The energy needed for life on our planet originates with the sun. As we have discussed, living organisms require a source of organic fuel molecules to provide energy for cell functioning. Organisms that can use energy from the sun and carbon from their physical surroundings to produce their own organic molecules, including fuel molecules, are autotrophs. The majority of autotrophs produce their organic molecules by the process of photosynthesis. Photosynthesis transforms light energy into chemical energy, and uses that chemical energy to produce organic molecules, typically glucose, from water and carbon dioxide.

Most photosynthetic organisms (or photoautotrophs) are plants or protists that contain chlorophyll. Many prokaryotes are also photosynthetic. The cyanobacteria have chlorophyll pigments. Some bacteria, such as the purple sulfur bacteria, have different light-capturing pigments and photosynthetic mechanisms. They are studied in microbiology. Recall that heterotrophs are organisms that obtain their organic fuel molecules pre-formed from the environment. Animals, fungi, many protists and many bacteria are heterotrophs.

Not all autotrophs are photosynthetic; a tiny proportion of living organisms, the chemoautotrophs, manufacture organic fuel molecules by chemosynthesis. Chemosynthetic autotrophs (or chemoautotrophs) use energy from chemical reactions involving inorganic atoms and molecules, such as S, Fe, H and N, to make organic compounds. Chemosynthesis sustains some deep seabed ecosystems that surround sulfur vents. To be complete, some green and purple non-sulfur bacteria are photoheterotrophs, using light to reduce organic compounds. Bacterial energy and carbon source versatility are discussed in microbiology.

The products of photosynthesis, along with a number of inorganic atoms and molecules, are the basis for the biological molecules used for structure and metabolism for all living organisms as well as the fuel molecules we use in cell respiration.

The process of photosynthesis also produces oxygen gas as a "by-product", the very same molecule that is used in aerobic cell respiration, without which most organisms on earth would not survive.

Photosynthesis produces about 160 billion metric tons of carbohydrate annually, an amount most of us can't even imagine. Plants being efficient organisms use less than they produce and we depend on the plants "leftovers" for survival. This dependence, for humans, is greater than just obtaining food directly from an autotroph or processed through the food chain by a series of heterotrophs, many of which use tremendous amounts of photosynthetic output to make those conversions. Our lifestyles depend on products of or past products of photosynthesis, too. As you read and learn the photosynthetic pathways, consider that you would not be here without it!
Photosynthesis - 2

The Process of Photosynthesis
As stated, photosynthesis involves the transformation of light energy to chemical energy. The chemical energy is then used to manufacture carbohydrate molecules, primarily glucose. In eukaryotic organisms, and in the Cyanobacteria, the process of photosynthesis also produces oxygen. The photosynthetic bacteria produce organic carbon molecules, but do not produce oxygen. We will focus on plants and photosynthesis in our discussions.

Photosynthesis occurs in all parts of plants that contain the green pigment, chlorophyll, which is located on the thylakoid membranes of chloroplasts. In most plants, however, chloroplasts are concentrated in leaves so most photosynthesis occurs in leaves. In the laboratory, we shall look closely at the leaf structure as it relates to its function in photosynthesis.

Photosynthesis involves two stages or pathways, occurring in separate locations within chloroplasts. In the light-dependent reactions of photosynthesis, light energy is transformed into chemical energy that is "trapped" by energy transfer molecules. A series of re-dox reactions transfers electrons and hydrogen from water to the energy transfer molecule NADP⁺ to form NADPH. (Recall that a number of metabolic processes, including cell respiration, involve oxidation-reductions with electrons being "carried" along a gradient of electron transfer molecules.) The light-dependent reactions are known as photophosphorylations, because they also involve producing ATP. The light-dependent reactions of photosynthesis take place on the thylakoid membranes of the grana.

In the Calvin cycle (sometimes called the light-independent reactions or sometimes the Calvin – Benson cycle or the carbon-fixation reactions), the energy-rich products of the light reactions, ATP and NADPH, are used to manufacture carbohydrate molecules, some of which form glucose. The Calvin cycle first "fixes" CO₂ (carbon fixation) and then uses NADPH to reduce the "fixed" carbon to carbohydrate molecules. These endergonic (ATP consuming) reactions occur in the stroma of the chloroplast. The two photosynthetic pathways are linked by the products of the light-dependent reactions.
The overall chemical equation for photosynthesis* is:

\[
\begin{align*}
\text{Chlorophyll} & \quad 6\text{CO}_2 + 12\text{H}_2\text{O} + \text{light energy} \rightarrow \text{C}_6\text{H}_12\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2 \\
\text{Chlorophyll} & \quad \text{Carbon dioxide + water + light energy} \rightarrow \text{glucose + water + oxygen}
\end{align*}
\]

* The process of photosynthesis directly produces 12 molecules of the 3-carbon compound, glyceraldehyde-3-phosphate (G3P). Two of these molecules can be metabolized to glucose during a "complete" photosynthesis. The remaining 10 molecules of G3P are recycled in the photosynthetic process.

**Photosynthetic Requirements**

In order to do photosynthesis, \( \text{H}_2\text{O}, \text{CO}_2, \) chlorophyll and light energy must be available (as well as the appropriate staging, i.e., the chloroplast). We shall briefly discuss each of these requirements before discussing how photosynthesis is accomplished.

