Chapter 22: Homeostasis at the Cellular Level

UN Figure 1 Chloroplast photograph of Norman E. Good, pioneer in photosynthesis research. Light areas reveal where chloroplasts moved away from the brightest light.

Learning Objectives

1. Describe how plants capture energy from sunlight and convert that energy into new forms of potential energy.
2. Distinguish the three parts of photosynthesis and explain the overall chemical and physical processes involved in each one.
3. Explain the relationship between photosynthesis and global warming.
4. Discuss how carbon fixation is regulated by homeostasis of metabolic reactions.
5. Review the diversity of habitats and energy sources exploited by extremophiles.
6. Draw a picture that shows the flow of energy at thermal vents starting with the abiotic sources and ending with organisms that eat microbes.

Bio-Math Exploration Learning Objectives

Ethical, Legal and Social Implications Learning Objectives

1. Assess a range of environmental polices that were created by one country but affect multiple countries.
2. Argue why environmental policies require an understanding of biology at all five levels of organization – molecular, cellular, organismal, population and ecological systems.

Chapter 22 Outline

Introduction

22.1 Why is paraquat used in American but illegal in Europe?

22.2 How does Brazil's rainforest affect Greenland's glaciers?

ELSI Box 22.1 How do you compromise when a policy hurts one country but helps another?

22.3 Is there anywhere on earth devoid of life?

Conclusions

You Are Here		Big Ideas of Biology				
		Information	Evolution	Cells	Emergent Properties	Homeostasis
Levels of the Biological Hierarchy	Molecules	Chapter 1	6	11	16	21
	Cells	2	7	12	17	Chapter 22
	Organisms	3	8	13	18	23
	Populations	4	9	14	19	24
	Ecological Systems	5	10	15	20	25

Vignette here

22.1 Why is paraquat used in American but illegal in Europe?

- Context: Plants and animals are both eukaryotes so our cells contain many similar enzymes. Herbicides are designed to kill only plants, but does our common eukaryotic ancestry present challenges that could affect your health?
- Major Themes: Biological systems utilize feedback mechanisms to regulate and maintain optimal conditions; time-dependent processes regulate biological systems; life requires organization which is energy dependent; and biological system's size and environment influence how it addresses physical and chemical challenges.
- Bottom Line: Harvesting light energy into new chemical bonds shares many components found in cellular respiration which makes it difficult to produce herbicides that do not harm animals.

In 2003, farmers in the European Union (EU) began using the most common herbicide on the planet – paraquat (Figure 22.1a). Paraquat is used in over 100 countries to kill unwanted plants, especially weed that reduce the crop yield for farmers. In 2007, the EU Court of First Instance overturned the use of paraquat for EU-member countries. Farmers in the European Union are not allowed to use paraquat because it, "Fails to satisfy the requirement of protection of human health, which prohibits any exposure higher than the acceptable operator exposure level." Most cases of paraquat poisoning are due to oral ingestion but some people have died due to absorption through the skin. How does paraquat work on plants? Why does an herbicide designed to kill plants also kill humans? Pathologists quantified the level of paraquat in lab rats who had consumed potentially lethal doses of the herbicide (Figure 22.1b). Physicians can try to lower the harm of ingested paraquat with a few interventions, but lowering the blood level of paraquat is easier than stopping the accumulation in lungs.

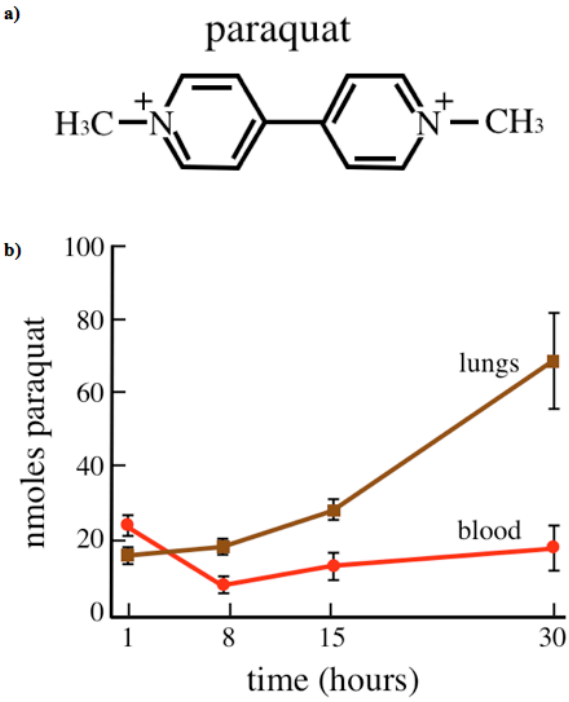


Figure 22.1 Parquat and its mammalian consequences. (a) Molecular structure of the herbicide paraquat. (b) Paraquat levels in the blood (per mL) and lungs (per gram) of rats fed paraquat. Error bars are standard error, n = 5 rats per time point.

To understand how paraquat works in plants and how this might affect humans, you need to understand photosynthesis first. You have been taught that plants photosynthesize to capture the sun's energy to produce their own food, but what does this really mean? You might be surprised to know that research into photosynthesis began before the existence of the United States. In 1774, the British theologian and scientist Joseph Priestley was experimenting with the composition of air. What was in the air we breathe that supports the flame of a burning candle

(Figure 22.2a)? Priestley placed an air-tight glass dome over a burning candle and a sprig of mint picked from his garden. After a few minutes, the flame went out because it had consumed something in the air. The experiment was placed in the sunlight for many days before Priestley used a magnifying glass to relight the candle. If the equipment was kept in the dark, or lacked the mint leave, the candle could not be lit again. The light and the leaves combined to replenish what the flame had consumed.

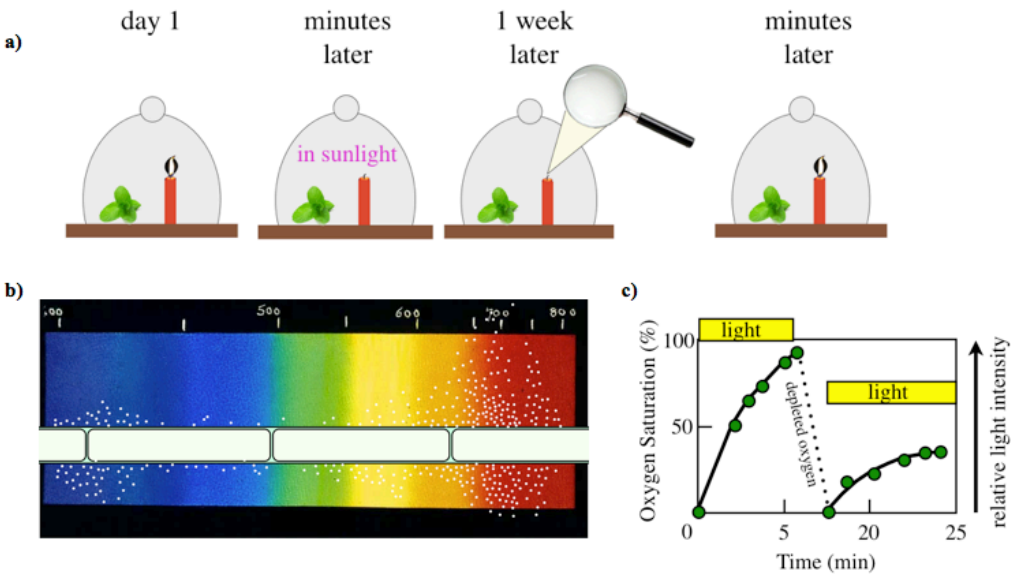


Figure 22.2 Plants produce oxygen. (a) Priestely's 1774 experiment showing that plants in the sunlight can replenish depleted air. (b) Engelmann's 1882 experiment showed oxygen-loving bacteria (white spots) moved to the portion of algae cells exposed to particular wavelengths of light. The original drawing was in black and white – color has been added for clarity. (c) Hill's experiment measured oxygen production from purified chloroplasts exposed to light.

More than 100 years after Priestley, a German physiologist, Theodor Wilhelm Engelmann, conducted experiments to understand what happened to plants when exposed to sunlight (Figure 22.2b). Engelmann worked with a rod-shaped algae and a species of bacteria he called *Bacterium termo* that exhibited a surprising behavior. Engelmann had discovered that *B. termo* could detect very small quantities of oxygen and swam towards areas of higher oxygen when presented with two solutions of different dissolved oxygen levels. Engelmann used a specially designed microscope, built by his friend Carl Zeiss who founded the famous microscope company. The microscope could separate the different colors of light as seen in a rainbow. Engelmann knew of Priestley's research and wanted to determine what the plants produced when exposed to sunlight. Engelmann exposed algae to the rainbow of light under a microscope and watched to see what *B. termo* would do. Engelmann did not have a camera to capture the image, so he drew his observations that documented the first example of plants producing a substance that attracted bacteria.

Approximately 50 years after Engelmann's experiments with *B. termo*, Robin Hill, a biochemist working at Cambridge University in the UK, made significant discoveries about the process of photosynthesis (Figure 22.2c). Hill determined that the green colored chloroplasts were sub-cellular organelles and used sunlight to produce what flames consume. Hill disrupted spinach leaf cells and used centrifugation to isolate the intact chloroplasts. Hill exposed the chloroplasts to light and measured the concentration of dissolved oxygen in the buffer containing the chloroplasts. Hill demonstrated that the rate of oxygen production could be influenced by the

intensity of light. Between the two experiments shown in the figure, Hill used a chemical that rapidly consumed all the oxygen.

Integrating Questions

1. Describe what happens to the tissue distribution of the herbicide for rats that consume paraquat. What impact might paraquat's tissue distribution have on attempts to save human patients exposed to lethal doses of the poison? Based on its chemical structure, is paraquat water soluble? Explain your answer - the chemical structure will be critical to understanding how paraquat works and why it is harmful to animals.
2. Summarize Priestley's experimental design and his findings. Though Priestley did not know the name used today, you should be able to determine what molecule was produced by the mint leaves when exposed to light.
3. Engelmann used the ability to separate the colors of light produced by the sun to refine Priestley's results. Explain What Engelmann learned by using *B. termo* and the photosynthetic algae.
4. Summarize Hill's discoveries as illustrated in Figure 22.2c. What new information did Hill provide that you did not know from Priestley or Engelmann?

When rats consume paraquat, the poison steadily accumulates in their lungs and even blood transfusions fail to lower the concentration of paraquat from the lungs. It would be unethical to perform similar experiments on humans but it is reasonable to deduce that all mammals would accumulate paraquat in their lungs. Paraquat is water soluble which you could predict given its +2 net charge. Paraquat is sold as a chloride salt which makes the powder form extremely dangerous to breathe. The electrical charge of paraquat is central to its role in plants and humans.

Priestley realized that a gas in the air we breathe is consumed by fire. Of course, you know the gas is oxygen, O_2, but Priestley called it something else at the time. More importantly, Priestly discovered that plants can replenish the air with oxygen if and only if the plants are exposed to light. Engelmann added to the understanding of photosynthesis by discovering that some wavelengths of light are more effective than others at stimulating plants to produce oxygen. From his histogram-like drawing, you can see that blue light and red light stimulated the most oxygen production while blue-green into yellow stimulated the least amount of oxygen production. By dividing light into is color spectrum, Engelmann's results raised a new set of questions about the nature of light absorption by plants.

You know from your own experience of the world that most plants appear green and Engelmann demonstrated that plants do not use the green spectrum of light. Objects appear a certain color because they do not absorb that color. Hill focused his attention on the only organelle that looked green inside plants – chloroplasts. Hill realized that if plants cannot use green light to produce oxygen, and chloroplasts are green, this organelle is the likely source of oxygen production in response to light. The results of Figure 22.2c confirmed Hill's hypothesis and demonstrated the rate of oxygen is proportional to the intensity of light.

