

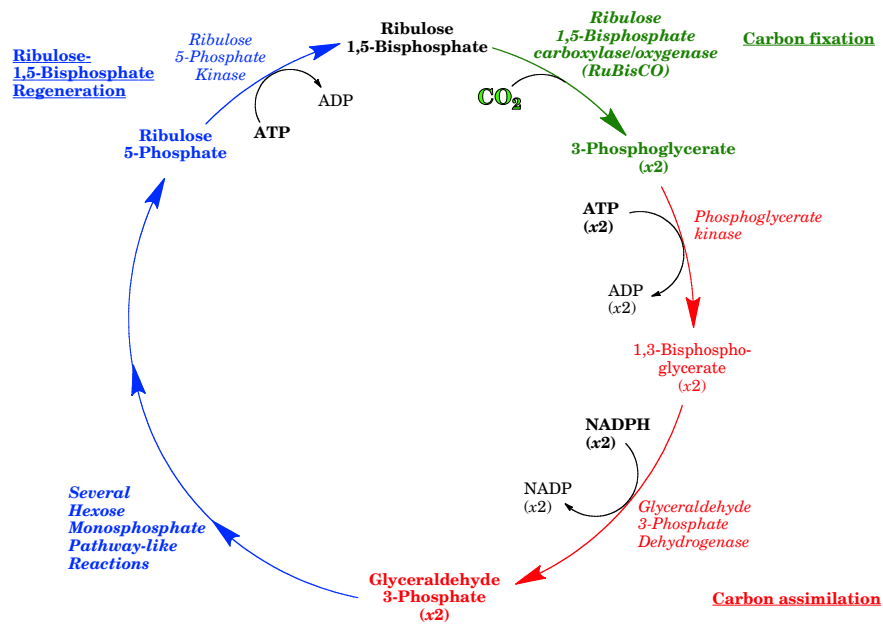
Photosynthesis (Carbon Assimilation)

The light reactions result in the formation of the high-energy compounds ATP and NADPH. While these compounds can be used to drive metabolic processes, one additional critical reaction must occur: the fixation of carbon dioxide. Without CO₂ fixation, the respiratory processes necessary to generate energy at night would result in an irreversible conversion of carbon compounds to CO₂. In addition, carbon fixation is required to provide energy to non-photosynthetic tissues within the plant, and to supply the raw material required for growth of the plant.

Animals lack the ability to perform net carbon dioxide fixation; reactions such as the pyruvate carboxylase reaction only result in temporary increases in the number of carbon atoms in the compound.⁷ Plants, however, have the capability of using the energy obtained from light to drive the permanent incorporation of carbon dioxide into structural molecules. The process for fixing and assimilating carbon is called the Calvin cycle in honor of the 1961 Nobel Laureate Melvin Calvin.

Overview of the Calvin cycle

The Calvin cycle begins with the five-carbon carbohydrate ribulose-1,5-bisphosphate.



Ribulose-1,5-bisphosphate acts as the carbon dioxide acceptor; following carbon dioxide addition, the resulting six-carbon compound is cleaved into two three-carbon 3-phosphoglycerate molecules. The 3-phosphoglycerate is then converted to

⁷ The pyruvate carboxylase reaction is the formation of the four-carbon oxaloacetate from the three-carbon pyruvate. Oxaloacetate may remain in the TCA cycle, or may be used for biosynthetic processes. When used for biosynthetic processes such as gluconeogenesis, the oxaloacetate loses the additional carbon. However, even carbon dioxide added to oxaloacetate that remains in the TCA cycle is effectively only fixed temporarily.

