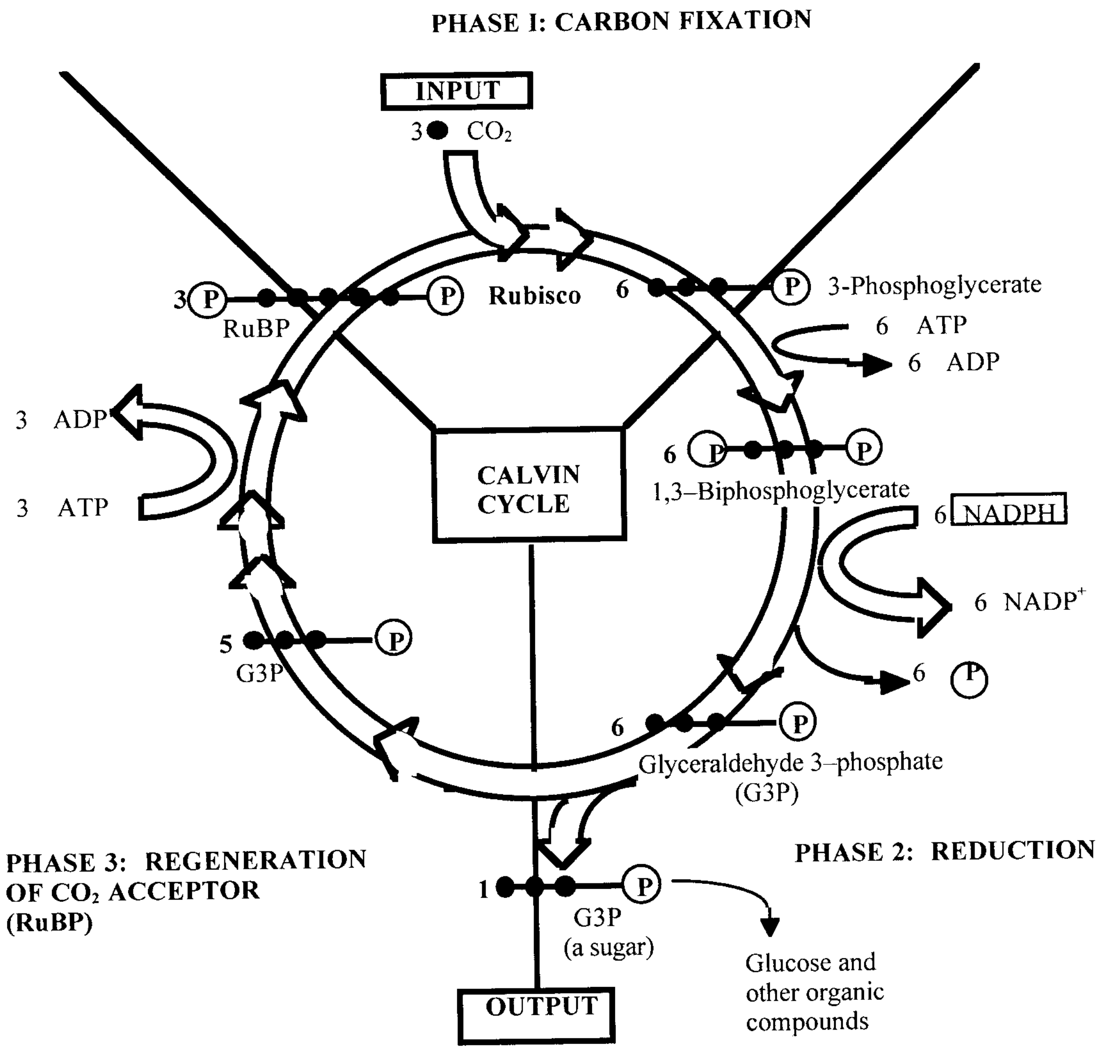
- Produces ATP
- Unlike noncyclic electron flow, does *not* produce NADPH or O_2

D. The Calvin cycle uses ATP and NADPH to convert CO_2 to sugar: *a closer look*

ATP and NADPH produced by the light reactions are used in the Calvin cycle to reduce carbon dioxide to sugar.

- The Calvin cycle is similar to the Krebs cycle in that the starting material is regenerated by the end of the cycle.
- Carbon enters the Calvin cycle as CO_2 and leaves as sugar.
- ATP is the energy source, while NADPH is the reducing agent that adds high-energy electrons to form sugar.
- The Calvin cycle actually produces a three-carbon sugar *glyceraldehyde 3-phosphate* (G3P).

> Students can easily follow the Calvin cycle if you use a diagram for reference, such as Figure 10.16. This figure is especially helpful because you can go through the cycle twice; once to count carbons and once to follow the reactions pointing out where ATP and NADPH are used, where glyceraldehyde phosphate is produced and how RuBP is regenerated.