By the 1940s, botanists and biochemists focused their attention to the pigments in chloroplasts that absorbed light and gave plants their green appearance (Figure 22.3). You know from your own observations that different types of plants can vary in their shade of green, but essentially all plants share a common set of 4 pigments – **chlorophyll a**, **chlorophyll b**, **β-carotene**, and **lutein**. When cell biologists visualized chloroplasts in detail, they could distinguish internal membranes called **thylakoids** that floated in the liquid **stroma**. Thylakoids

are hollow and flattened membrane vesicles that enclose the **thylakoid space**. As you learned in Chapter 6, chloroplasts contain their own chromosomal DNA and two layers of external membranes which are evidence of their evolutionary origin as photosynthetic bacteria. In chapter 21, you learned that some proteins cluster together to facilitate a faster transfer of metabolic intermediates. In chloroplasts, photosynthetic pigments also cluster together in structures referred to as **antenna complexes**. The antenna complex contains many pigments in a ring of proteins that surrounds the central core proteins and pigments. The central core is where light energy is converted to chemical energy. To better understand the antenna complex, view this interactive Jmol tutorial. {Definitions: **Chlorophyll a** and **chlorophyll b** are the two most abundant photosynthetic pigments in green plants. **β-carotene** is the orange photosynthetic pigment that gives carrots their color while **lutein** is more yellow in appearance. **Thylakoids** are membrane vesicles inside chloroplasts that are green in appearance. **Stroma** is the liquid-filled space inside chloroplast where thylakoids reside. **Thylakoid space** is the liquid-filled lumen of thylakoids. **Antenna complexes** are collections of photosynthetic pigments and proteins that surround the main site for capturing light energy.}

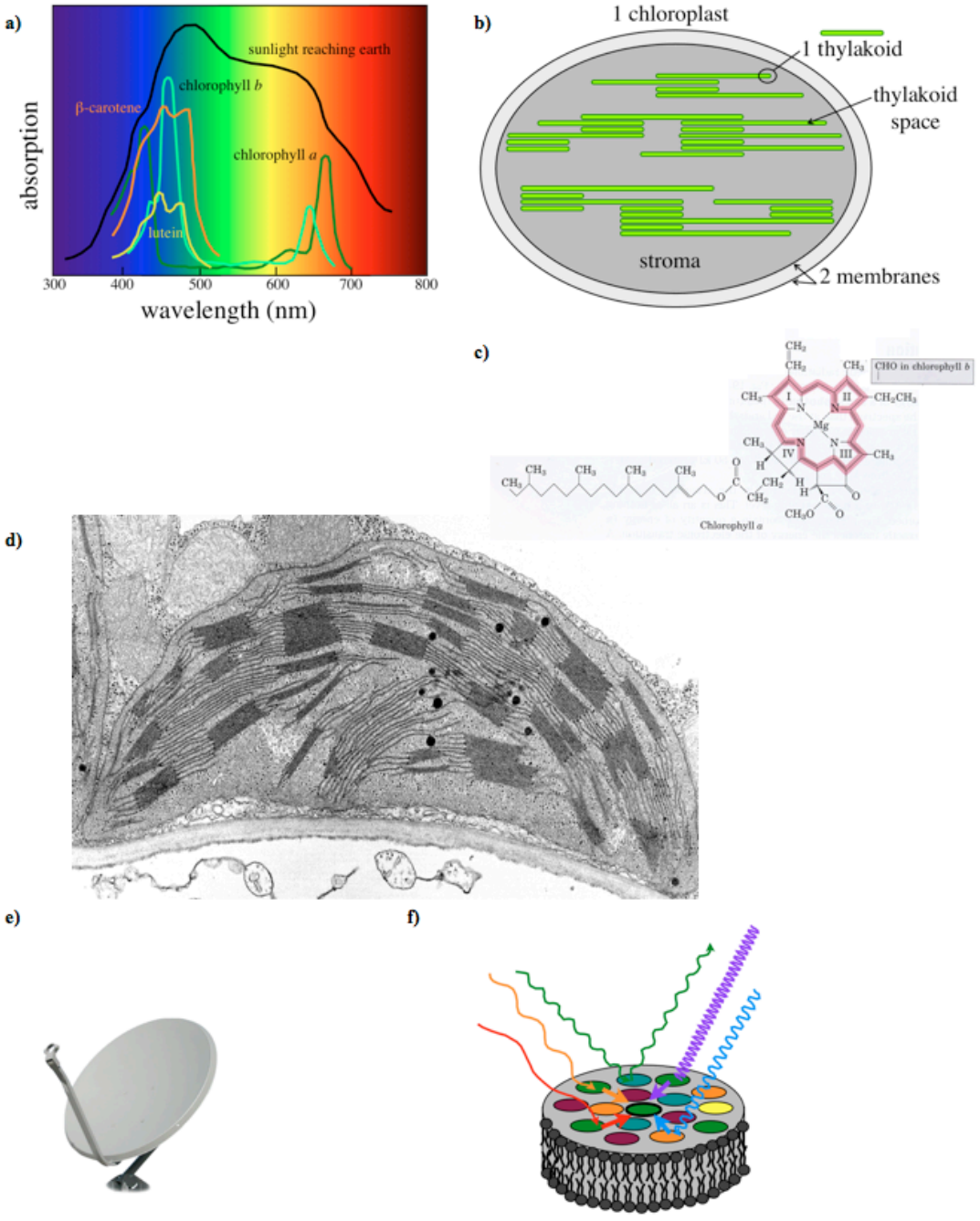


Figure 22.3 Chloroplast pigments absorb light and direct it to the reaction

center. (a) Pigments and the colors they absorb compared to all the sunlight reaching earth. (b) Diagram of chloroplast and its parts. (c) Structure of chlorophyll *a* and *b*. (d) Electron micrograph of spinach chloroplast; dark circles are artifact and not biologically relevant. (e) Satellite antenna collects signals and points them to a central location. (f) Diagram of antenna complex of photosynthetic pigments and the colors absorbed. Reaction center is outlined in black in the middle of the structure.

The inner core of the antenna complex are proteins that position two chlorophyll a molecules that form the key to this **reaction center**. The reaction center is a combination of proteins and chlorophyll a molecules and is the precise location where light **photons** are converted into biochemical energy carried by other proteins. You have experienced the energy of photons every time you sat in the warm sunlight. Red light has long wavelengths but carries less energy than blue light with its shorter wavelengths (see Figure 22.3a for the color spectrum and wavelengths). Light can be described as both a wave and tiny, massless particles or units of energy. The term photon is used to describe this packet of light, derived from the familiar atomic terms of electron, proton and neutron. When the reaction center chlorophyll a molecules absorb light, one electron in the outer most shell of the magnesium ion is boosted to a higher energy level, or an **excited state**. Photosynthesis requires this excited state electron to be harness into new forms of chemical energy. {Definitions: **Reaction centers**, inside antenna complexes, are proteins and paired chlorophyll a pigments that harness light energy into biochemical energy. **Photons** are units of light energy that can be quantified as a function of their wave lengths. **Excited state** describes a brief moment when a molecule has absorbed energy and an electron is boosted to a higher energy level.}

Integrating Questions

5. What colors do the chlorophylls absorb and what color would they appear by eye? Why do plants appear green if they contain more pigments than just chlorophyll a and b? What time of year can you observe these other pigments? What colors do you perceive when looking at them?
6. Where in plant cells are the photosynthetic pigments located? Name the structures and the precise location of the pigments within chloroplasts. Use the structure of chlorophyll in Figure 22.3c to predict the pigment's orientation within the chloroplast.
7. The diagram in Figure 22.3b represents the major components of a chloroplast but the electron micrograph in Figure 22.3d is more accurate. Estimate the ratio of thylakoid membranes in stacks or versus not in stacks. Round to the nearest 25%. Do you think this ratio changes with time in living plants, or does thylakoid architecture remain relatively constant?
8. Use the analogy of a satellite dish to explain how the antenna complex of plant pigments works to capture light and send the absorbed light energy to the reaction center. Include in your answer why plants have more than just chlorophyll pigments in their antenna complexes. Look at UN Figure 22.2 and fill in the table with the appropriate values from this list of energy levels in kJ/mole of photons: 199, 239, 171, 299.

wavelength	color	kJ/mole
700	far red	
600	orange	
500	green	
400	violet	

UN Figure 22.2 Table for Integrating Question #8.

9. Click on the buttons of the Jmol tutorial to see how many pigment molecules are present. Notice the gap in the outer ring of proteins. Hypothesize the function of this gap. What ion is in the center of chlorophyll's ring (see Figure 22.3c) and what molecule does chlorophyll remind you of that you have studied before?

Using Figure 22.3, you can see that photosynthetic pigments absorb violet through blue-green light and orange through red light. In particular, the four photosynthetic pigments do not absorb green light which is reflected back to your eyes so the leaves appear green to you. By not absorbing organ and red, the non-chlorophyll pigments appear yellow to orange, depending on their concentration. Each autumn, you can see non-chlorophyll pigments in the colorful display of tree leaves. All photosynthetic pigments have structures similar to chlorophyll with a hydrophobic tail and a hydrophilic head. The difference between chlorophyll a and b is a simple substitution of a –COH group in chlorophyll b instead of CH_3 found in chlorophyll a (Figure 22.3c). The hydrophobic tails force pigments to seek out places such as membranes and hydrophobic portions of proteins while the portion containing the charged Mg^{+2} ion must be positioned in an aqueous environment. Inside chloroplasts, the photosynthetic pigments are embedded in the thylakoid membrane. The stroma does not contain any pigments, nor does the thylakoid space. It is difficult to quantify the percentage of thylakoid membrane that is stacked vs. unstacked, but you may have guessed about 50% of each, or 75% unstacked. The exact number is not important for now but it is important that you realize thylakoids can be stacked or unstacked inside chloroplasts. Later you will learn whether the percentage is fixed or dynamic in living plants.

When you interacted with the Jmol tutorial on the antenna complex, you might have been surprised to see so many proteins and photosynthetic pigments arranged in neat circles. What was not evident in the Jmol but you can see in Figure 22.3f is that the antenna complex and the reaction center are all embedded in the phospholipid bilayer of thylakoid membranes. As the name implies, the antenna complex traps light of different wavelengths and shuttles the energy towards the reaction center. Satellite dishes perform a similar function energy beams collected by the disc are bounced to the receiver in the middle (Figure 22.3e). Violet light carries more energy (299 kJ/mole of photons) than orange or far red light (171 kJ/mol) and the different pigments allow different levels of light energy within white light to be captured. The function of the antenna complex is to shuttle energy to the reaction center and the reaction center must be able to transfer the energized electron removed from chlorophyll a. Biochemists working on the mechanism of energy trapping hypothesized that the gap in the outer ring of antenna complex proteins permits another molecule to gain access to the excited state electron of chlorophyll a. Removing an electron with a protein is similar to what you learned in Chapter 21 about the electron transfer chain taking place in a hydrophobic environment. Before you follow the pathway of the excited state electron, you need to understand a bit more about how photosynthesis works.