1. **Water (\( \text{H}_2\text{O} \))**

   Water is the hydrogen and electron donor for the process of photosynthesis. Light energy is used to split water molecules, forming \( 2\text{H}^+ \), \( 2\text{e}^- \) and Oxygen (\( \text{O}_2 \)) during the process of photosynthesis, as proposed by C.B. van Neil in the 1930's and demonstrated using radioactive oxygen isotopes in \( \text{H}_2\text{O} \) and \( \text{CO}_2 \) in separate experiments in 1941 by Ruben and Kamen. Only the radioactive \( \text{H}_2\text{O} \) resulted in radioactive \( \text{O}_2 \) production.

   Water is obtained from the environment, absorbed by roots and conducted throughout the plant in the **xylem** tissue of the vascular system. Water needed for photosynthesis is but one of the demands for water in plants. *(How water moves within plants is discussed in Biology 213.)*
2. **Carbon Dioxide (CO₂)**
Carbon dioxide provides the carbon source for manufacturing the carbohydrates in photosynthesis. Carbon dioxide (CO₂) diffuses from the atmosphere through pores in leaf surfaces called **stomata**, which are formed by a pair of guard cells. CO₂ then diffuses to the photosynthetic cells of the **leaf mesophyll**. The rate of diffusion of carbon dioxide and availability of carbon dioxide often limit the rate of photosynthesis. *(The mechanisms of stomatal operation are discussed in Biology 213, although we will observe stomata in the laboratory.)*

3. **Chloroplasts**
The pigments needed for photosynthesis are located in the chloroplasts. Recall that the chloroplast has a double-layered outer membrane with a series of internal membranes, the **thylakoids**. The thylakoids are folded into disk-shaped stacks called **grana**. Light energy is captured by pigments located on the thylakoid membranes. The interior compartments of the thylakoids serve as reservoirs for **hydrogen ions** (H⁺) that are needed for producing ATP.

![ Chloroplast Diagram ]

The reactions of photosynthesis that are involved in the transformation of light energy to chemical energy, the **light-dependent reactions**, occur on the thylakoid membranes of the chloroplast.

The **Calvin cycle** reactions needed to produce carbohydrates occur in the **stroma** region of the chloroplast. Enzymes needed for the Calvin cycle are found in the stroma.

4. **Light and Photosynthesis**
Light is a form of electromagnetic radiation. Visible light is a combination of many wavelengths that we can see as different colors (of the rainbow) in the range of 380 - 750 nm. Each wavelength is associated with a specific photon, or particle of energy. A photon of a shorter wavelength has more energy than a photon of longer wavelengths.

The light absorbing photosynthetic pigments do not absorb all wavelengths of light equally. Some light energy cannot be absorbed (and is reflected instead) and some is transmitted, or passed through the chloroplasts. To be useful, a photon must be absorbed and have sufficient energy to do the work required.
The absorption of different wavelengths, or absorption spectrum, of light by the photosynthetic pigments can be demonstrated in the laboratory using spectrophotometers. The light waves most absorbed and most useful to photosynthesis are reds and blues. Not surprisingly, green light is absorbed poorly. One can also measure rates of photosynthesis in different wavelengths to generate an action spectrum. This is done by growing plants in light boxes exposed to just one wavelength of light.

Engelmann’s Action Spectrum of Spirogyra and Bacteria attracted to the Oxygen evolved.
5. **Light Absorbing Pigments of Photosynthesis**

**Chlorophyll** is the primary pigment that absorbs light energy in photosynthesis. In plants, there are two forms of chlorophyll (\(a\), which has a methyl group, and \(b\), which has an aldehyde group). There are additional chlorophyll forms in some protists and in the cyanobacteria. There are also important **accessory pigments**, the carotenes, and in some protists, phycobilins, in chloroplasts. Each pigment absorbs and concentrates light energy for the photosynthetic process.

In addition, the carotenes may function to protect chlorophyll molecules from being damaged by intense light. The red and blue phycocyanin pigments also absorb light and serve as accessory pigments in photosynthesis.

When pigment molecules absorb photons of energy from light, electrons in the molecules are excited and move to a higher energy orbital in an excited state. The energy level of a photon absorbed and the rise in energy level to the higher energy orbital must match, which is why only certain wavelengths can be absorbed by certain pigments. This rise in energy is temporary. Electrons fall back to their ground state if the energy is not transferred elsewhere. When the electrons fall back to their ground state energy is given off as heat, or sometimes as light (fluorescence). Chlorophyll \(a\) does both, fluorescing as red light.
The Photosystems
The photosynthetic pigment molecules do not work alone. They are arranged on the thylakoids of the chloroplast in clusters of about 300 pigment molecules, mostly chlorophyll a, within a protein matrix to form a light harvesting or antenna complex (system). The pigment complex gathers and transfers energy to a photochemical reaction center, embedded in a transmembrane protein complex. As the photon energy is transferred from pigment molecules that absorb from shorter to longer wavelengths. The reaction center has a special pair of chlorophyll a molecules that absorb longer wavelengths, as well as the primary electron acceptor (which accepts the electrons released from chlorophyll a). There are two different types of pigment complexes found in the chloroplasts, called Photosystem I and Photosystem II. Each has a unique electron acceptor and a different geometry for the pigment molecules.