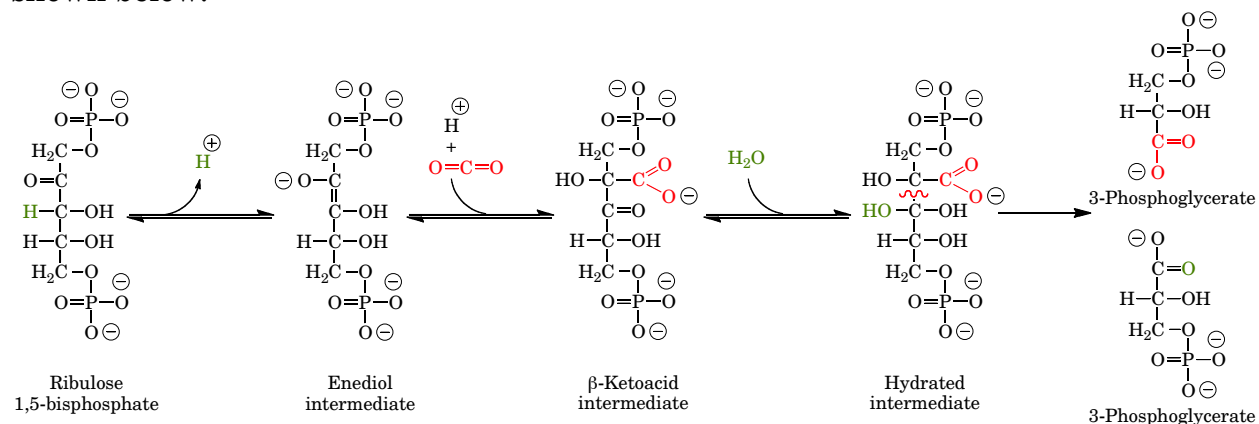
glyceraldehyde-3-phosphate using energy obtained from the light reactions of photosynthesis. The glyceraldehyde-3-phosphate has several possible fates; however, the continuation of the carbon fixation process requires regenerating the ribulose-1,5-bisphosphate starting material. The cycle thus has three phases: carbon fixation, glyceraldehyde-3-phosphate formation and ribulose-1,5-bisphosphate regeneration.

Rubisco

The rate-limiting enzyme in the Calvin cycle is **ribulose 1,5-bisphosphate carboxylase/oxygenase** (better known as **rubisco**). In most plants, rubisco is a complex of 8 large (53 kDa) and 8 small (14 kDa) subunits ($\alpha_8\beta_8$). The large subunit is coded by a chloroplast gene, while the small subunit gene is located in the nucleus. In photosynthetic bacteria, rubisco is usually a dimer of proteins homologous to the plant large subunit.

Rubisco is an intensively studied protein, because it is one of the most important enzymes in existence. Rubisco is present at a concentration of ~ 250 mg/ml in the chloroplast stroma, comprising $\sim 50\%$ of the total chloroplast protein. Plants production of rubisco exceeds that of any other enzyme, and probably any other protein, on earth. Current estimates suggest that the biosphere contains ~ 10 kg of rubisco per human (this works out to about 0.02 moles of the enzyme per person). This tremendous amount of protein is necessary because the catalytic efficiency of rubisco is fairly low: it has a k_{cat} of 1 to 3 sec^{-1} , and a rather low affinity for carbon dioxide. It is also necessary because rubisco catalyzes the wasteful oxygenase side reaction (discussed later); fixing sufficient carbon to support growth requires large amounts of this enzyme.

The rubisco reaction is moderately complex. The general reaction mechanism is shown below.



The enzyme has a basic residue that abstracts a proton from the 3-position carbon of the ribulose-1,5-bisphosphate substrate, producing the enzyme-bound enediol intermediate. The enediol acts as the carbon dioxide acceptor, with the carbon dioxide molecule forming a bond to the 2-position carbon. The six-carbon β -ketoacid intermediate is then attacked by a water molecule, resulting in cleavage of the bond between the former 2-position and 3-position carbons of the ribulose-1,5-

bisphosphate molecule. This results in the release of two 3-phosphoglycerate molecules (one of which, as released, contains a negatively charged carbon ion which then obtains a proton from the aqueous solvent).