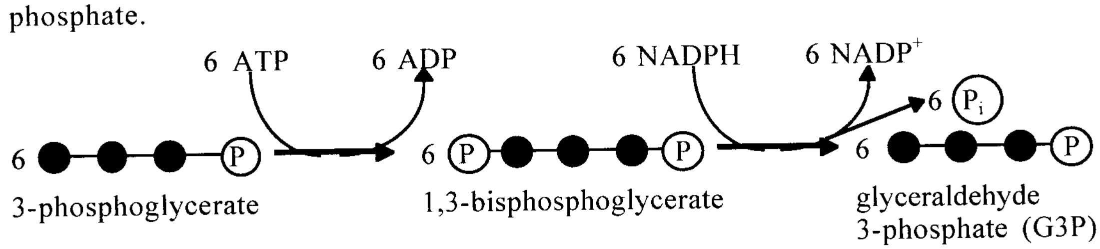


For the Calvin cycle to synthesize one molecule of sugar (G3P), three molecules of CO_2 must enter the cycle. The cycle may be divided into three phases:

Phase 1: Carbon Fixation. The Calvin cycle begins when each molecule of CO_2 is attached to a five-carbon sugar, *ribulose biphosphate* (*RuBP*).

- This reaction is catalyzed by the enzyme *RuBP carboxylase* (*rubisco*) – one of the most abundant proteins on Earth..
- The product of this reaction is an unstable six-carbon intermediate that immediately splits into two molecules of 3-phosphoglycerate.
- For every three CO_2 molecules that enter the Calvin cycle via rubisco, three RuBP molecules are carboxylated forming six molecules of 3-phosphoglycerate.

Phase 2: Reduction. This endergonic reduction phase is a two-step process that couples ATP hydrolysis with the reduction of 3-phosphoglycerate to glyceraldehyde phosphate.



- An enzyme phosphorylates 3-phosphoglycerate by transferring a phosphate group from ATP. This reaction:
  - Produces 1, 3-bisphosphoglycerate
  - Uses six ATP molecules to produce six molecules of 1,3-bisphosphoglycerate.
  - Primes 1,3-bisphosphoglycerate for the addition of high-energy electrons from NADPH.
- Electrons from NADPH reduce the carboxyl group of 1,3-bisphosphoglycerate to the aldehyde group of glyceraldehyde 3-phosphate (G3P).
  - The product, G3P, stores more potential energy than the initial reactant, 3-phosphoglycerate.
  - G3P is the same three-carbon sugar produced when glycolysis splits glucose.
- For every three CO_2 molecules that enter the Calvin cycle, six G3P molecules are produced, only one of which can be counted as net gain.
  - The cycle begins with three five-carbon RuBP molecules – a total of 15 carbons.
  - The six G3P molecules produced contain 18 carbons, a net gain of three carbons from CO_2.
  - One G3P molecule exits the cycle; the other five are recycled to regenerate three molecules of RuBP.

Phase 3: Regeneration of CO_2 acceptor (RuBP). A complex series of reactions rearranges the carbon skeletons of five G3P molecules into three RuBP molecules.

- These reactions require three ATP molecules.
- RuBP is thus regenerated to begin the cycle again.

For the net synthesis of one G3P molecule, the Calvin cycle uses the products of the light reactions:

- 9 ATP molecules
- 6 NADPH molecules

G3P produced by the Calvin cycle is the raw material used to synthesize glucose and other carbohydrates.

- The Calvin cycle uses 18 ATP and 12 NADPH molecules to produce one glucose molecule.