After World War II, biologists made many discoveries about how plants function. Daniel Arnon, working at UC Berkeley, was a pioneer in photosynthesis research. By the mid-1950s, it was clear that plants could **split** (or consume) **water**, produce ATP, and convert CO_2 into multi-carbon sugars (**carbon fixation**). Arnon measured the rate of all three processes when exposed to different concentrations of a particular inhibitor (Figure 22.4). In the first experiment, he placed

chloroplasts in a solution containing 1.5 x 10^{-4} M inhibitor and compared the rate of water splitting to chloroplasts without any inhibitor. For the remaining two experiments, Arnon measured the rate of ATP production and the rate of carbon dioxide fixation over a range of inhibitor concentrations. By testing the ability of chloroplasts to perform all three functions in the presence of a single inhibitor, Arnon made an important discovery about the overall process of photosynthesis. {Definitions: **Splitting water** is a enzymatic reaction during photosyntheiss that pulls the hydrogen atoms from water to produce O_2 and H^+ plus an electron. **Carbon fixation** is the enzymatic process during photosynthesis of covalently linking CO_2 with H^+ plus an electron to form 3-carbon sugars.}

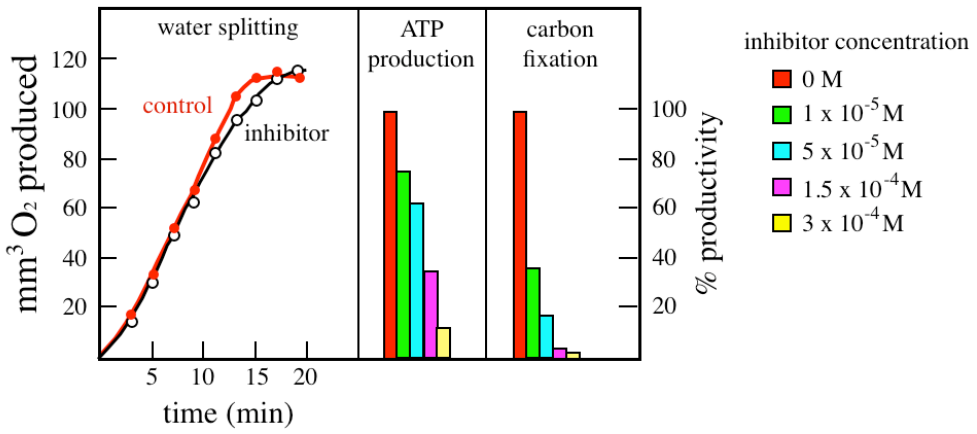


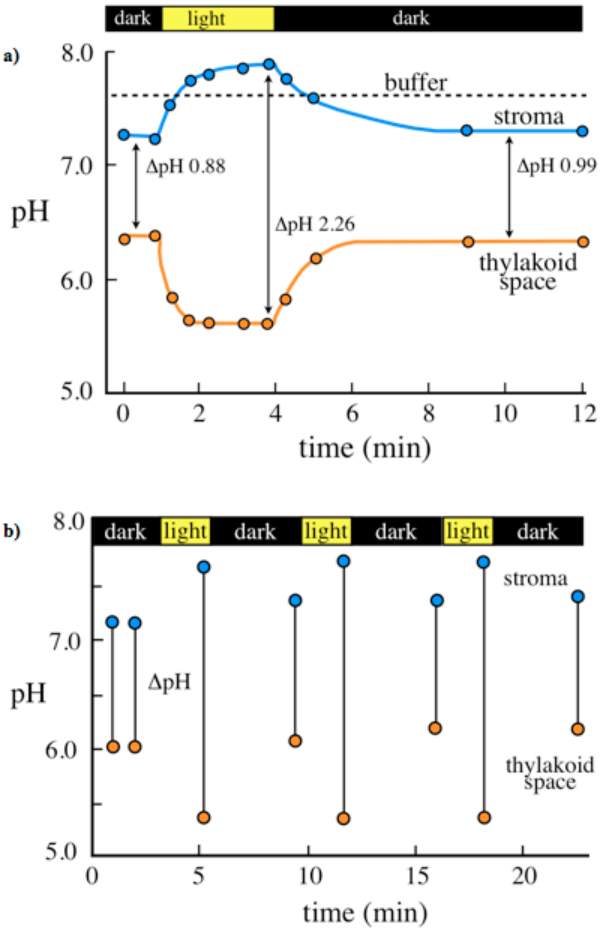
Figure 22.4
Photosynthesis is a summation of multiple parts. Using a general enzyme inhibitor, botanists dissected photosynthesis by measuring the production of oxygen, ATP and carbon fixation.

Integrating Question

10. Look at the data in Figure 22.4. Are all three photosynthetic processes affected equally by the inhibitor? Based on these results, what can you conclude about the interdependence of water splitting, ATP synthesis and carbon fixation? Are they all linked together or can they be biochemically independent?

Arnon made several major contributions to our understanding of photosynthesis, and the data in Figure 22.4 catalyzed a wave of new experiments. You don't need to memorize which processes were inhibited and which one was not. The key finding is that the three processes were inhibited differently from each other. The conclusion from Arnon's work was that splitting water, producing ATP and fixing carbon are each produced by different biochemical pathways. The significance of his findings was apparent to his colleagues because now they could design experiments to test one pathway at a time in order to understand how each pathway worked on its own. Once the three separate pathways were isolated and characterized, investigators could examine how water splitting, ATP synthesis and carbon fixation were interrelated in the overall process of photosynthesis.

Working in Munich Germany, Hans Heldt and his colleagues wanted to focus on ATP production. You have already learned that mitochondria use a proton gradient of H^+ ions to drive ATP synthase, so you might expect similar experiments on chloroplasts producing ATP (Figure 22.5a). The German biochemists simultaneously measured the pH inside thylakoids and pH in the stroma of chloroplasts. After one minute of measuring in the dark, the investigators turned on the light and monitored the pH in both compartments. Three minutes later, they turned off the bright light and continued to record the pH in both compartments inside chloroplasts. A good scientists asks questions after every experiment. Are my data reproducible? Does the pH change



more than once for a given thylakoid? How quickly can the change in pH be reestablished after turning on or off the lights? You can see the answers to these questions in Figure 22.5b. Keep in mind that the chloroplasts used in Heldt's experiments were penetrated by microelectrodes and so they would not be performing quite as well as undisturbed chloroplasts.

Figure 22.5 Comparison of pH in stroma and thylakoid space. (a) Botanists used pH microelectrodes to measure the pH of a single chloroplast exposed to dark or light, as indicated. (b) Repeated exposures of a chloroplast to light and dark cycles within minutes of each other.

By the early 1970s, biochemists had discovered that like mitochondria, chloroplasts produce a pH gradient across a phospholipid bilayer. However, light was the source of the energy in chloroplasts, not 2-carbon products of cellular respiration as found in mitochondria. {*Connections: You learned about cellular respiration and the conversion of carbon sources into pH gradient in Chapter 21.*} Once again, Robin Hill and his collaborators provided critical data and insights into how light is converted into a pH gradient (Figure 22.6). Although we know many more details now than Hill did, he correctly deduced the flow of electrons from chlorophyll a in reaction centers. Working with spinach chloroplasts, biochemists had discovered that the chlophyll a in the reaction centers came in two slightly different forms because they could bleach the pigments with intense lights at two different wavelengths of 680 nm and 700 nm due to slight differences in their surrounding proteins. Once biochemists could distinguish the two forms of chlorophyll a based on their bleaching wavelengths, the two reaction centers were named accordingly – **P680** and **P700**, P for pigment. {Definition: **P680** and **P700** are the paired chlorophyll a molecules that absorbed slightly different wavelengths of light.}

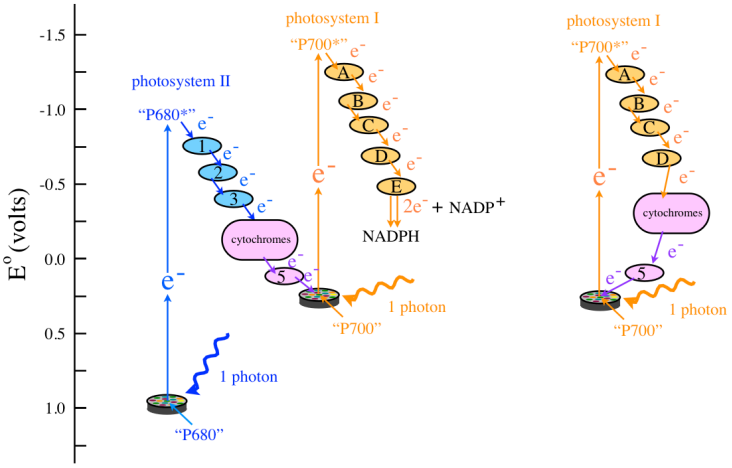


Figure 22.6 Flow of electrons excited by light. (a) Photosystems I (orange) and II (blue) absorb two photons and use electron carrier molecules to pass excited electrons that eventually form a new covalent bond in

NADPH formed in the stroma. (b) When photosystem II is not nearby, photosystem I has the ability to pass its electron back to its own reaction center via the shared components of photosystem I and II (pink).

Antenna complexes and their reaction centers can trap many different wavelengths of light but the P680 pigments can capture light with slightly higher energy levels than the P700 version. {*Connections: You learned about the relationship between voltage and energy in Chapter 21; see Figure 21.3a.*} For historical reasons, the proteins associated with P700 were collectively called **photosystem I** (PSI) and those associated with P680 were called **photosystem II** (PSII). When the chlorophyll a in P680 absorbs the energy of a photon, one of its electrons becomes excited and boosted to a higher energy level. The gap in the antenna complex probably enables another protein to be located nearby so that the excited state electron from P680 can be pulled away from the chlorophyll a molecule (Figure 22.6a). The energized electrons are passed from one molecule to another, including a cytochrome similar to those found in mitochondria. At this same time, P700 has also be stimulated by light and one of its electrons has been excited and pulled away to an electron acceptor. In both P680 and P700, their chlorophyll a molecules are **photooxidized**, meaning light has caused them to give up an electron. As in mitochondria, the electrons flow to successive proteins but the two photosystems have different outcomes. The excited electron from PSII travels to P700 and replaces the missing electron from oxidized P680 chlorophyll a. The electron that began at P700 combines with H^+ and forms a new covalent bond onto $NADP^+$ to produce NADPH. Sometimes PSI is not near PSII and the excited state electron travels to some of the same proteins but jumps over to a cytochrome and then right back to P700 again, in a cyclic pathway over and over (Figure 22.6b). PSI can either produce NADPH or not, depending on what proteins are nearby at any given time. You will learn more about the homeostatic balance between cyclic PSI by itself and PSII interacting with PSI a bit later. {Definitions: **Photosystem I** and **photosystem II** include the antenna complex, P700 and P680, respectively, and electron transport chains. **Photooxidation** is the removal of an electron from chlorophyll a when the pigment is excited by a photon.}

Biochemists and botanists performed thousands of experiments in order to elucidate the pathways summarized in Figure 22.6. However, you still have not seen how the flow of electrons is converted into a pH gradient across the thylakoid membranes (Figure 22.7). When electrons move from PSII to P700 in PSI, they pass through the cytochromes where H^+ ions are transported across the thylakoid membrane. Similarly, when PSI is moving its electrons in the cyclic pathway, the electrons are shuttled through the thylakoid membrane with the assistance of cytochromes which pump H^+ ions across the membrane again.

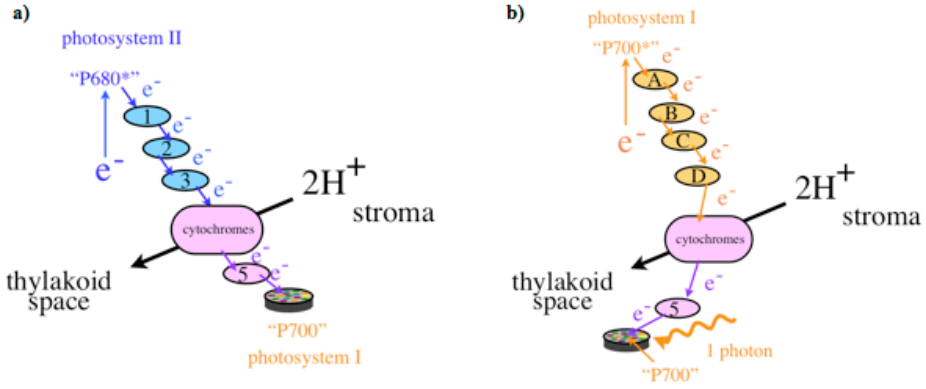


Figure 22.7 Balancing electron and H^+ ion flow in chloroplasts. (a) The movement of electrons from photosytem II to photosystem I converts high energy electrons into a H^+ ion gradient. (b) Cyclic electron flow within photosystem I also converts high energy electrons into a H^+ ion gradient.