The overall reaction is therefore:

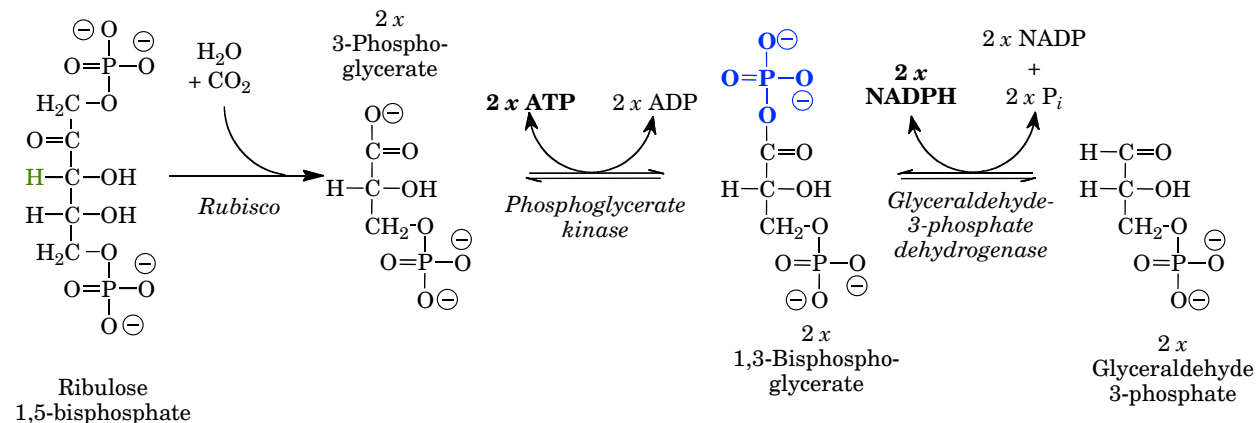


The rubisco reaction thus yields two triose phosphate molecules from the single pentose bisphosphate. Note that ATP is **not** required for the rubisco reaction, a property that differs from most other carboxylase reactions; in this case, the reaction has a large negative ΔG° because of the release of the two triose phosphates.

Carbon assimilation reactions

The carbon-fixing rubisco reaction thus results in the release of 2 three-carbon 3-phosphoglycerate molecules. The 3-phosphoglycerate molecules are converted to 1,3-bisphosphoglycerate by **phosphoglycerate kinase**; this reaction requires ATP as a phosphate donor. The 1,3-bisphosphoglycerate is then converted to glyceraldehyde-3-phosphate by **glyceraldehyde-3-phosphate dehydrogenase**.

These two reactions use chloroplast isozymes of the glycolytic enzymes phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase (note that the glyceraldehyde-3-phosphate dehydrogenase uses NADPH as a substrate rather than NADH). Because two 3-phosphoglycerate molecules are released by rubisco, two ATP and two NADPH are required to convert all of the triose phosphate to glyceraldehyde-3-phosphate. The ATP and NADPH required are produced by the light reactions, which therefore drive the entire process.