E. Alternative mechanisms of carbon fixation have evolved in hot, arid climates

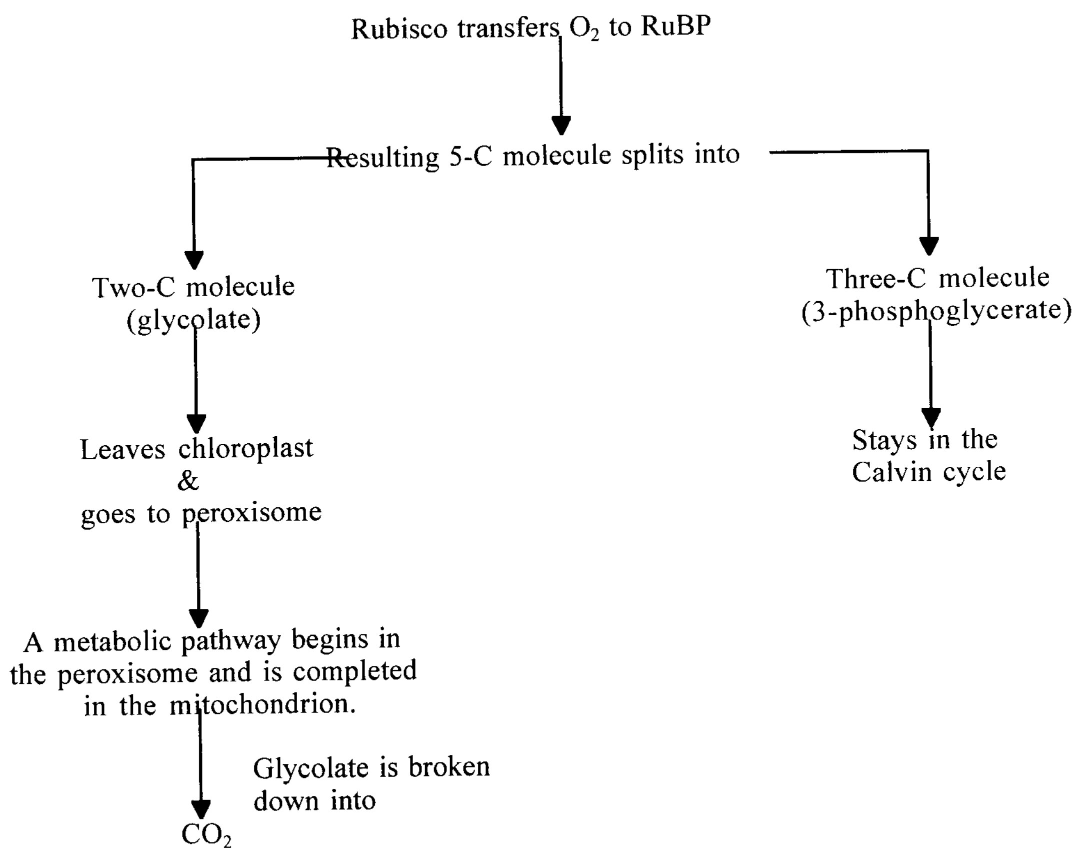
1. Photorespiration: an evolutionary relic?

A metabolic pathway called *photorespiration* reduces the yield of photosynthesis.

Photorespiration = In plants, a metabolic pathway that consumes oxygen, evolves carbon dioxide, produces no ATP and decreases photosynthetic output.

- Occurs because the active site of rubisco can accept O_2 as well as CO_2
- Produces no ATP molecules
- Decreases photosynthetic output by reducing organic molecules used in the Calvin cycle

When the O_2 concentration in the leaf's air spaces is higher than CO_2 concentration, rubisco accepts O_2 and transfers it to RuBP. (The "photo" in photorespiration refers to the fact that this pathway usually occurs in light when photosynthesis reduces CO_2 and raises O_2 in the leaf spaces.)



(The "respiration" in photorespiration refers to the fact that this process uses O_2 and releases CO_2.)

Some scientists believe that photorespiration is a metabolic relic from earlier times when the atmosphere contained less oxygen and more carbon dioxide than is present today.

- Under these conditions, when rubisco evolved, the inability of the enzyme's active site to distinguish carbon dioxide from oxygen would have made little difference.
- This affinity for oxygen has been retained by rubisco and some photorespiration is bound to occur.

Whether photorespiration is beneficial to plants is not known.

- It is known that some crop plants (e.g., soybeans) lose as much as 50% of the carbon fixed by the Calvin cycle to photorespiration.
- If photorespiration could be reduced in some agricultural plants, crop yields and food supplies would increase.

Photorespiration is fostered by hot, dry, bright days.

- Under these conditions, plants close their stomata to prevent dehydration by reducing water loss from the leaf.
- Photosynthesis then depletes available carbon dioxide and increases oxygen within the leaf air spaces. This condition favors photorespiration.

Certain species of plants, which live in hot arid climates, have evolved alternate modes of carbon fixation that minimize photorespiration. C_4 and CAM are the two most important of these photosynthetic adaptations.

2. C_4 plants

The Calvin cycle occurs in most plants and produces 3-phosphoglycerate, a three-carbon compound, as the first stable intermediate.

- These plants are called *C_3 plants* because the first stable intermediate has three carbons.
- Agriculturally important C_3 plants include rice, wheat, and soybeans.

Many plant species preface the Calvin cycle with reactions that incorporate carbon dioxide into four-carbon compounds.

- These plants are called *C_4 plants.*
- The C_4 pathway is used by several thousand species in at least 19 families including corn and sugarcane, important agricultural grasses.
- This pathway is adaptive, because it enhances carbon fixation under conditions that favor photorespiration, such as hot, arid environments.

Leaf anatomy of C_4 plants spatially segregates the Calvin cycle from the initial incorporation of CO_2 into organic compounds. There are two distinct types of photosynthetic cells:

1. Bundle-sheath cells
   - Arranged into tightly packed sheaths around the veins of the leaf
   - Thylakoids in the chloroplasts of bundle-sheath cells are not stacked into grana.
   - The Calvin cycle is confined to the chloroplasts of the bundle sheath.
2. Mesophyll cells are more loosely arranged in the area between the bundle sheath and the leaf surface.