The final data you need to know in order to connect the light-induced pH gradient with ATP production is in Figure 22.8. ATP production is very tightly coupled with exposure to light. As in mitochondria, ATP-synthase is embedded in the thylakoid membrane such that the entrance to the H^+ ion channel faces the lower pH compartment while the proteins that create the new covalent bond between ADP and P_i is located in the high pH compartment. The proteins involved in the chloroplast and mitochondrial ATP-synthases are very similar to each other which reflects their common prokaryotic origins. Bacteria and archaea living today also utilize a similar pH gradient and ATP-synthase because the two organelles and prokaryotes all have the same evolutionary heritage.

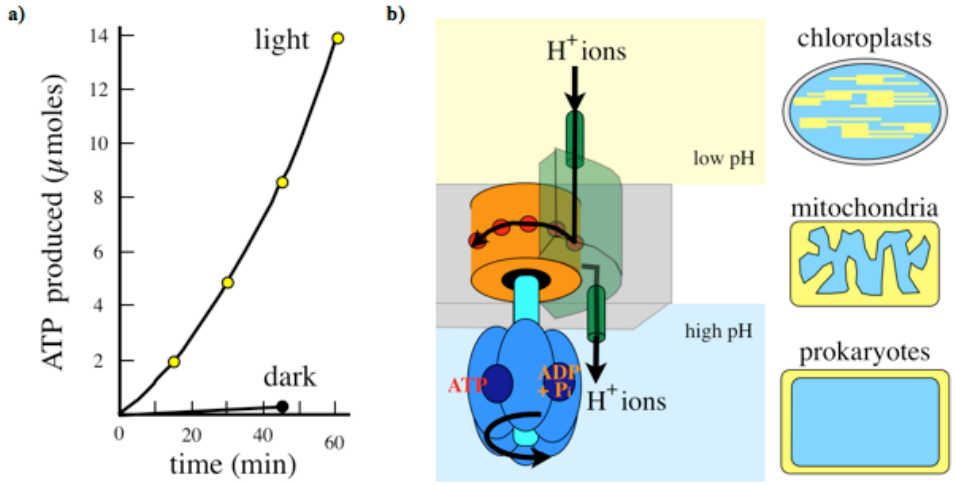


Figure 22.8 ATP production from light energy. (a) Purified chloroplasts produce ATP when exposed to light but not when they are kept in the dark. (b) Evolutionary comparison between three distinct but evolutionarily related ATP synthesis systems.

Integrating Questions

11. What is the difference in pH units between thylakoid spaces and stroma when kept in the dark? What is the fold difference in H^+ ions given that the pH uses a $\log_{10}$ scale? Quantify the difference in pH units and fold change when light is shone on thylakoids. Are the data in Figure 22.5 highly reproducible and can chloroplasts repeat the pH change multiple times with a few minutes?
12. Compare and contrast cyclic electron flow of PSI with the non-cyclic flow of PSII + PSI in Figure 22.5. What forms of energy enters these two subcellular pathways, and how is the energy stored? What common molecules do the cyclic and non-cyclic electron pathways share? Do you think the movement of electrons down the electron transport chain has a positive or negative ΔG? Explain your answer.
13. Summarize how light absorption in PSII and PSI results in a pH gradient? Where do the H^+ ions come from and where do they accumulate? For every one H^+ in the thylakoid space, how many are in the stroma when chloroplasts are absorbing light? Connect the data in Figure 22.5 with the diagram in Figure 22.7.
14. Compare and contrast mitochondria, chloroplasts and prokaryotes with respect to pH gradients, ATP synthesis and their energy sources.

Although the chloroplasts used in Figure 22.5 are not in the same conditions as normal chloroplasts due to the pH sensors, you can tell that in the dark, the pH difference is about 1.2 pH units. A pH differential of 1.2 is the same as a 16 fold difference in H^+ ions with the higher concentration of protons located in the thylakoid space. When exposed to lights, the thylakoid accumulates about 80 fold more H^+ ions which is the same as 1.9 pH units. More sophisticated pH measurements indicate that the difference in pH for a typical leaf cells is about 3 pH units,

which is 1000 fold H^+ ion difference in proton concentration. Cyclic and non-cyclic electron flow share many components: they both transport electrons down a chain of carrier molecules. The movement of electrons down the photosynthetic chain of carriers within the thylakoid membrane has a negative ΔG (-180 kJ/mole) as you should have predicted because there is less potential energy to perform work in NADPH or reduced chlorophyll than when the first accepting molecule is initially reduced by excited state chlorophyll a. Electrons do not flow "up hill" because the pathway strongly favors its directionality because the ΔG is a large negative number. In both pathways, the electron is energized by sunlight absorption of the antenna complex which is channeled to the reaction center chlorophyll a molecules. Both electron pathways produce a H^+ ion gradient when the electrons are passed through the cytochromes. However, the cyclic electron pathway does not produce an electron hole in the form of an oxidized chlorophyll a in the reaction center. Non-cyclic electron flow produces NADPH as the final electron carrier which is a chemical form of potential energy while the cyclic flow only produces a proton gradient as its conversion from light to chemical energy. Therefore, cyclic electron pathway produces only ATP while non-cyclic electron flow produces both ATP and NADPH. There are several other differences and similarities, but the key points so far are described above.

The production of the light-induced pH gradient is the consequence of an excited state electron in the reaction centers P680 and P700. Each electron that moves through the cytochromes causes the passage of 2 H^+ ions to move from the stroma to the thylakoid space. As indicated in Figure 22.8, light causes the pH to drop inside the thylakoids which is a functionally equivalent compartment to the intermembrane space of mitochondria or the cytoplasm of prokaryotes. H^+ ions move down their electrochemical gradient and in the process cause ATP synthases to spin and produce ATP from ADP and inorganic phosphate (P_i or PO_4^{-3}). Once the H^+ ion gradient is dissipated, ATP production is near zero in chloroplasts. Similarly, $NADP^+$ reduction to NADPH in the stroma is tied directly the H^+ ion accumulation inside thylakoids. During darkness, the ratio of H^+ ions inside the thylakoid to in the stroma is about 16:1 but when exposed to light, the ratio jumps to 100 or 1000:1. As the H^+ ions accumulate inside the thylakoids, H^+ ions will rush back into the stroma in the process of ATP synthesis which is analogous to bailing out a leaky boat. Photosynthesis produces ATP by harnessing the energy in photons while prokaryotes and mitochondria use the reducing power in $FADH_2$ and NADH that feed the electron transport chain and produce the H^+ ion gradients depicted in Figure 22.8b.

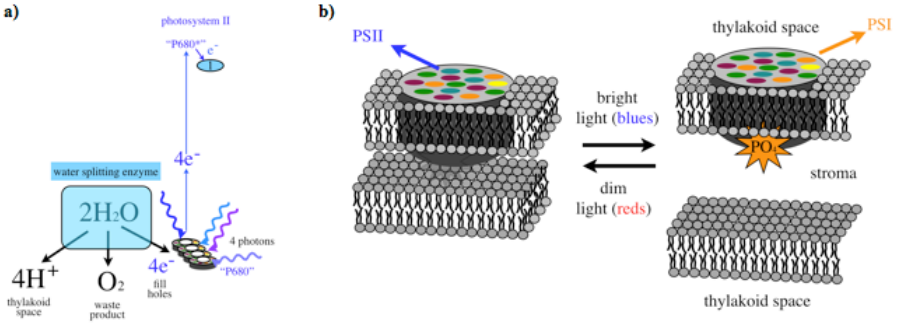


Figure 22.9 Homeostasis of photosynthetic processes. (a) A water splitting enzyme generates replacement electrons as well as beneficial waste products. (b) Stacking of thylakoid membranes is part of the homeostatic regulation of energy flow through plants.

At this point, two aspects of homeostasis need to be considered (Figure 22.9). The first aspect is oxidized P680. After the PSI reaction center has lost its excited state electrons, the entire photosystem would be silenced since light is not strong enough to produced a "doubly oxidized"

chlorophyll a molecule. Oxidized chlorophyll is very electronegative, which you remember means it has a strong affinity for electrons participating in covalent bonds of other molecules. Reaction centers contain multiple chlorophyll a molecules and when four of them become oxidized, two water molecules will be split with the assistance of an enzyme so that the four electrons are used to reduce chlorophyll back to its original charge:

$$4 \text{ photons} + 2H_2O \rightarrow 4 H^+ + 4e^- + O_2.$$

Each of four oxidized $P680^+$ receives a single electron and PSII is reset and ready to absorb light and contribute to the H^+ ion gradient.

At this point in your understanding of photosynthesis, you are probably wondering how PSI "knows" when to perform cyclic vs. non-cyclic electron flow (Figure 22.9b). Remember that non-cyclic electron flow results in split water, NADPH and ATP production while cyclic flow produces only ATP. Therefore, the regulation of cyclic vs. non-cyclic has profound consequences for the plant. To understand the regulation of non-cyclic and cyclic eletron flow, return to Figure 22.3d. You observed that some thylakoids are stacked while others are not. The regulation of stacked vs. non-stacked is the mechanism that governs cyclic vs. non-cyclic. PSI is primarily located in the non-stacked thylakoids along with ATP synthase but PSII is more numerous than PSI in the stacked membrane regions. The antenna complex associated PSII has the ability to move between stacked and non-stacked thylakoids. The location of antenna complex II is determined by the type of light reaching the chloroplasts. When bright light containing high levels of blue light reaches the chloroplast, a kinase is activated that covalently modulates the antenna complex which causes the thylakoids to separate and reduce the number of stacked membranes. Phosphorylated antenna complexes migrate within the thylakoid membrane and associate with PSI as a result of bright light. Conversely, dim light, which contains more red light, turns on a phosphatase the removes the phosphate from the antenna complex. In response to losing the phosphate, the antenna complex facilitates more stacking of thylakoids and reassociation with PSII.

Integrating Question

15. Based on what you know about photosynthesis so far, where do you think water is located when it is split to produce O_2 gas as a waste product? What two other products are produced when water is split and where do those products go?
16. In Figure 22.4, you learned that water splitting and ATP production are separate biochemical processes. Explain how they are separate and linked at the same time. How could a chemical inhibitor block ATP production but not water splitting?
17. How many protons are added to the thylakoid space for every 2 split water molecules? Count the number of H^+ ions and state how each one reached the low pH compartment inside thylakoids. How many photons are required to split 2 water molecules and produce NADPH from $NADP^+$ and H^+? How many NADPH molecules are produced when 2 water molecules are split to replace the "missing" electrons from oxidized P680?
18. How does sunlight regulate the ratio of cyclic vs. non-cyclic electron flow in chloroplasts? What happens to the rate of NADPH production when dim light reaches chloroplasts? Do you think thylakoids ever reach 100% stacked or 100% unstacked?