The reaction centers of Photosystems I and II also have different absorption peaks. The reaction center in Photosystem I absorbs maximum light at 700nm. The reaction center of Photosystem II absorbs maximum light at 680nm. Each thylakoid has thousands of the two different photosystems. Both are needed for photosynthesis. The two photosystems working in tandem, with each absorbing different wavelengths, increases the rate of photosynthesis.

The first step of photosynthesis occurs in the photosystem reaction centers. When the pair of chlorophyll a molecules in the reaction center absorb sufficient energy, an excited electron moves to an outer energy level. Chlorophyll is oxidized and the primary electron acceptor within the reaction center captures the electrons getting reduced. It is only within the reaction center of intact chloroplasts that the oxidized electrons are captured. Isolated chlorophyll molecules absorb light energy, oxidize, but the excess energy is lost to heat and fluorescence, and the electrons fall back to their "unexcited" state within the chlorophyll a molecule.
6. **Electron Transport System Molecules** (Energy Transfer Molecules)

As discussed previously, many chemical reactions of metabolism are coupled oxidation-reductions that utilize a chain of electron transport molecules that specialize in oxidizing and reducing at specific energy levels to minimize energy loss in energy transfers.

In the process of photosynthesis, the electron transport carriers are embedded in the thylakoid membranes. The electrons oxidized from chlorophyll are the source of the initial reduction in the electron transport chain. The downhill energy gradient of electrons passed through the electron transport chain is used to move $H^+$ from the stroma of the chloroplast through the thylakoid membranes into the inner thylakoid compartment. The accumulated $H^+$ proton motive force is used to generate ATP by chemiosmosis.

Molecules that specialize in energy transfers in the two photosystems (I and II) in the thylakoids are: NADP+, plastoquinone, two cytochromes, plastocyanin and ferredoxin.
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The Photosynthesis Pathways

Light (Dependent) Reactions
- The light-dependent reactions transform light energy into chemical energy that is trapped and carried by ATP and NADPH to the Calvin Cycle.
- The light-dependent reactions require chlorophyll and occur in the thylakoid membranes of the grana of the chloroplast.
- During the light-dependent reactions water is split in an enzyme-mediated reaction located in photosystem II into:
  \[ \text{H}_2\text{O} \rightarrow 2\text{H}^+ + 2\text{e}^- + \frac{1}{2}\text{O}_2 \]
  Splitting water produces oxygen and provides electrons that are transferred to photosystem II and ultimately used for the reduction of NADP⁺, along with the H⁺, to NADPH. NADP⁺ gains H⁺ and electrons; water is oxidized because it loses the H⁺ and 2e⁻.

There are two electron pathways in the light-dependent reactions: **Noncyclic electron transport** and **cyclic electron transport**. We will discuss both of these processes. (These processes are also called Noncyclic Photophosphorylation and cyclic photophosphorylation in case you want more names. And sometimes they use "flow" rather than "transport", or "linear" instead of "noncyclic").

Noncyclic Electron Transport
Noncyclic electron transport uses photosystem I, photosystem II and the electron transport system.

The **inputs** for noncyclic electron transport are:
- Water
- Light energy
- ADP and Pᵢ
- NADP⁺

The **outputs** for noncyclic electron transport are:
- ATP
- NADPH (reduced form) (from NADP⁺, the oxidized form)
- O₂
We often diagram the light reactions to show the relative energy levels of the electrons as they move throughout the pathway, even though the molecules of photosystems I and II and the electron transfer molecules are embedded within the thylakoid membranes. This is sometimes called the "Z scheme".

Noncyclic Electron Transport Details
- Light energy hitting pigment molecules in photosystem II is ultimately transferred to the chlorophyll a molecules in the $P_{680}$ reaction center causing the two now excited $P_{680}$ chlorophyll a molecules to lose an electron to the primary electron acceptor, leaving very unstable oxidized chlorophyll a.
- The now oxidized chlorophyll a molecules in photosystem II need to replace their electrons. This is done by splitting (oxidizing) water within photosystem II. $P_{680}$ chlorophyll a potent oxidizing agent, forces the oxidation. Water’s electrons are passed to the oxidized chlorophyll a molecules in photosystem II’s reaction center.
  - The water’s $H^+$ becomes part of the chloroplast pool of $H^+$ to be used for the $H^+$ gradient or to be passed to NADP$^+$ (see later).
  - The oxygen is released as oxygen gas molecules ($O_2$).
- The electrons from the primary electron acceptor in the reaction center are now passed to a set of electron transport molecules (plastoquinone, a cytochrome complex and plastocyanin) move “down” the chain by oxidation/reductions releasing energy. Some of this energy is used to actively transport $H^+$ from the stroma into the thylakoid compartment generating a $H^+$ gradient within the thylakoid. This gradient drives the ATP synthesis by chemiosmosis (see later).
- Light energy also hits photosystem I, and is ultimately transferred to the chlorophyll a molecules in the $P_{700}$ reaction center causing the now excited chlorophyll a molecules to lose an electron to its primary electron acceptor. The electrons are transferred to ferredoxin and then to NADP$^+$, which will pick up two $H^+$ forming NADPH + $H^+$. (We often assume the extra $H^+$ and just write NADPH)
- The electrons released from photosystem II, which actually originated in $H_2O$, (see above), once they have passed through the electron transport system, are used to replace the electrons lost by chlorophyll a in photosystem I.
To summarize, the low energy electrons that originated from water are elevated in energy by passing through both photosystem II and photosystem I, then trapped by NADP⁺ where their potential energy will be used for the high-energy reduction of carbon in the Calvin cycle. Along the way, ATP, needed for the endergonic Calvin cycle is produced by chemiosmosis, to be discussed soon.