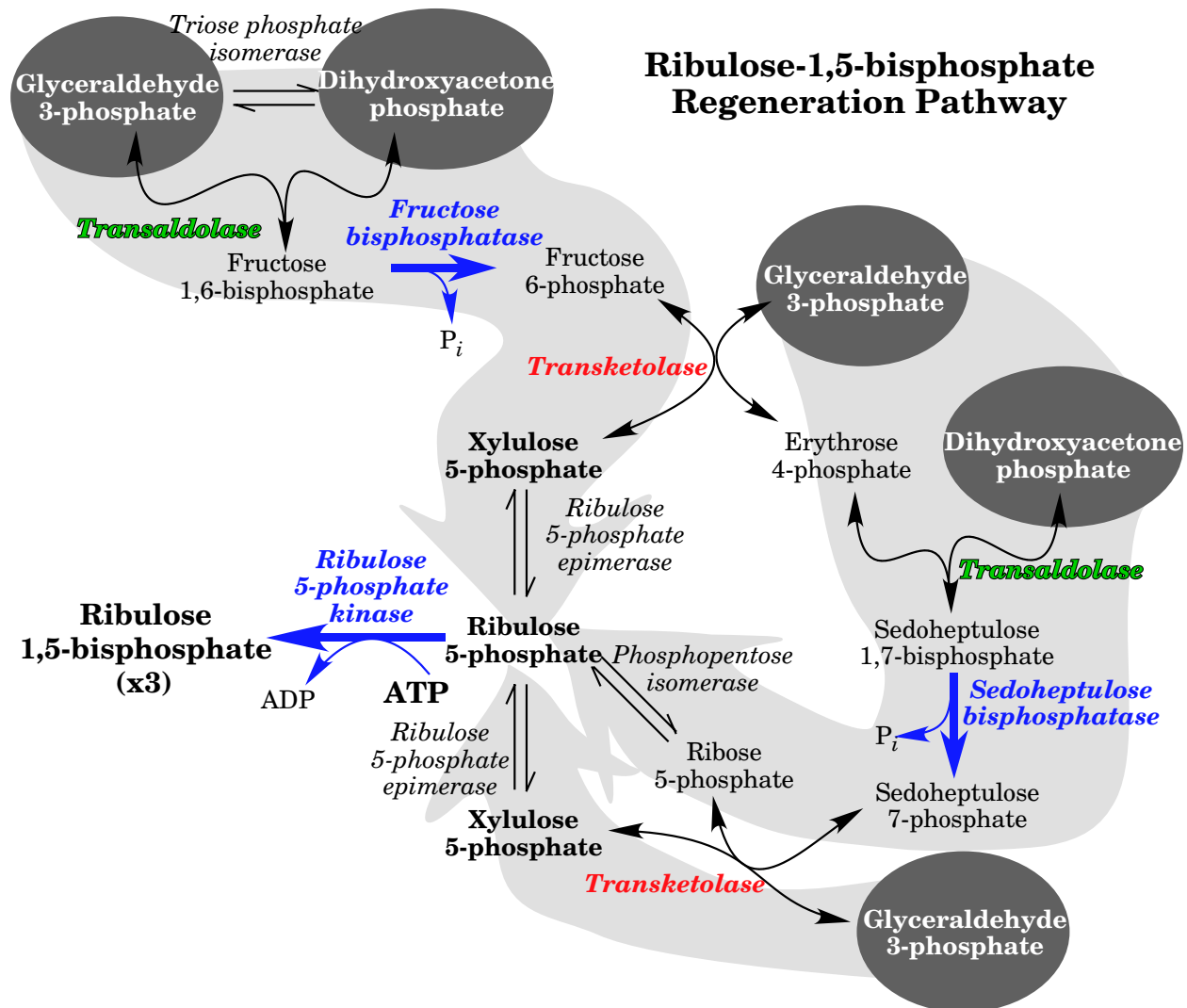
The phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase reactions are reversible. In chloroplasts exposed to light and carbon dioxide, these reactions result in glyceraldehyde-3-phosphate formation due to the high concentrations of ATP, NADPH, and 3-phosphoglycerate.

Regeneration of ribulose-1,5-bisphosphate

Glyceraldehyde-3-phosphate can be converted to glucose without further input of energy. However, unless the ribulose 1,5-bisphosphate is regenerated the rubisco reaction will be unable to continue.

The carbon assimilation process begins with three ribulose 1,5-bisphosphate (containing a total of 15 carbons); it ends with six glyceraldehyde-3-phosphate (containing a total of 18 carbons), a net gain of three carbons from carbon dioxide. Assuming that five triose phosphates are used to regenerate the starting ribulose 1,5-bisphosphate, the remaining triose phosphate can then be diverted to other processes.

The regeneration process uses enzymes related to those of the hexose monophosphate pathway. Three-carbon units in the form of glyceraldehyde-3-phosphate or dihydroxyacetone phosphate enter the pathway in a total of five places. The result of the pathway is the formation of three ribulose 1,5-bisphosphate molecules from the five trioses.