Water is in every subscellular compartment of plant cells, but water is only split in one place. If you predicted that water is split in the thylakoid space, you were correct. Splitting water inside the thylakoid is more efficient that splitting in the stroma since the H^+ ions are stockpiled inside

the thylakoid in order to produce ATP. Oxygen is non-polar, so it can diffuse across all the membranes of the chloroplast and be released to the air via the stomata. {*Connections: You learned about stomata and guard cells in Chapter 17.*} The third and final product of water splitting is 4 electrons which reduce the chlorophyll a molecules in the P680 reaction center. Water splitting occurs when electrons flow via the non-cyclic pathway and P680 is photooxidized. ATP production is the consequence of accumulating a H^+ ion gradient which is formed when the electrons from P680 and P700 are shuttled by cytochromes. Therefore, ATP production and water splitting are related, but not necessarily. If the inhibitor used in Figure 22.4 were able to block the pumping of H^+ ions into the thylakoid space but not inhibit the photooxidation of P680, then the water splitting enzyme would still function normally but ATP would not be produced as efficiently compared to no inhibitor.

As you learned when you studied cellular respiration, photosynthesis is partly a bookkeeping exercise. You know that the first law of thermodynamics states energy cannot be created or destroyed, only changed. Similarly, atoms are not lost in biochemical processes. Consider the summary equation for water splitting at P680:

$$4 \text{ photons} + 2H_2O \rightarrow 4\ H^+ + 4e^- + O_2.$$

With every two waters split, 4 protons are added to the low pH thylakoid space. With the production of 4 electrons, 8 H^+ ions are pumped through the cytochromes into the thylakoid space. Therefore 12 H^+ ions accumulated when 2 waters are split by P680. The equation states 4 photons are required to split 2 waters, but this count ignores the photons required for the second half of the non-cyclic electron flow. Four more photons must be absorbed by P700 to reenergize 4 electrons to reduce NADP+ and H+ to form 2 molecules of NADPH and a new covalent bond. Therefore, 8 photons are required to split 2 water molecules and form 2 NADPH molecules with 12 H^+ ions added to the thylakoid space. You can view an interactive molecular structure of PSII and the water splitting center online (similar to http://molvis.sdsc.edu/fgij/fg.htm?mol=1S5L).

One dynamic example of homeostasis in photosynthesis is the regulation of cyclic to non-cyclic electron flow. When leaves absorb bright light with high energy blue wavelengths (~ 450 nm), the antenna complexes associated with PSII become phosphorylated. The addition of a phosphate to the antenna complex causes the stacked thylakoids to separate and the covalently modulated antenna complex moves closer to PSI electron transport chains which favors cyclic electron flow. When lower energy (~ 650 nm) red light predominates, the phosphates are removed from antenna complexes, the thylakoids form more stacks, and the captured light energy is more frequently delivered PSII and the non-cyclic electron flow. Therefore, dim light favors the production of NADPH. During the heat of the day when blue light would be most abundant, electrons tend to move in the cyclic pathway which reduces the need for water. However, biological systems are very rarely 100% of anything, so cyclic electron transport can happen when light is dim and non-cyclic electron flow is possible when the sun is brightest. It is important that you remember absolutes are very rare in the biological world.

You began this section learning that the European Union banned paraquat though it is used in over 100 countries including the United States. How does paraquat work and why is it toxic to humans (Figure 22.10). If you review the structure of paraquat, you will see it contains two positive charges which makes it very electronegative. Paraquat's structure allows it to bind very close to the electron binding site on the cytochrome complex of proteins and its strong electronegativity draws the electrons away from the cytochromes. Paraquat poisoning in humans most often happens through oral ingestion, but some people have died through skin exposure. Only 10 mL of a 20% paraquat solution is sufficient to kill an adult. Unlike plants, the mode of

toxicity is the production of oxygen radicals (O_2^-) which are highly reactive molecules. The European Public Health Alliance (http://www.epha.org/a/2757) wants all pesticides and herbicides to be evaluated for their toxicity to humans. Furthermore, they want animal tests to be used only as "a last resort". The company that produces and sells paraquat, Syngenta, has begun to redesign paraquat to reduce its toxicity in humans but the data on its success are not available when this book was written. Syngenta argues the public benefits from increased crop production when farmers use paraquat (http://paraquat.com/benefits).

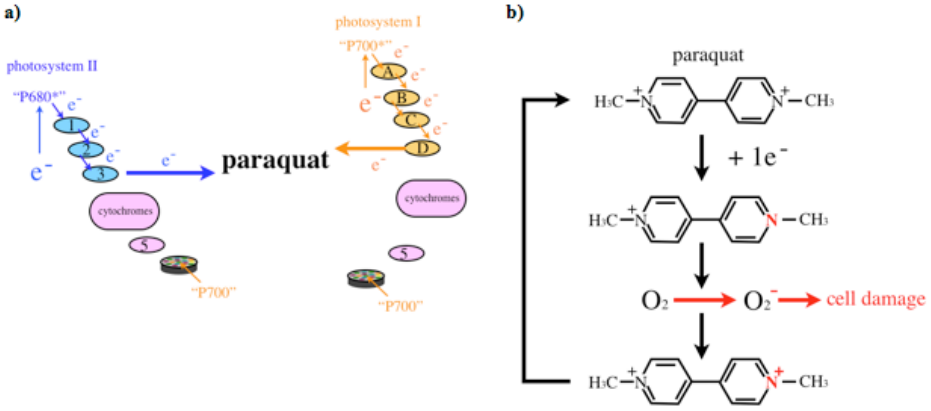


Figure 22.10 Effects of paraquat. (a) Paraquat binds near the cytochromes and diverts electrons towards itself and away from the biological flow of energy. (b) Reaction pathway of paraquat in human tissues.

Integrating Questions

19. What photosynthetic processes are blocked by paraquat? Why would this kill plants? How can paraquat harm humans since we don't photosynthesize?
20. The potential energy to perform work for 12 of H^+ accumulated inside the thylakoid space is about 200 kj/mole and a chloroplast produces about 3 moles of ATP for each 8 moles of photons absorbed and 2 moles of waters split. Given that the ΔG for ATP is 30.5 kJ/mole, what is the percent efficiency of photosyntheticly produced ATP compared to cellular respiration which averages about 35% efficiency?
21. How safe does a product need to be before it can be used? How much benefit to food production would be necessary to justify a health risk with low probability of direct harm? How do you define "last resort" for the use of animals in toxicity tests? If you survived paraquat poisoning, do you foresee any long-term health risks based on paraquat's mode of action in humans?

Plants are very efficient at converting the low pH inside thylakoids into ATP. The ΔG of 3 ATPs is 91.5 kJ/mole which means plants capture about 46% of the free energy available in the electrochemical gradient produced by photosynthetic accumulated protons. Animal cells are less efficient at converting their pH gradients into ATP which tells you that plants are models of efficient energy conversion. The amount of energy from the sun that reaches Earth in one hour is more than the amount of energy the world consumes in a year. You can search the Internet for the phrase "artificial photosynthesis" to learn how biochemists are trying to change how humans obtain energy to run our machines. The key step is mimicking the conversion of light into a electrochemical gradient and come close to nature's efficiency chemical storage of the trapped energy.

Paraquat kills plants by absorbing the electrons intended for the cytochromes in cyclic and non-cyclic electron transport pathways. When electrons are diverted from the cytochromes, H^+ ions do not accumulate in thylakoid spaces and ATP cannot be produced. Furthermore, NADPH

would not be produced since PSII would not be able to supply PSI with electrons for non-cyclic electron transport. Green plants cannot survive in the absence of photosynthesis. Treating plants with paraquat is functionally the same as trying to grow plants in total darkness. Paraquat accumulates in the endothelial cells of mammalian lungs. Parquat can absorb the electrons from the electron transport chain of cellular respiration and produce highly reactive oxygen radicals which destroy the structure and function of proteins, lipids and nucleic acids. Human death is the result of tissue damage in the lungs and subsequent loss of oxygen. If someone survived a sublethal dose, DNA damage by O_2^- might lead to long-term health problems such as cancer.

Everyone agrees paraquat is a very effective herbicide that kills all plants. Likewise, all interested parties agree that paraquat can kill humans if consumed. You are exposed to many substances that are considered both beneficial and toxic to humans. What is the best way to evaluate risks and benefits of chemicals designed to improve the quality of human life? Herbicides are not intended to be consumed by people, so should they be evaluated for their safety when accidentally ingested? How little exposure to paraquat is safe and how much exposure should we tolerate? Is animal testing appropriate to ensure human safety, or should animals be spared from the potential harm caused by deliberate exposure of potentially toxic compounds? Formulating public policy is a very difficult task but policy must be based on scientific understanding of biochemical processes.

Photosynthesis utilizes feedback mechanisms to regulate the ratio of cyclic and non-cyclic electron transport in chloroplasts. Bright light produces more ATP while dim light produces more NADPH plus ATP. The consumption of water to reduce P680 is also regulated by light as is the stacking of thylakoid membranes. Before ATP can be produced, electrons must be energized by the absorption of energy in photons. The movement of energized electrons generates a pH differential in chloroplasts that is the potential energy used to produce ATP. ATP production is a time-dependent process regulated by organelles inside plant cells. The extremely small space of the thylakoid lumen accelerates the rapid drop in pH which increases the potential energy of H^+ ions to diffuse unidirectionally towards the stroma. The compact nature of thylakoid membranes enables the antenna complex from PSII to quickly bump into PSI and minimize the down time of the light capturing pigments. Every activity of cells requires energy and thus the homeostasis of photosynthesis is essential to life. In the next section, you will learn how plants convert ATP and NADPH into sugars for use throughout the plant as well as sustaining all animal life.

22.2 How does Brazil's rain forest affect Greenland's glaciers?

- Context: Plants produce O_2 and consume CO_2 to produce sugars as precursors to proteins, carbohydrates and lipids.
- Major Themes: Biological systems utilize feedback mechanisms to regulate and maintain optimal conditions; time-dependent processes regulate biological systems; life requires organization which is energy dependent; and biological system's size and environment influences how it addresses physical and chemical challenges.
- Bottom Line: Plants convert the energy stored in ATP and NADPH to reduce carbon in CO_2 and produce multi-carbon molecules required for all plant and animal life.

Almost every week you hear news about global warming and atmospheric CO_2 levels. The

amount of CO_2 released into the atmosphere as a direct consequence of human activity has increased dramatically over the last 150 years (Figure 22.11a). Petroleum, coal and natural gas are the three leading sources of new CO_2 in the atmosphere. These three sources of CO_2 are called fossil fuels because they originate from organisms that died millions of years ago and due to pressure and chemical reactions, their bodies have produced a variety of hydrocarbons rich in covalent bonds. As human consumption of fossil fuels have increased, so have atmospheric concentration of CO_2, in parts per million (ppm; Figures 22.11b-c). The current level of atmospheric CO_2 is approaching 400 ppm which is nearly 50% higher than it was when America became a new country. Over the last 400,000 years, the level of CO_2 has fluctuated between 290 and 190 ppm (Figure 22.11d). Carbon dioxide levels have not reached 400 ppm since 13 million years ago (Figure 22.11e). Look at the seasonal fluctuations of atmospheric CO_2 in Hawaii (Figure 22.11f). CO_2 levels peak in May every year and are their lowest in September and October. You can go online and view the most recent month's CO_2 levels in Hawaii as well as CO_2 levels in the same month of the past two years.