As mentioned, noncyclic electron transport is sometimes referred to as the "Z" scheme, because the electrons do an energy "zig-zag" as they move from water to photosystem II to photosystem I to NADP⁺. Using both photosystems has an enhancement effect so that the overall rate and efficiency of light capture is higher than if there were just one photosystem or if the two photosystems worked independently of each other.

Cyclic Electron Transport (Cyclic Photophosphorylation)
Cyclic electron transport uses photosystem I and the electron transport system, and produces only ATP. Cyclic electron transport dates to the earliest photosynthetic bacteria as a mechanism to generate ATP, but not to facilitate the synthesis of organic fuel molecules. The electrons captured were not used to reduce carbon or an intermediate molecule. In plants, cyclic electron transport still functions to generate ATP as a complement to noncyclic electron transport. There is some evidence that it also protects plants from light damage.

The inputs for cyclic electron transport are:
- Light energy
- ADP and Pᵢ

The output for cyclic electron transport is:
- ATP

Cyclic electron transport only uses Photosystem I and produces only ATP. However the Calvin cycle requires more ATP than NADPH, so both cyclic and noncyclic electron transport ATP is available for the reactions of the Calvin cycle.

In cyclic electron transport, electrons released from chlorophyll a P₇₀₀ are returned back to Photosystem I after passing through the chain of electron transport molecules (chlorophyll a P₇₀₀ → primary electron accepter → ferredoxin → plastoquinone → plastocyanin → cytochromes → chlorophyll a P₇₀₀) ATP is produced by chemiosmosis.
Comparing Noncyclic and Cyclic Electron Flow

<table>
<thead>
<tr>
<th>Ultimate electron source</th>
<th>Noncyclic photophosphorylation</th>
<th>Cyclic photophosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen released?</td>
<td>Yes (from H₂O)</td>
<td>None</td>
</tr>
<tr>
<td>Terminal electron acceptor</td>
<td>NADP⁺</td>
<td>None</td>
</tr>
<tr>
<td>Form in which energy is temporarily captured</td>
<td>ATP (by chemiosmosis); NADPH</td>
<td>ATP (by chemiosmosis)</td>
</tr>
<tr>
<td>Photosystem(s) required</td>
<td>I &amp; II</td>
<td>I only</td>
</tr>
</tbody>
</table>

Chemiosmotic ATP Synthesis in the Chloroplast: Photophosphorylation
The electron transport systems are located in the thylakoid membrane. Energy released from reducing ferredoxin and plastoquinone in the electron transport system is used to move Hydrogen ions (H⁺) from the stroma into the inner thylakoid compartments. This concentration of H⁺ in the inner compartment of the thylakoid establishes a concentration, pH and electrical gradient (the proton-motive force) in the thylakoid compartment that has an inherent (potential) energy value. This is not insignificant. The pH gradient goes from pH 5 within the thylakoid membrane to pH 8 in the stroma (a 1000X difference).

The accumulated H⁺ ions diffuse through the ATP synthase protein complex channels in the thylakoid membranes that are coupled to ATP synthesis. As the H⁺ ions flow down the gradient in the protein channels, their energy is used to make ATP from ADP and P₀ on the other side of the thylakoid membrane in the stroma, a process called chemiosmosis, or photophosphorylation because the originating source of energy is light.

Peter Mitchell won the 1978 Nobel Prize in chemistry "for his contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory". ATP is synthesized in the mitochondria during cell respiration (called oxidative photophosphorylation because the originating energy is the oxidation of organic carbon molecules) by a similar mechanism. It is of interest to note that chloroplast ATP synthase and human mitochondrial ATP synthase are 60% identical in composition, an example of the unity of life.
The Calvin (Calvin–Benson) Cycle and Carbon Fixation

The second set of reactions for photosynthesis is known as the Calvin cycle, or sometimes, the light independent reactions. The Calvin cycle is highly endergonic but does not directly use light energy for its energy source. The ATP produced in the light reactions provides energy for the Calvin cycle and NADPH from the light reactions provides hydrogen and electrons for the reduction of carbon.

Carbohydrate molecules are produced in Calvin Cycle in the stroma of the chloroplast.

In the Calvin cycle carbon dioxide combines with a 5-carbon sugar (Ribulose bisphosphate) and undergoes a reduction to form 3-carbon molecules. These 3-carbon intermediates can be used to regenerate the 5-carbon sugar, metabolized to form the carbohydrate, glucose, or used for the synthesis of the organic molecules needed for cell structure and function.