The Calvin cycle of C4 plants is preceded by incorporation of CO_2 into organic compounds in the mesophyll (see Campbell, Figure 10.18)

Step 1: CO_2 is added to phosphoenolpyruvate (PEP) to form oxaloacetate, a four-carbon product.

- *PEP carboxylase* is the enzyme that adds CO_2 to PEP. Compared to rubisco, it has a much greater affinity for CO_2 and has *no* affinity for O_2.
- Thus, PEP carboxylase can fix CO_2 efficiently when rubisco cannot — under hot, dry conditions that cause stomata to close, CO_2 concentrations to drop and O_2 concentrations to rise.

Step 2: After CO_2 has been fixed by mesophyll cells, they convert oxaloacetate to another four-carbon compound (usually malate).

Step 3: Mesophyll cells then export the four-carbon products (e.g., malate) through plasmodesmata to bundle-sheath cells.

- In the bundle-sheath cells, the four carbon compounds release CO_2, which is then fixed by rubisco in the Calvin cycle.
- Mesophyll cells thus pump CO_2 into bundle-sheath cells, minimizing photorespiration and enhancing sugar production by maintaining a CO_2 concentration sufficient for rubisco to accept CO_2 rather than oxygen.

3. CAM plants

A second photosynthetic adaptation exists in succulent plants adapted to very arid conditions. These plants open their stomata primarily at night and close them during the day (opposite of most plants).

- This conserves water during the day, but prevents CO_2 from entering the leaves.

- When stomata are open at night, CO_2 is taken up and incorporated into a variety of organic acids. This mode of carbon fixation is called *crassulacean acid metabolism (CAM)*.
- The organic acids made at night are stored in vacuoles of mesophyll cells until morning, when the stomata close.
- During daytime, light reactions supply ATP and NADPH for the Calvin cycle. At this time, CO_2 is released from the organic acids made the previous night and is incorporated into sugar in the chloroplasts.

The CAM and C_4 pathways:

- Are similar in that CO_2 is first incorporated into organic intermediates before it enters the Calvin cycle.
- Differ in that the initial steps of carbon fixation in C_4 plants are structurally separate from the Calvin cycle; in CAM plants, the two steps occur at separate times.

Regardless of whether the plant uses a C_3, C_4 or CAM pathway, all plants use the Calvin cycle to produce sugar from CO_2.

F. Photosynthesis is the biosphere's metabolic foundation: *a review*

On a global scale, photosynthesis makes about 160 billion metric tons of carbohydrate per year. No other chemical process on Earth is more productive or is as important to life.

- Light reactions capture solar energy and use it to:
  - Produce ATP
  - Transfer electrons from water to $NADP^+$ to form NADPH
- The Calvin cycle uses ATP and NADPH to fix CO_2 and produce sugar.

Photosynthesis transforms light energy to chemical bond energy in sugar molecules.

- Sugars made in chloroplasts supply the entire plant with chemical energy and carbon skeletons to synthesize organic molecules.
- Nonphotosynthetic parts of a plant depend on organic molecules exported from leaves in veins.
  - The disaccharide *sucrose* is the transport form of carbohydrate in most plants.
  - Sucrose is the raw material for cellular respiration and many anabolic pathways in nonphotosynthetic cells.
- Much of the sugar is *glucose* – the monomer linked to form *cellulose*, the main constituent of plant cell walls.

Most plants make more organic material than needed for respiratory fuel and for precursors of biosynthesis.

- Plants consume about 50% of the photosynthate as fuel for cellular respiration.
- Extra sugars are synthesized into starch and stored in storage cells of roots, tubers, seeds, and fruits.
- Heterotrophs also consume parts of plants as food.

Photorespiration can reduce photosynthetic yield in hot dry climates. Alternate methods of carbon fixation minimize photorespiration.

- C4 plants spatially separate carbon fixation from the Calvin cycle.
- CAM plants temporally separate carbon fixation from the Calvin cycle.

REFERENCES

Atkins, P.W. *Atoms, Electrons, and Change*. New York, Oxford: W.H. Freeman and Company, 1991. Chapter 9, "Light and Life" is a witty, imaginative description of photosynthesis. Though written for a lay audience, it is probably best appreciated by someone already familiar with photosynthesis.

Campbell, N., et al. *Biology*. 5th ed. Menlo Park, California: Benjamin/Cummings, 1998.

Lehninger, A.L., D.L. Nelson and M.M. Cox. *Principles of Biochemistry*. 2nd ed. New York: Worth, 1993.

Matthews, C.K. and K.E. van Holde. *Biochemistry*. 2nd ed. Redwood City, California: Benjamin/Cummings, 1996.