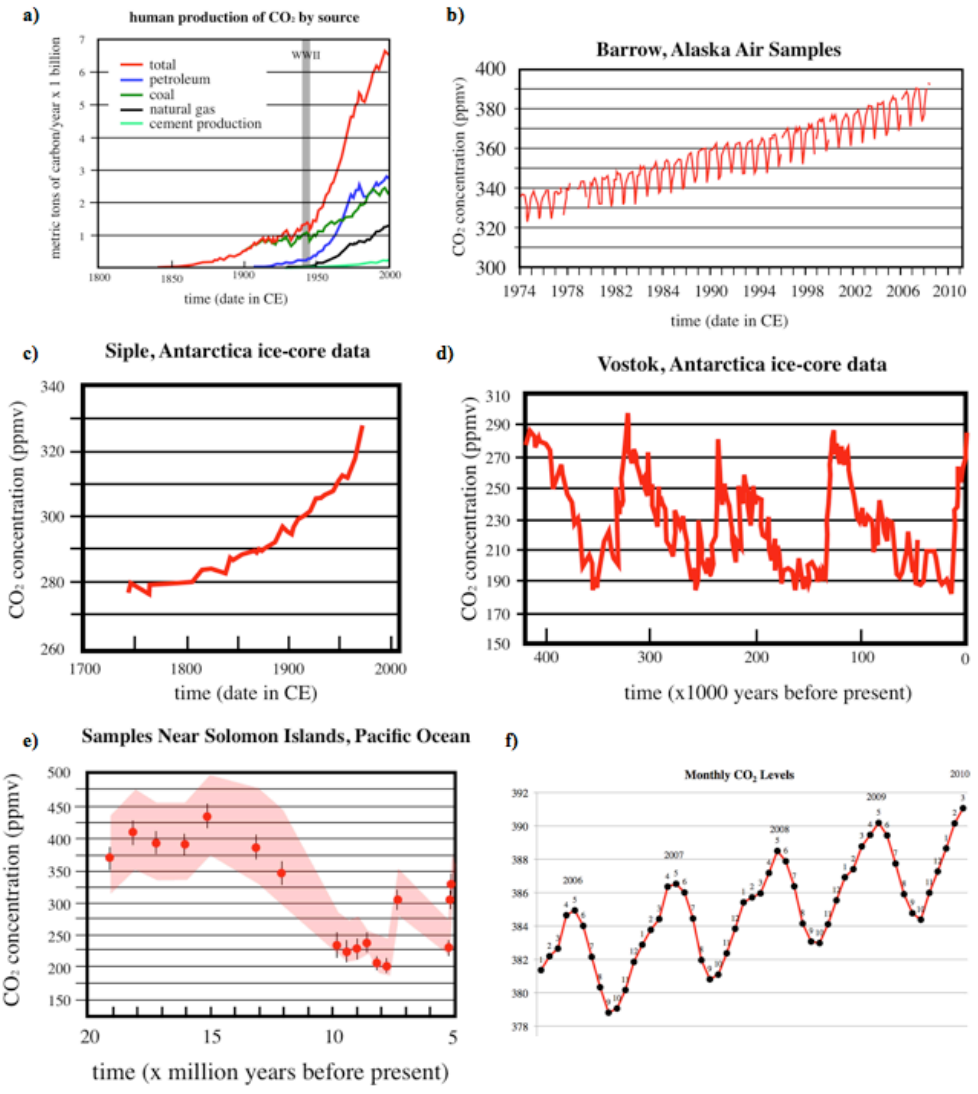


Figure 22.11 Energy consumption and CO_2 levels over time. (a) The total production of carbon dioxide and its sources over the last 200 years. The gray stripe denotes the five years of World War II. Manufacturing cement produces CO_2 as well. (b) The amount of CO_2 measured directly from air in Alaska. (c) CO_2 levels over the past 300 years measured from ice cores taken Antarctica. (d) CO_2 levels over the past 425,000 years measured from ice cores taken Antarctica. (e) CO_2 levels over the past 20 million years measured from ocean floor sediment in the South Pacific ocean. Error bars are +/- standard deviation and the red shaded area indicates the full range of calculated values. (f) Monthly CO_2 atmosphere levels taken from Mauna Loa, HI. Numbers indicate the month for each year, with 1 being January, etc.

When you consume food for energy, you produce CO_2 as a gaseous waste product. Burning fossil fuels for energy produces CO_2 as a gaseous waste product, too. In Chapter 25, you will learn about the connection between atmospheric CO_2 levels and global climate change. In this Section, you will learn how the rain forests in Brazil are connected to the glaciers of Greenland via carbon dioxide.

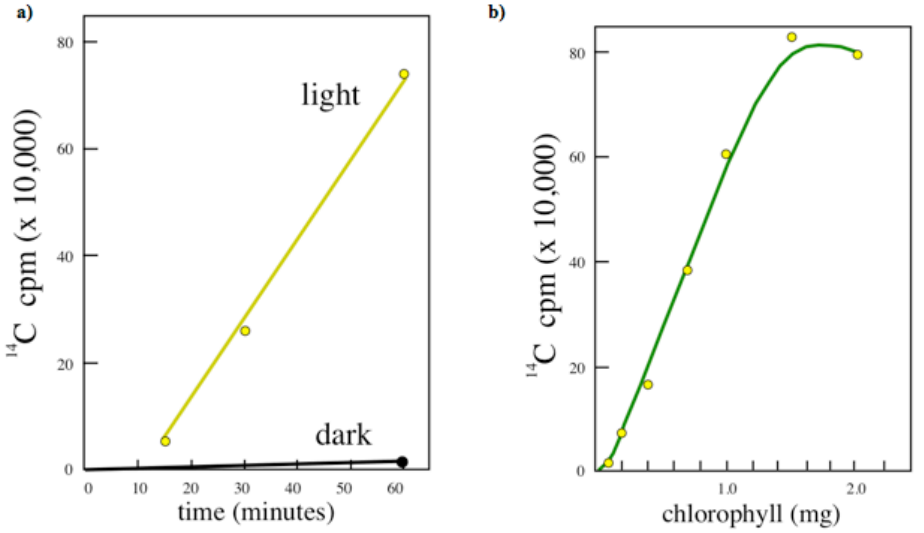


Figure 22.12 Carbon fixation capacity. (a) When exposed to light for various periods of time, chloroplasts incorporated radioactive CO_2 inside the organelles. (b) Investigators tested different concentrations of chloroplasts to determine the effect more chlorophyll has on carbon fixation.

In Figure 22.4, you saw that plants can fix carbon dioxide as a distinct biochemical process from splitting water and producing ATP. In 1955, M. B. Allen and his colleagues at UC Berkeley measured the rate of carbon dioxide fixation as a function of time and chloroplast concentration (Figure 22.12). Allen and his team isolated spinach chloroplasts and suspended them in a buffered solution. At time zero, they added radioactive $^{14}CO_2$, containing ^{14}C, to the chloroplast mixture and either exposed the organelles to light or not. After the indicated amount of time, they determined how much radioactive carbon was incorporated into carbohydrates by adding acid to the chloroplasts and allowing them to dry completely. Any remaining $^{14}CO_2$ gas would have diffused away leaving only radioactive carbon incorporated into larger sugars remaining for quantification. In the second experiment, the biochemists quantified the amount of radioactive carbohydrate produced by different concentrations of chloroplasts under constant illumination.

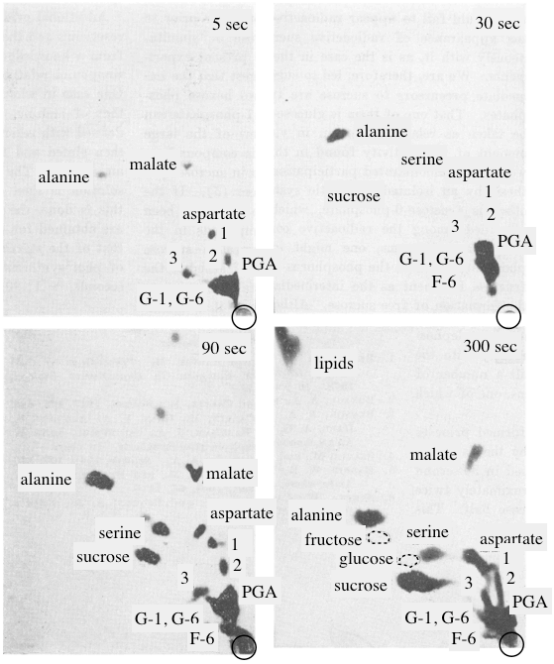


Figure 22.13 Incorporation of radioactive carbon into sugars and other molecules. Algae cells *Chorella pyrenoidosa* cells produce increasingly more complex organic molecules with longer exposure times to light and CO_2. Black circles in the bottom right corner indicate the location of the spotted materials placed on the TLC surface. Dashed lines denote the position of sugars that are not radioactive. PGA= phosphoglyceric acid; G-1 = glucose-1-phosphate; G-6 = glucose-6-phosphate; 1,2 and 3 are additional sugars.

During the 1940s and 1950s, UC Berkeley was home to some of the best photosynthesis research

in the world. Working with entire plant cells, a different pair of biochemists Melvin Calvin and Andrew Benson wanted to know how quickly the carbohydrates were produced by photosynthesis. The two biochemists exposed algae cells to radioactive $^{14}CO_2$ and light for different amounts of time and then used two-dimensional thin layer chromatography (2D TLC) to separate and identify which carbohydrates were radioactive (Figure 22.13). The investigators killed the algae cells rapidly by pouring hot ethanol onto them before harvesting the cellular contents for 2D TLC analysis. The four panels represent four separate experiments where the algae cells photosynthesized in the presence of light and CO_2 for the indicated times.

Integrating Questions

22. Hypothesize what causes the seasonal variation in CO_2 levels in Hawaii in Figure 22.11f. Based on your hypothesis, what would you expect to see in the Southern Hemisphere such as in New Zealand or South Africa?
23. Is carbon fixation by chloroplasts light dependent? Is the rate of carbon fixation constant over time, or did it show signs of stopping after an hour? What waste gas would you expect to be produced during carbon fixation? Was the amount of radioactive carbohydrate formation linear with increasing amounts of chloroplasts? Explain your answer using the data in Figure 22.12b.
24. What carbohydrate is the first molecule to acquire the carbon from CO_2? What other types of molecules incorporate the radioactive carbon besides sugars? Do you think the non-sugars are produced directly from $^{14}CO_2$, or are these other molecules assembled from the newly produced sugars?

The month-to-month variation of atmospheric CO_2 is caused by the seasonal variation in photosynthesis. Plants photosynthesize the least during the winter because some plants lose their leaves, the amount of sunshine is reduced, and the chemical reactions are slowed down by the cooler temperatures. When plants stop consuming CO_2, the gas accumulates more in the atmosphere until the new growth season begins in spring. In Hawaii, May begins the growth season but in the Southern hemisphere you would expect the peak CO_2 levels 6 months later, in October or November.

You know from Figure 22.12a that light is required for photosynthesis, including carbon fixation. From the data, it appears that plants can photosynthesize continuously as long as they have CO_2 and water to replace the electrons in oxidized reaction centers. If the electrons are replaced, then you would expect O_2 gas to be produced and the biochemists were able to measure a constant production of waste O_2 while the plants were fixing CO_2. However, the data in Figure 22.12b are not what you might have expected. You probably thought the amount of carbon fixation would scale proportionally with the amount of chlorophyll but after 1.5 mg, the level of carbon fixation does not increase. To understand this apparent paradox, think about a very crowded beach where more and more people congregate. At some point, the amount of sun any one person can receive begins to diminish simply because the people are casting shadows on each other. Similarly, if you put too many chloroplasts in a tube, some of them will get less light and the rate of carbon fixation will not increase any more. The data from Allen's research was the first time anyone had determined that all aspects of photosynthesis was contained in chloroplasts and not dependent upon other plant cell parts.