The requirements for the Calvin cycle are:
- Carbon dioxide (CO₂)
- NADPH from the light-dependent reactions
- ATP from the light-dependent reactions
- Ribulose bisphosphate, regenerated in the cycle
- Appropriate enzymes for each step in the cycle. Of these, Ribulose bisphosphate carboxylase/oxidase (Rubisco) is especially important.

The Metabolic Intermediate in the Calvin cycle is:
- G3P (Glyceraldehyde-3-Phosphate)

The Calvin cycle produces:
- Glucose or 2 G3P
- Ribulose bisphosphate, regenerated in the cycle
- Water

The parts of the Calvin cycle are
- CO₂ Fixation
- Reduction
- Regeneration

Plus the Output or Product
- The carbohydrate product that goes out of the cycle after the reduction

The Calvin cycle pathway was determined using the ¹⁴C radioisotope. The researchers (Calvin, Benson and others) won a Nobel prize for their work, a summary of which is below. This pathway is called 3-carbon photosynthesis (C₃), (because of the 3-C G3P intermediate) to distinguish it from an alternative pathway known as 4-carbon (C₄) photosynthesis, to be discussed in a bit. To some, the Calvin cycle looks like a carbon cut-and-paste dance. It is. It's easy to follow the maze if one counts carbons. It also helps to remember that without this happening, you'd starve!
Calvin's work to determine the steps of the Calvin–Benson cycle

1. Radioactively labeled carbon in CO₂ was used to identify the products generated by photosynthesis in the green alga, *Chlorella*.
2. After a 30-second exposure to ¹⁴CO₂, the cells were killed and carbohydrates and amino acids were extracted, and many compounds, including monosaccharides and amino acids, contained radioactive carbon.
3. Calvin decreased the exposure to ¹⁴CO₂ to just 3 seconds and only one compound was found labeled with ¹⁴C, the three-carbon sugar 3-phosphoglycerate (3PG).
4. Other products of the cycle were found by increasing the ¹⁴CO₂ exposure time in a stepwise manner until all intermediates, and products were revealed.
5. Calvin’s group was not expecting a three-carbon sugar, because the initial reaction of the Calvin–Benson cycle fixes one CO₂ with the five-carbon compound, ribulose 1,5-bisphosphate (RuBP). But the intermediate six-carbon compound that forms is unstable and breaks down to form the two three-carbon molecules of 3PG that Calvin discovered.
6. The enzyme that catalyzes the fixation of carbon dioxide is ribulose bisphosphate carboxylase/oxygenase, called Rubisco, the most abundant protein in the world, comprising 20 percent of all the protein in every plant leaf.
Overview of the Calvin – Benson Cycle

For the production of the typical end product of photosynthesis, glucose, the Calvin cycle must consume 6 molecules of CO2, "turning" 6 times.

The Calvin Cycle – Details
Activation
The enzyme, Rubisco is activated by the decrease in H⁺ in the stroma by the pumping of H⁺ from the stroma into the thylakoid lumen during electron transport of the light-dependent reactions.

In addition, ferredoxin, one of the electron transport chain molecules, transfers some electrons to the protein, thioredoxin, which reduces disulfide bonds in four of the enzymes used in CO₂ fixation, converting them from inactive to active forms.
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Carbon (CO₂) Fixation

\[ 6\text{CO}_2 + 6 \text{RuBP} \rightarrow 6 \text{Hexose Phosphate} \rightarrow 2 \text{3PG} \]

Reduction Phase of the Calvin Cycle – Reduction of 3PG to G3P

Summary (CO₂ Fixation and Reduction of 3PG to G3P)

\[ 6\text{CO}_2 + 6 \text{RuBP} + 12 \text{ATP} + 12 \text{NADPH} \rightarrow 12 \text{G3P} \text{ (or PGAL).} \]

The 3-carbon intermediates, 3PG and G3P (3-phosphoglycerate and glyceraldehyde-3-phosphate) are also called PGA and PGAL (phosphoglyceric acid and phosphoglyceraldehyde).

Regeneration of Ribulose Bisphosphate (RuBP)

The 12 molecules of G3P produced will be used for two purposes: Regeneration of ribulose bisphosphate (RuBP) and synthesis of glucose or other carbon molecules for structure and function of the cell (the output from the cycle).

- The regeneration of RuBP will use 10 of the 12 G3P molecules from the reduction phase. Water and ADP are given off in the process.

Summary

\[ 10 \text{G3P} + 6 \text{ATP} \rightarrow 6 \text{Ribulose-1,5-bisphosphate} + 6\text{ADP} + 6 \text{H}_2\text{O} \]
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How the regeneration of RuBP works:

- The Regeneration phase of the Calving cycle uses 10 molecules of G3P in 2 sets of 5 each.
- The 3-carbon G3Ps are combined, beheaded, combined differently to form RuMP, which is then converted into the 5-carbon RuBP.
- One ATP is required to convert 1 RuMP to 1 RuBP.
- For each set of 5 G3P molecules: CCC CCC CCC CCC CCC

Combine 2 G3P to get a 6-C intermediate

\[ \text{CCC + CCC} \rightarrow \text{CCCCCC} \]

Remove 2 carbons from the 6-C intermediate \( \rightarrow \) 2-C + 4-C

\[ \text{CCCCCC} \rightarrow \text{CC + CCCC} \]