Three **irreversible steps**, fructose biphosphatase, sedoheptulose biphosphatase, and ribulose-5-phosphate kinase make the regeneration pathway irreversible. Note that the regeneration pathway only uses five trioses; this means that **the sixth triose formed can be used for other purposes**. Animals lack rubisco, sedoheptulose biphosphatase, and ribulose-5-phosphate kinase, and therefore are incapable of the carbon assimilation reactions (in addition to lacking the requisite, non-metabolic, energy source).

Glyceraldehyde-3-phosphate and dihydroxyacetone phosphate are effectively interchangeable due to the action of the reversible glycolytic enzyme triose phosphate isomerase. Glyceraldehyde-3-phosphate and dihydroxyacetone phosphate can be combined in an aldol condensation reaction by **transaldolase** (an isozyme of the glycolytic aldolase) to produce fructose-1,6-bisphosphate. The fructose-1,6-bisphosphate is then converted to fructose-6-phosphate by **fructose biphosphatase**, the first irreversible enzyme in the pathway.

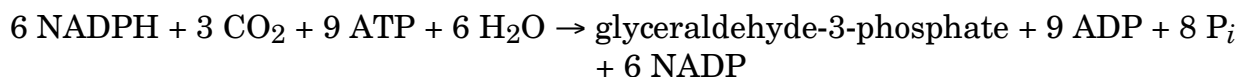
The fructose-6-phosphate then acts as a substrate for transketolase. **Transketolase** is a thiamin pyrophosphate-dependent enzyme that catalyzes two-carbon transfer reactions. In this case, it transfers two carbons from fructose-6-phosphate to glyceraldehyde-3-phosphate, yielding the four-carbon erythrose-4-phosphate, and the five-carbon xylulose-5-phosphate. Ribulose-5-phosphate epimerase converts the xylulose-5-phosphate to the first of the ribulose-5-phosphate molecules produced by the regeneration pathway.

The erythrose-4-phosphate, an aldotetrose, acts as a substrate for another **transaldolase** reaction, condensing with dihydroxyacetone phosphate to form the seven-carbon compound sedoheptulose-1,7-bisphosphate. (Note that the transaldolase reactions of the regeneration pathway both result in bisphosphate carbohydrates.) **Sedoheptulose biphosphatase** catalyzes the second irreversible reaction of the regeneration pathway, releasing sedoheptulose-7-phosphate.

The sedoheptulose-7-phosphate acts as a substrate for a second **transketolase** reaction, combining with the final glyceraldehyde-3-phosphate to yield two pentose phosphates: ribose-5-phosphate, and a second xylulose-5-phosphate. Both of these pentose phosphates are then converted to ribulose-5-phosphate molecules.

The pathway produces a total of three ribulose-5-phosphate from the five triose phosphates. Ribulose-5-phosphate kinase catalyzes the final irreversible step of the regeneration pathway, the ATP-dependent phosphorylation of ribulose-5-phosphate to produce the rubisco substrate, ribulose-1,5-bisphosphate.

The net reaction of running the Calvin cycle to produce one excess triose phosphate (in the form of glyceraldehyde-3-phosphate) is:

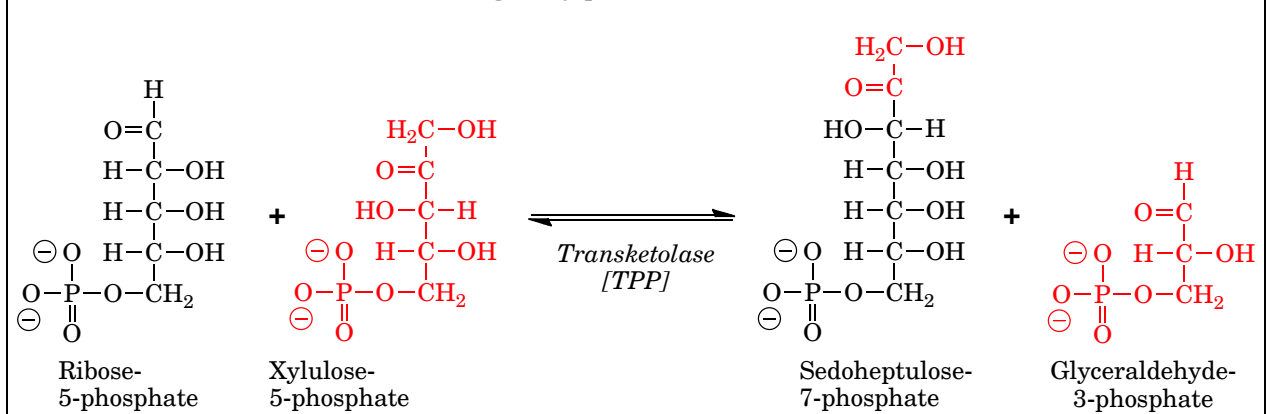


The light reactions produce roughly three ATP for every two NADPH; thus, *in*

principle, one complete oxygen-evolving light reaction results in fixation of one molecule of carbon dioxide. In practice, however, some ATP and NADPH are used for other purposes, and therefore the light reactions and carbon assimilation reactions are not always stoichiometrically coupled.

Side Note: The transketolase reaction

The enzyme transketolase contains a thiamin pyrophosphate cofactor. As in most enzymes that use this cofactor, in transketolase, the thiamin pyrophosphate cofactor becomes transiently covalently modified during the reaction. In transketolase the two-carbon unit is removed from the ketose (in the example below, from xylulose-5-phosphate) and given to the aldose acceptor (in the example, to ribose-5-phosphate). The reaction generates an aldehyde in the remaining portion of the donor molecule, and maintains the ketone originally present in the transferred two-carbon unit.



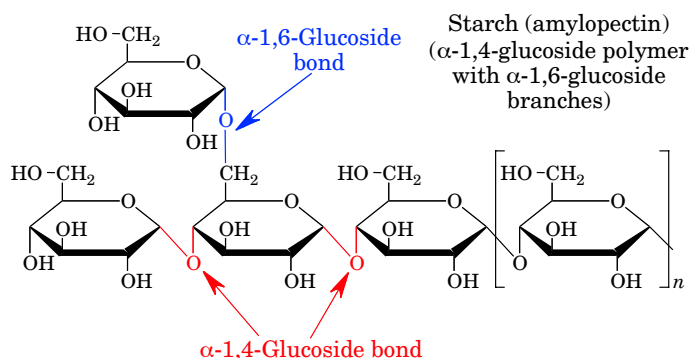
Utilization of assimilated carbon

The excess trioses produced have several possible fates. These fates must involve release of phosphate from the carbohydrate, otherwise phosphate becomes limiting. (Light allows carbon fixation, but does not increase the amount of phosphate present.)

1) **Starch production:** Starch is an energy storage form of glucose. The starch produced and stored within the chloroplast acts as an energy source to maintain metabolism during the night.

Starch is a mixture of **amylose**, a linear α -1,4-glucose polymer and **amylopectin**, an α -1,4-glucose polymer with α -1,6 branches. Amylopectin differs from glycogen only in having fewer branch points.

The diagram at right shows the structural features of the glucose polymers.