Calvin and Benson successfully measured the rate of carbohydrate production after very short exposures to light. With only 5 seconds of light, the algae cells converted CO_2 into the 3-carbon

sugar phosphoglycerate (**PGA**). A few other sugars are produced from PGA but the bulk of the radioactive carbon fixed in the first 5 seconds is PGA. With increasing amounts of time, more carbohydrates become radioactive as enzymes shuttled ^{14}C from PGA to other more complex molecules. Within five minutes, lipids with many carbons become radioactive as the plant's biochemical synthesis converts PGA into much larger molecules. {*Definition:* **PGA** is the abbreviation for the first sugar formed by photosynthetic carbon fixation.}

Calvin and Benson published many papers describing the biochemical pathway for carbon fixation until biologists clearly understood how plants produce their organic molecules from CO_2 - the waste product of cellular respiration (Figure 22.14a). Because of their critical discoveries, the cycle of carbon fixation is sometimes referred to as the Calvin, or Calvin and Benson, cycle. Plant cells contain many sugars, including a 5-carbon sugar ribulose that has two phosphates covalently linked to it on the number 1 and number 5 carbons (ribulose 1,5-bisphosphate). The most abundant enzyme in the entire world is **rubisco** that covalently links CO_2 and the doubly phosphorylated ribulose sugar. The newly formed 6-carbon sugar is unstable, and just as happened in glycolysis (see Figure 21.14), the sugar splits in half and forms two copies of PGA. {*Definition:* **Rubisco** is the enzyme that covalently attaches CO_2 onto ribulose bisphosphate that leads to the production of PGA.}

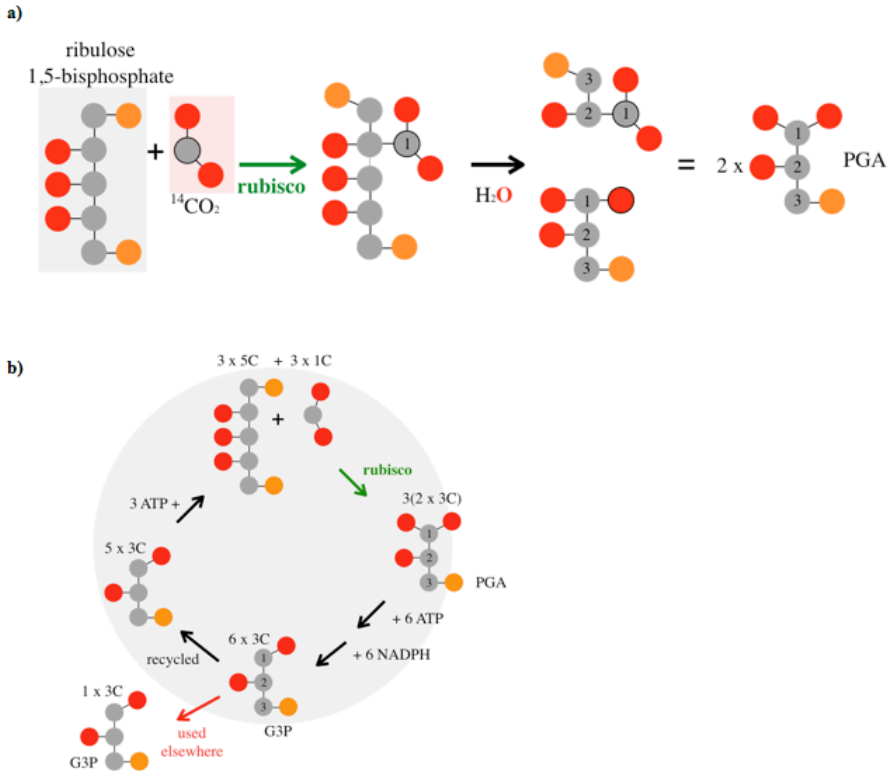


Figure 22.14 Cellular carbon fixation and carbon recycling. (a) In carbon fixation, the enzyme rubisco joins one CO_2 and ribulose bisphosphate to produce two copies of the 3-carbon sugar PGA. The outlined carbon represents the radioactive atom. The outlined oxygen came from the consumed water. (b) Three rounds of carbon fixation from panel a allows chloroplasts to recycle carbons and regenerate ribulose bisphosphate.

With insight similar to Krebs' who realized the metabolic pathway was circular, plant biochemists deduced that carbon fixation was cyclical as well (Figure 22.14b). You recall that 2 water molecules are split when 4 electrons are excited from the reaction centers. In carbon fixation, the critical number is three. When 3 carbon dioxide molecules are consumed by rubisco to form 6 PGA molecules, 2 PGAs for each CO_2, the carbon fixation cycle has accumulated enough new PGA to perform its recycling pathway. When 6 PGAs are produced, 6 ATPs and 6 NADPHs are consumed to convert PGA into G3P (glyceraldehyde 3-phosphate). At this step, the chloroplast has produced 6 G3P molecules containing a total of 18 carbons. Of these 18 carbons, 15 were

already in the 3 ribulose bisphosphate molecules and the other 3 carbons are from the consumed CO_2. If you removed one G3P molecule from the population of 6 G3Ps produced, you would have 5 G3Ps remaining which contain 15 carbons. An enzyme inside chloroplasts binds to 5 copies of G3P and 3 ATP molecules to produce 3 copies of ribulose 1,5-bisphosphate which brings the carbon fixation cycle back to where it began.

Integrating Questions

25. Do green plants consume CO_2 and produce waste O_2? Do green plant cells produce CO_2 and consume O_2? Do plant cells contain mitochondria, chloroplasts, or both?
26. Consult Figure 22.14a and determine to which carbon on ribulose does the carbon of CO_2 covalently attach? Other than CO_2, what is added to the ribulose bisphosphate sugar before producing 2 molecules of PGA? Where within chloroplasts would you expect rubisco to be located and for carbon fixation to take place?
27. What does the 3 tell you in the name G3P? What is the structural difference between PGA and G3P as shown in Figure 22.14b? Quantify the consumed energy in the form of ATP and the reducing agent NADPH. What is the ratio of ATP to NADPH consumed in the recycling process of carbon fixation? What does this ratio tell you about the proportion of cyclic to non-cyclic electron flow during photosynthesis?

A common misconception among biology students is that only plant cells photosynthesize and only animal cells perform cellular respiration. Both plant and animal cells contain mitochondria, so both types of eukaryotic cells perform cellular respiration. Although animal cells cannot photosynthesize, many prokaryote cells can photosynthesize even though they do not have chloroplasts. Prokaryote cells also conduct cellular respiration and they use their entire cells as the functional equivalent of mitochondria which is consistent with the bacterial origins of mitochondria (see Figures 21.25 and 22.8). {*Connections: You learned about photosynthetic prokaryotes in Chapter 7 when you studied coral bleaching. You studied the evolutionary origins of mitochondria in Chapter 6.*} Therefore, plant cells consume O_2 and produce waste CO_2 from their mitochondria at all times, while their chloroplasts consume CO_2 and produce waste O_2 when their chloroplasts are exposed to light.

Rubisco, the most abundant enzyme on the planet, produces a covalent bond between the carbon of CO_2 and the second carbon in ribulose bisphosphate. To facilitate the splitting of the 6-carbon sugar into 2 copies of PGA, water is consumed and its oxygen is covalently bound to the short-lived 6 carbon sugar immediately prior to the formation of 2 PGA molecules. The two H^+ ions accumulate in the stroma where rubisco is located and carbon fixation takes place. These H^+ ions are capable of being pumped into the thylakoid during the cyclic and non-cyclic electron pathways you learned about in Section 22.1. Rubisco's stromal location means that it functions where ATP and NADPH are formed, thus eliminating the need to transport these two substrates of carbon fixation.

A pair of enzymes convert PGA into G3P at the expense of ATP and NADPH. The 3 in G3P tells you that the phosphate is covalently attached to carbon number 3 in G3P, but the phosphate was attached to the same carbon in PGA. The structural change that necessitates a new chemical name for G3P is not the relocation of the phosphate, but the loss of an oxygen from carbon number 1 in PGA. You remember that the loss of oxygen is an indication of reduction which makes sense given NADPH is oxidized to $NADP^+$ when PGA is reduced to G3P. Reducing PGA to G3P is requires a lot of energy in the form of ATP and NADPH. The production of 3 ribulose

bisphosphate molecules from 5 G3P molecules consumes a total of 9 ATP and 6 NADPH. Because chloroplasts require 50% more ATP than NADPH, you should have predicted that the cyclic electron pathway of PSI occurs about 50% more often than the non-cyclic electron pathway of PSII and PSI. The non-cyclic pathway produces NADPH and ATP in a 1:1 ratio while the cyclic pathway produces only ATP. You learned in Figure 22.9b that bright light produces more cyclic electron flow and thus more ATP while dim light produces ATP and NADPH equally. The intensity of light influences the homeostatic regulation of carbon fixation by influencing the ratio of cyclic and non-cyclic electron pathways. The production of new PGA via the addition of a new carbon onto ribulose bisphosphate is the conversion of light energy into covalent bonds which is consistent with the first law of thermodynamics. Energy is not lost or gained during photosynthesis, only converted from one form to another. The reduction of entropy, or randomness, by fixing carbon dioxide is consistent with the second law of thermodynamics because within this system, more order is created with the input of energy.

You have learned how photosynthesis stores light energy as ATP and NADPH and how these two molecules are used in carbon fixation. However, this chapter focuses on homeostasis at the cellular level so you need to understand how the cell regulates the concentration of ATP and NADPH with the production of PGA after carbon fixation. Rubisco is perhaps the most important enzyme in the world since this protein is responsible for the fixation of carbon from CO_2 into usable sugars, amino acids and lipids. Therefore, plant cells must have a way to regulate rubisco's activity (Figure 22.15). Plant biochemists quantified the effect of pH on the rate of carbon fixation by rubisco. The investigators placed rubisco into buffers with pH ranging from 7, the approximate pH of the cytoplasm and stroma in the dark, to pH 10. You can see from the data that rubisco has a very clear pH optimum range for its activity. Plant physiologists knew from previous work that when photosynthesis reduces the pH of the thylakoid space, magnesium ions, Mg^{+2}, move from the thylakoid into the stroma as a result of the increased positive charge inside thylakoids. As a result, investigators wanted to measure the effects of both pH and Mg^{+2} concentration on the activity of rubisco (Figure 22.15b). The investigators prepared 3 concentrations of Mg^{2+} ions in buffers ranging from pH 7 to pH 9.5. You can see from the data that pH and Mg^{2+} concentration affect the activity of rubisco. Plant physiologists determined the Mg^{2+} concentration of stroma during photosynthesis is about 5 mM compared to about 1 mM in the dark. You should not be surprised to learn that the other enzymes involved in carbon fixation/recycling have similar pH requirements for maximum activity.

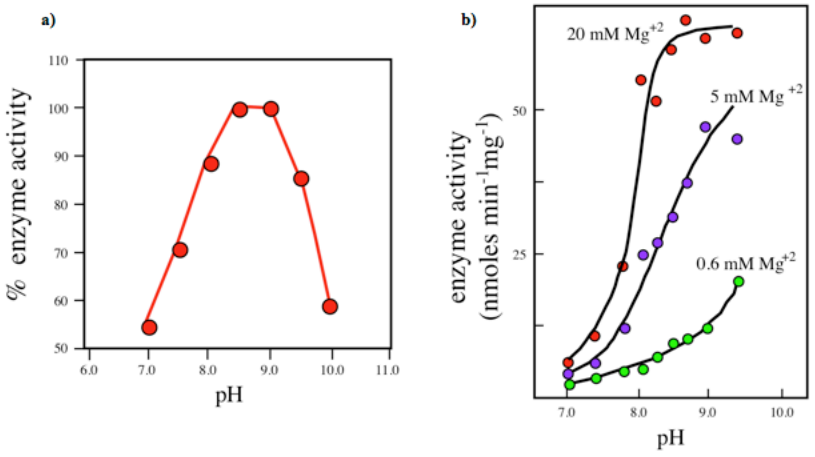


Figure 22.15 Physiological regulation of rubisco. (a) Rubisco activity is sensitive to pH. (b) Rubisco activity is sensitive to Mg^{+2} concentration and pH.