Combine the 2-C fragment with 1 G3P \( \rightarrow \) 5-C RuMP

\[ \text{CC + CCC} \rightarrow \text{CCCCC} \]

The 5-C RuMP + ATP \( \rightarrow \) RuBP

\[ \text{CCCCC + ATP} \rightarrow \text{RuBP} \]

Combine the 4-C fragment with 1 G3P \( \rightarrow \) 7-C

\[ \text{CCCCC + CCC} \rightarrow \text{CCCCCCC} \]

Remove 2 carbons from the 7-C \( \rightarrow \) 5-C RuMP + 2-C

\[ \text{CCCCCCC} \rightarrow \text{CC + CCCCC} \]

The 5-C RuMP + ATP \( \rightarrow \) RuBP

\[ \text{CCCCC + ATP} \rightarrow \text{RuBP} \]

Combine the 2-C fragment with 1 G3P \( \rightarrow \) 5-C RuMP

\[ \text{CC + CCC} \rightarrow \text{CCCC} \]

The 5-C RuMP + ATP \( \rightarrow \) RuBP

\[ \text{CCCCC + ATP} \rightarrow \text{RuBP} \]

Repeat the process to regenerate the needed 6 RuBPs.
Calvin Cycle - a Biochemistry Version:

Ribulose-1,5-bisphosphate

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow \text{Glycerate-3-phosphate} \]

\[ \text{Glycerate-3-phosphate} \rightarrow \text{Glycerate-1,3-bisphosphate} \]

\[ \text{Glycerate-1,3-bisphosphate} \rightarrow 6 \text{NADPH} + 6 \text{Pi} \]

Ribose-5-phosphate

\[ \text{Ribose-5-phosphate} \rightarrow \text{Ribulose-5-phosphate} \]

\[ \text{Ribulose-5-phosphate} \rightarrow \text{Ribulose-1,5-bisphosphate} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow 3 \text{ADP} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow 3 \text{ATP} \]

Fructose-6-phosphate

\[ \text{Fructose-6-phosphate} \rightarrow \text{Fructose-1,6-bisphosphate} \]

\[ \text{Fructose-1,6-bisphosphate} \rightarrow \text{Dihydroxyacetone phosphate} \]

\[ \text{Dihydroxyacetone phosphate} \rightarrow \text{Glyceraldehyde-3-phosphate} \]

\[ \text{Glyceraldehyde-3-phosphate} \rightarrow \text{Glycerate-3-phosphate} \]

\[ \text{Glycerate-3-phosphate} \rightarrow \text{Glycerate-1,3-bisphosphate} \]

\[ \text{Glycerate-1,3-bisphosphate} \rightarrow 6 \text{NADPH} + 6 \text{Pi} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow \text{Ribulose-5-phosphate} \]

\[ \text{Ribulose-5-phosphate} \rightarrow \text{Ribulose-1,5-bisphosphate} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow 3 \text{ADP} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow 3 \text{ATP} \]

Xylose-5-phosphate

\[ \text{Xylose-5-phosphate} \rightarrow \text{Sedoheptulose-1,7-bisphosphate} \]

\[ \text{Sedoheptulose-1,7-bisphosphate} \rightarrow \text{Sedoheptulose-7-phosphate} \]

\[ \text{Sedoheptulose-7-phosphate} \rightarrow \text{Sedoheptulose-1,7-bisphosphate} \]

\[ \text{Sedoheptulose-1,7-bisphosphate} \rightarrow \text{Dihydroxyacetone phosphate} \]

\[ \text{Dihydroxyacetone phosphate} \rightarrow \text{Glyceraldehyde-3-phosphate} \]

\[ \text{Glyceraldehyde-3-phosphate} \rightarrow \text{Glycerate-3-phosphate} \]

\[ \text{Glycerate-3-phosphate} \rightarrow \text{Glycerate-1,3-bisphosphate} \]

\[ \text{Glycerate-1,3-bisphosphate} \rightarrow 6 \text{NADPH} + 6 \text{Pi} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow \text{Ribulose-5-phosphate} \]

\[ \text{Ribulose-5-phosphate} \rightarrow \text{Ribulose-1,5-bisphosphate} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow 3 \text{ADP} \]

\[ \text{Ribulose-1,5-bisphosphate} \rightarrow 3 \text{ATP} \]
The Output - Producing Glucose and Metabolic Intermediates

The remaining 2 molecules of G3P are converted into one Glucose molecule.

\[
2 \text{ G3P} \rightarrow 1 \text{ Glucose}
\]

Although glucose is a typical end product in discussions of photosynthesis, plants do not store or transport glucose. Sucrose (synthesized from fructose-phosphate and glucose-phosphate) is the typical solute translocated throughout the plant. Plants typically store starch in amyloplasts in storage cells, and also accumulate starch short term within the chloroplasts for rapid conversion to sucrose for transport throughout the plant during non photosynthetic time.

In addition, plants are capable of synthesizing all of their organic molecules (amino acids, lipids, etc.) from photosynthetic intermediates, notably G3P and glucose phosphate. Plants are very versatile in their synthetic abilities compared to animals, and particularly with the common intermediates found in photosynthesis and cell respiration pathways. There are also plant products, called secondary metabolites, that are synthesized directly or perhaps as by-products of plant activity. Some secondary metabolites are protective in nature, such as toxins; some, such as lignin, are used in plant structure.