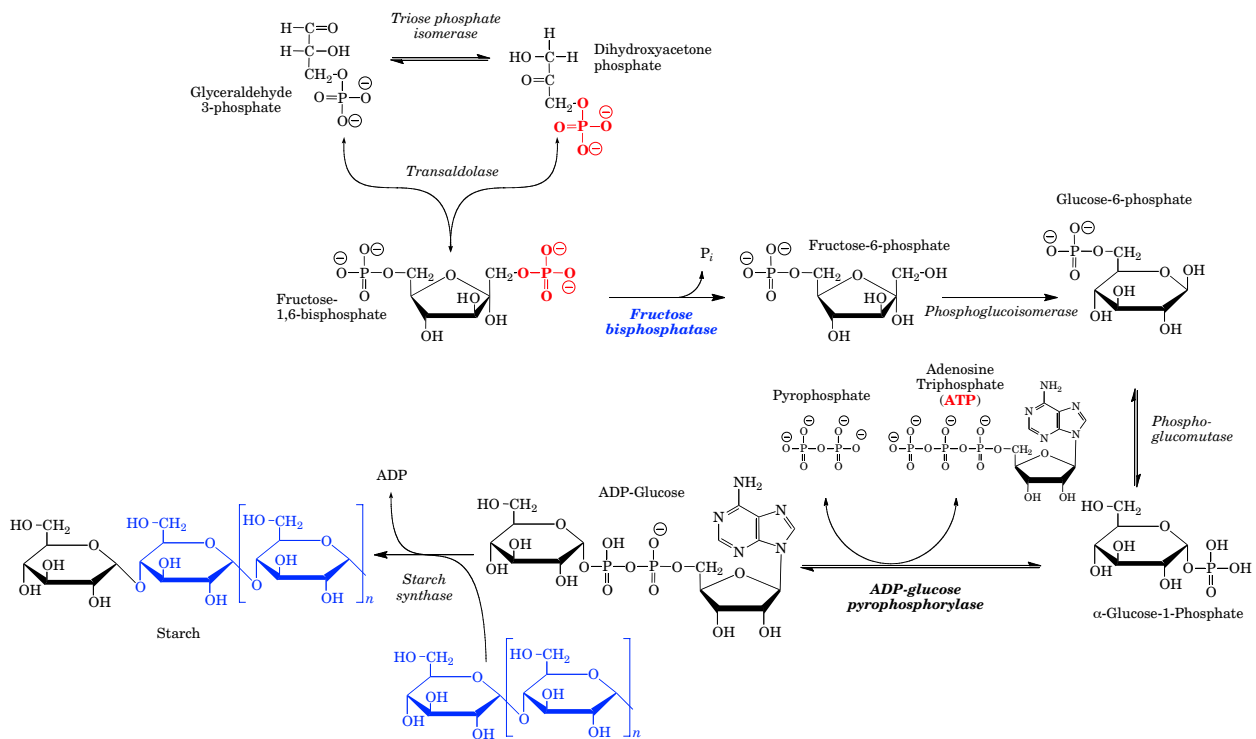


The starch synthetic system is generally similar to glycogen synthesis in animals. However, the starch synthase uses ADP-glucose rather than UDP-glucose as the

glucose donor.

Production of starch is regulated primarily by **ADP-glucose pyrophosphorylase**, which acts as the control point and rate-limiting step for starch synthesis. The enzyme uses ATP and glucose-1-phosphate as substrates, yielding ADP-glucose and pyrophosphate as products. The reaction is reversible, with pyrophosphate hydrolysis acting as the driving force. The ADP-glucose produced acts as a substrate for the starch synthase that adds the glucose unit to the growing starch chain. ADP-glucose pyrophosphorylase is allosterically regulated; in most plants it is stimulated by 3-phosphoglycerate (an indicator that the cell is actively fixing carbon) and is inhibited by phosphate (which is elevated when ATP levels are low).