Integrating Questions

28. What is the optimum pH range in Figure 22.15a for rubisco activity and carbon recycling? What is the pH of stroma when chloroplasts are kept in the dark? Look at Figure 22.5 to

remind yourself the pH of stroma and thylakoid spaces when chloropasts are illuminated and punctured with pH detectors. Is rubisco's location appropriate for fixing carbon during the day when photosynthesis produces the pH gradient?

29. Does rubisco increase or decrease its activity when the stroma is flooded with Mg^{+2} ions from the thylakoid space? What is the difference in rubisco activity at 0.6 mM vs. 5 mM Mg^{+2} when the pH is 7, 8 and 9? What is the approximate pH of stroma for intact chloroplasts?

The pH optimum of rubisco is between 8.5 and 9 which is the same pH as the stroma of intact chloroplasts while photosynthesizing. When dark, the stroma returns to 7 – 7.5 based on the data for experimentally altered chloroplasts in Figure 22.5. Therefore, the pH of the stroma, where rubisco is located, helps regulate the carbon fixing enzyme. Based on the data in Figure 22.15b, you can see that elevated Mg^{+2} enhances the activity of rubisco in addition to elevated pH. The difference in activity between 5 and 0.6 mM Mg^{+2} changes with the pH. At pH 7, the difference in activity is about 1%, but at pH 8, the difference is about 20%. At pH 9, about the pH of stroma during photosynthesis, the difference is about 38%. Therefore, during photosynthesis, the stroma's ionic composition is ideal for increasing the activity of rubisco for both H^+ and Mg^{+2} ions.

This Section began with the question asking how rainforests are connected to Greenland glaciers. Green plants in the rainforest fix carbon during the spring and summer months which reduces the global levels of atmospheric CO_2. CO_2 is a greenhouse gas, meaning it traps heat and contributes to the elevation of temperatures on earth which causes the glaciers of Greenland to melt faster than they accumulate during the Northern hemisphere winter. You will learn more about the environmental effects of CO_2 and greenhouse gases in Chapter 25, but for now, you can see how photosynthetic plants that fix carbon and atmospheric CO_2 levels are intimately connected. The term photosynthesis makes more sense now that you understand light (photo-) leads to the building (-synthesis) of new carbohydrates.

Biological systems like plant cells use feedback mechanisms to regulate carbon fixation. Mg^{+2} and pH regulate rubisco as a consequence of light-induced changes in the stroma to maintain an appropriate balance of rubisco activity. Rubisco consumes CO_2 and the harvested light energy is converted to covalent bonds in ATP and NADPH. The timing of carbon fixation is carefully regulated by the activation of rubisco and its time-sensitive optimal reaction conditions. Rubisco works best when plants are making ATP. The size and location of thylakoid membranes and the surrounding stroma minimizes the energy needed to collect the substrates for carbon fixation. Photosynthesis, like all of life, requires organization and energy. Plants make larger molecules by reducing CO_2 and producing carbohydrates such as PGA that can be converted into all the building blocks of life – proteins, lipids and carbohydrates. Animals depend on plants for carbon sources but plants do not need animals to live. Without plants, animals would die, but would all life cease to exist? In the next Section, you will read about some unusual forms of microbial life whose diets defy traditional definitions of food.

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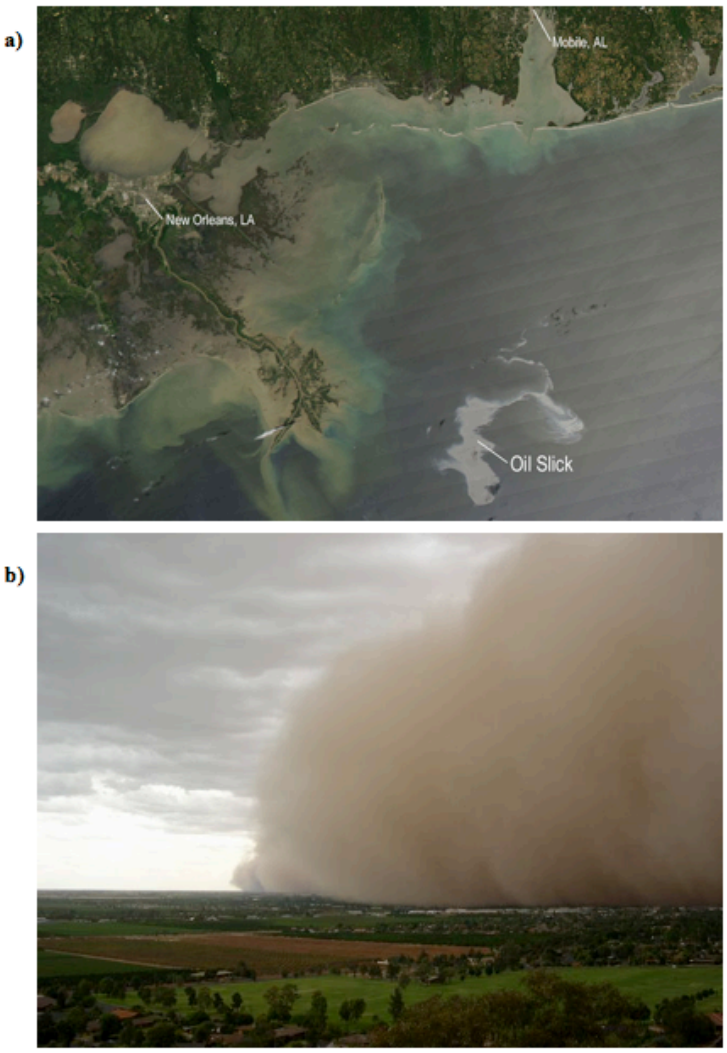
ELSI Box 22.1 *How do you compromise when a policy hurts one country but helps another?*

Every country on the planet has laws and policies that were produced to help someone. Countries regulate how much foreign currency can be brought into their economies, they regulate import and export rates and tariffs, and they regulate who can enter or leave the country. Europe

banned the use of paraquat but a majority of the world still permits its use. However, some policies intended for one nation wind up affecting others. For example, if the United States passed a law that permitted US farmers to remove all the water from rivers that flow through their states, Texans in Laredo might be fine, but their Mexican counterparts on the other side of the Rio Grande in Nuevo Laredo might suffer from the lack of water.

When it comes to homeostasis, Earth is one giant ecosystem that maintains a delicate balance defined by geology rather than politics. Wind, water, weather, animals, plants, pathogens… all natural resources are part of a network that connects every country to one another. In the spring of 2010, you may remember two global reminders of how closely connected everyone is. In Iceland, a volcano erupted under a glacier which sent steam and ash high into the atmosphere. The jet stream carried the ash over northern Europe which closed airports in at least a dozen countries.

Volcanoes are not regulated by governments, but oil reserves are. Closer to home, British Petroleum was pumping oil from one mile below the ocean surface when something went wrong. On the night of April 20, 2010, an explosion followed by a fire left the drilling platform sinking in the water and three large holes in the pipeline that used to deliver oil (ELSI Figure 22.1a). An estimated 180 million gallons of petroleum oil gushed into the water for five months and currents carried the pollution eastward towards many independent nations. The United States has a national energy policy that permits drilling in some locations but not others. Some politicians chanted "Drill baby drill," while others in the same political party call for a moratorium in waters near their homes. Policies also govern safety procedures and frequencies of inspections. The oil was being extracted by a British company, from an area claimed by the US but the oil spread east and affected fishing and tourism in many countries.



ELSI Figure 22.1 National policies can affect many nations. (a) NASA satellite image of the 2010 Gulf of Mexico oil spill. (b) Dust storms can blow from one country to another.

A more widespread problem is erosion caused by farming practices and water use (ELSI Figure 22.1b). Sand storms in China move east and affect North and South Korea. Dust carried from the Sahara Desert blows over the Pacific Ocean and affects the weather in North America

by altering weather patterns influenced by *el Niño*. Sand storms are caused by poor farming practices. When people cut down forests and follow bad agricultural practices, wind storms can lift hundreds of tons of dirt into the air, carrying away valuable top soil. Prevailing winds carry the dust great distances and can influence other countries that try to minimize wind erosion of soil.

The cost-benefit analysis of national polices are difficult when you only consider one country's perspective. But countries have neighbors, and neighbors sometimes have competing interests. Should an herbicide be legal or illegal everywhere, or is it OK for neighboring countries to disagree on its safety? If paraquat fumes from one farm drift over to another country, should the farmer be punished by another country's laws? Because all ecosystems are interconnected, it is nearly impossible to craft a law in one country that does not produce consequences in another country. Everyone agrees that paraquat can be lethal to humans, but governments disagree on whether it should be used or not. What should be done to balance competing interest?

End of ELSI 22.1

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End Of Chapter Review Material

Review Questions

1. Relate paraquat's structure to its toxic function in plants.
2. Compare the tissue accumulation after consumption of paraquat in lungs vs. blood.
3. Explain the historical experiments that revealed oxygen is produced as a byproduct of photosynthesis.
4. Discuss the structure function relationships that explain how plants capture different colors of light and shuttle the energy to a reaction center within a chloroplast.
5. List the three distinct parts of photosynthesis and summarize the overall chemical processes.
6. Illustrate the connection between light and thylakoid pH.
7. Diagram the flow of energy in cyclic and non-cyclic electron flow of photosynthesis.
8. Compare the similarities between bacteria, mitochondria and chloroplasts with regards to pH driven ATP synthesis.
9. Review the homeostatic aspect of water splitting during photosynthesis.
10. Restate the homeostatic balance between cyclic and non-cyclic electron flow within plant chloroplasts.
11. Sketch the change over time of carbon dioxide in the atmosphere as known for different time scales.
12. Arrange a general hierarchy showing the progression of carbon polymer synthesis.
13. State the general steps of carbon fixation during photosynthesis.
14. Indicate how photosynthesis is regulated by the same pH gradient generated during photosynthesis.
15. List some extreme environments where microbes live and extract energy.
16. Summarize the evidence supporting the claim that some of the earliest microbes to evolve ate rocks for energy.
17. Characterize the vertical profile of microbes living in the sediment under oceans.
18. Construct a progression of information that led to the discovery of photosynthesis at thermal vents.
19. Review the evidence that supports the claim that photosynthesis first evolved near thermal vents and not on land.
20. Categorize the adaptations halophiles exhibit that allows them to live in very high salt environments.
21. Formulate an explanation that addresses the genomic data for the cyanobacterium that has lost many genes involved in cellular respiration.
22. Recall examples of environmental polices that affect more than one country. You may use the Internet to find current examples.

Apply What you Know

1. One level of homeostasis concerns gene activation and repression. Quickly read the abstract to this freely available journal article (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC43748/?tool=pubmed) that describes the evolutionary origins of a transcription factor. Did eukaryotic transcription evolve from bacteria or archaea? Now read this abstract to another freely available journal article (http://mbe.oxfordjournals.org/cgi/reprint/24/4/893). Is the timing of translation in archaeal cells more similar to bacteria or eukaryotes?

2. Search the internet for diabetes mellitus. There two main types of diabetes. Describe each type and explain why this disease is essentially a disease of homeostasis.
3. In a forest, some plants tower above the others and the tallest plants get the most sunlight. If energy from the sun is a limiting factor, then how can some plants survive if they live in the shade? Based on what you know about homeostatic regulation of photosynthesis, speculate how shade-loving plants can survive.
4. Read this news story at National Geographic's web site (http://news.nationalgeographic.com/news/2005/02/0203_050203_deepest.html) about an amazing discovery at the bottom of the ocean. Formanifera are unicellular eukaryotes, sometimes referred to as protists. What physical characteristic must these cells contend with in a homeostatic capacity? You can see photos of these organisms at WileyPlus (see 2005 paper by Gooday et al. *Zoological Journal of the Linnean Society*, 2008, **153**, 399–423. I have put it on our FTP site in Chapter 22 folder.)

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