It's important to understand the covalent bonds of the carbohydrate molecules synthesized in the Calvin cycle represent the light energy captured to provide the energy needs for all life!
Photosynthesis Productivity, C₃, C₄ and Photorespiration
All chlorophyll-containing plants have the light-dependent reactions and the Calvin cycle as part of their photosynthetic process. However, some plants have variations on how, when and where the Calvin cycle, particularly CO₂ fixation occurs relative to the light-dependent reactions in response to the environmental conditions in which they have evolved.

As a process, photosynthesis is not highly efficient (although it is all we have to provide energy for life on earth). A 5% efficiency relative to the potential energy of light is remarkable for plants, and efficiencies are often less.

Limits to Photosynthetic Productivity
It takes 18 ATP and 12 NADPH to make one molecule of glucose. Relative to the potential energy coming from the sun, much of the light energy that hits the surface of plants is not absorbed and not available. And much of the light that hits earth does not hit photosynthetic surfaces of plants.

However, the common limit to photosynthesis is not light, but the availability of carbon dioxide, which diffuses from the atmosphere into the leaves through the stomata on the leaf surfaces. Stomata also permit diffusion of water from the interior of the leaf out, which can be a significant source of water loss to the plant. As much as 90% of the water absorbed by roots can be lost this way and plants are often at risk of dehydration from water loss through stomata.

This evaporation of water through the stomata (called transpiration) is also used by the plant to generate a tension that serves to pull water up through the xylem from the roots to stems and leaves, so this water loss is not a completely negative thing for the plant. (Transpiration will be studied in Biology 213.)

Under hot and dry conditions, many plants close their stomata to minimize water loss, preventing diffusion of CO₂ into the leaf. Naturally, this impacts photosynthesis productivity.

The light reactions of photosynthesis are not affected by stomatal closure so the ratio of oxygen to carbon dioxide in the leaf increases without CO₂ entering the chloroplast. This favors a process that competes with CO₂ fixation called photorespiration.
**Photosynthesis and the Plant Solutions**

Rubisco (or properly: ribulose bisphosphate carboxylase/oxygenase), the enzyme that brings CO₂ and RuBP together, can also catalyze O₂ fixation of RuBP, which explains the "oxygenase" part of the enzyme’s name, although its affinity for CO₂ is about ten times greater than for O₂. When the concentration of CO₂ is high relative to the level of O₂, RuBP combines with CO₂, but when CO₂ levels drop, Rubisco can combine RuBP with O₂ to form phosphoglycolate, a 2-carbon molecule and just one molecule of 3PG that can continue in the Calvin cycle.

Photorespiration decreases the photosynthetic output of the plant by about 24% and can reduce it as much as 50%. This can be significant!

![Comparison of CO₂ Fixation and Photorespiration with Rubisco](image)

**Glycolate "Recovery"**

The glycolate molecule produced in photorespiration is not completely wasted. Plants are able to make use of it. The 2-carbon phosphoglycolate is converted to glycolate in the chloroplast, which is exported to peroxisomes where the glycolate is converted to glycine. Glycine diffuses into mitochondria where it can be converted into glycerate, which can be passed back to the Calvin cycle or used in cell respiration. Alternatively, the glycolate can be degraded in the mitochondria and peroxisomes releasing CO₂ some of which can be captured in the Calvin cycle.

However, photorespiration always diminishes the photosynthetic output by plants. Since several of the major agricultural crops on earth, including rice, wheat and soybeans, use the C-3 pathway, photorespiration impacts food production.

We also know that some plants genetically incapable of photorespiration are more susceptible to excess light damage leading researchers to conclude that photorespiration products may be protective.
Alternative CO₂ fixation

The process of photosynthesis discussed up to this point is called C₃ photosynthesis because the first organic product of CO₂ fixation in the Calvin cycle is the 3-carbon compound, 3-phosphoglycerate (3PG). Chloroplasts are concentrated in the mesophyll cells of the leaf where both light-dependent and the Calvin cycle take place. The vast majority of plants have only the C₃ photosynthetic pathway.

But some plants, particularly those living in hot and dry environments, have evolved mechanisms to minimize the effects of photorespiration, typically by changing the method or timing in which CO₂ is "fixed" by the plant. The two most common methods are C₄ Photosynthesis and Crassulacean Acid Metabolism (CAM). We share discuss and compare both of these plant modifications.
C₄ Plant Modifications - CO₂ Fixation and Kranz Anatomy

The name, C₄ photosynthesis is derived from the manner in which C₄ plants can "fix" CO₂ prior to the Calvin cycle. C₄ plants have a second enzyme, PEP carboxylase, in their mesophyll cells that can combine CO₂ with the 3-carbon phosphoenolpyruvate (PEP), to form the 4-carbon acid, oxaloacetic acid, which is then converted to malate.

PEP carboxylase is not affected by O₂ concentration, as is Rubisco. PEP can bind to CO₂ in the high O₂ conditions that occur when there is intense sunlight and hot temperature, the conditions that favor both maximum photorespiration and transpiration water loss (and diffusion rate of CO₂). For C₄ plants, which are tropical grasses, including two important crop grasses, sugar cane and corn, photosynthesis can occur even when stomata are closed to prevent transpiration loss, and photorespiration is minimal. The malate produced acts as a CO₂ reservoir in the mesophyll cells of the leaf.

In addition to having a CO₂ reservoir, C₄ plants have an internal anatomy that separates the oxygen producing light-dependent reactions of photosynthesis from the Calvin cycle. These two stages of photosynthesis occur in modified chloroplasts located in different cells within the leaves. (C₃ and C₄ leaf structure will be reviewed in lab.)

Light reactions occur in leaf mesophyll cells of C₄ plants, just as in C₃ plants. The chloroplasts of the C₄ plant mesophyll cells have lots of grana. Recall that O₂ is produced only in the light reactions. The enzymes for the Calvin cycle are absent in the stroma of the mesophyll cells.

The Calvin cycle can proceed with stomata partially or fully closed when there is a reservoir of malate so C₄ plants can take advantage of the hot sun for maximum photosynthesis without water loss.
The Calvin Cycle of C\textsubscript{4} plants occurs in the chloroplasts of special bundle sheath cells. The bundle sheath is the outer layer of the leaf veins, which normally adds strength to the leaf. The chloroplasts in the bundle sheath cells of C\textsubscript{4} plants have few thylakoids and only photosystem I, so no O\textsubscript{2} is produced in the chloroplasts of the bundle sheath cells, although some ATP through cyclic electron transport is produced.

Malate, along with ATP and NADPH from the light reactions produced in the chloroplasts of mesophyll cells passes through plasmodesmata that connect mesophyll cells to bundle sheath cells.

CO\textsubscript{2} is released from malate in the bundle sheath cells for re-fixation by Rubisco in the Calvin cycle forming a 3-carbon pyruvate by-product. Pyruvate is returned to the mesophyll cells where it is converted back to PEP in an energy-consuming step.

**C\textsubscript{4} Efficiency**

The separation of the Calvin cycle from O\textsubscript{2} production in the light reactions raises photosynthesis efficiency when O\textsubscript{2} levels are high because photorespiration is greatly reduced. However, regenerating PEP requires additional ATP, so C\textsubscript{4} photosynthesis is not always be more productive than the C\textsubscript{3} pathway, as shown when temperatures are moderate and moisture levels are adequate. The pathway is genetic; plants can't choose.

The tropical C\textsubscript{4} grasses evolved about 12 million years ago possibly during a CO\textsubscript{2} decline on earth. When CO\textsubscript{2} levels are higher, C\textsubscript{3} plants have advantages. Our temperate food crops, such as wheat and soybeans, are C\textsubscript{3} plants whose photosynthetic rates in moderate climates are only modestly affected by photorespiration most of their growing season, and the loss of productivity is tempered by not having to use ATP as the C\textsubscript{4} plants must.
Photosynthesis rate comparisons for light intensity, temperature, CO₂ and O₂ levels.

C₃ plants perform better at lower temperatures.

CAM – A Water Conservation Mechanism

Water loss by transpiration affects most plants, and closed stomata during daylight hours, as discussed, can dramatically lower photosynthetic productivity. To conserve water, some C₃ plants have "reverse stomata operation". They close stomata during the daylight hours to conserve water and open them at night for CO₂ uptake. Plants cannot accumulate gaseous CO₂. CO₂ will diffuse along its gradient, no matter which direction that gradient favors.

CAM plants have PEP carboxylase to fix CO₂ at night when their stomata are open, to form oxaloacetate that is converted to malate. They store the accumulated malate in mesophyll cell vacuoles. In the daytime, the CO₂ is released for C₃ photosynthesis, while the stomata can remain closed to prevent excessive water loss. Recall that the release of CO₂ from the 4-carbon acids and recovery of PEP is endergonic. CAM plants pay an ATP cost to minimize water loss.

The name, CAM, or Crassulacean Acid Metabolism, is derived from the types of plants in which it was first discovered, Crassuleans, and that the CO₂ trapped forms acids. A significant difference between C₄ plants and CAM plants is that CAM plants do not have a specialized anatomy with separate cells for the Calvin cycle and the light reactions as the C₄ plants do. CAM plants have C₃ photosynthesis. They perform both the Calvin cycle in the same mesophyll cells as the light reactions. It is strictly a water conservation benefit for photosynthesis.

CAM plants are typically succulent plants of arid environments.
Special Note on Energy and Carbon Sources for Life on Earth

We are accustomed to thinking about autotrophs, those who can manufacture their own organic carbon molecules from inorganic carbon (CO₂), and heterotrophs, whose carbon sources are pre-formed organic carbon molecules, which the heterotrophs digest and reform into their own organic carbon molecules. We also learn that most autotrophs use light as their energy source to synthesize organic carbon in the process of photosynthesis. Most photoautotrophs use H₂O to reduce CO₂ in the process of photosynthesis. Some bacteria (the H, S, Fe and nitrifying bacteria) are chemoautotrophs. They use inorganic compounds to reduce CO₂.

There is, however, among bacteria, versatility in carbon and energy sources, as well as the final electron acceptor in their re-doxy reactions in respiratory pathways, so the terms heterotroph and autotroph are further modified, as shown in the diagram below and studied in microbiology.