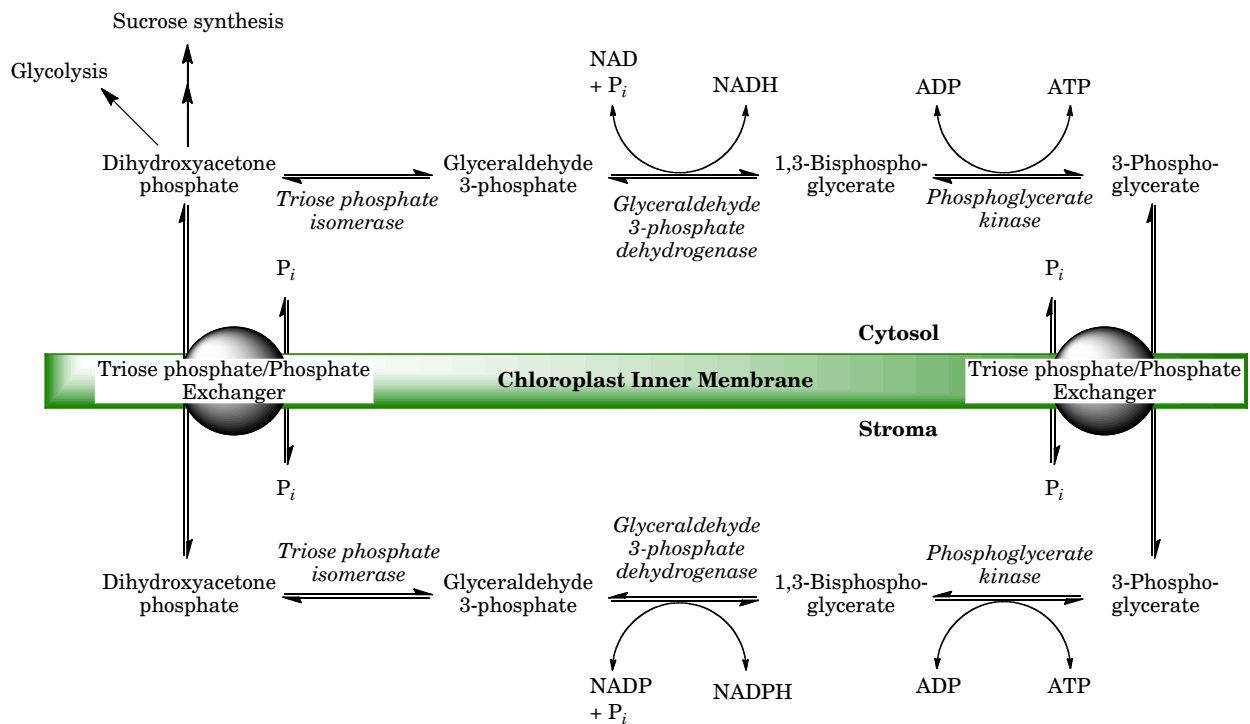
The synthesis of starch from triose phosphates is summarized in the chart below.



2) Triose Export: Utilization of the fixed carbon requires translocation of the triose phosphate molecules synthesized from the chloroplast to the cytoplasm. This process is performed by a phosphate/triose phosphate antiport located in the chloroplast inner membrane. Dihydroxyacetone phosphate produced in the carbon assimilation reactions leaves the chloroplast in exchange for phosphate (which is required for the synthesis of additional triose phosphates). The cytoplasmic phosphate is released from the carbohydrate during both glycolysis and sucrose synthesis (note that neither pyruvate nor sucrose contain phosphate).

The triose transporter can also be used as part of a shuttle system for moving ATP and reducing equivalents generated during the light reactions out of the chloroplast. The ATP and reduced nicotinamide cofactors can then be used to support

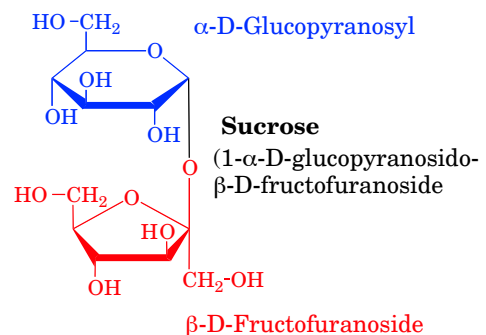
metabolism in the other parts of the cell. To allow this, dihydroxyacetone phosphate leaves the chloroplast in exchange for phosphate (as above). However, the dihydroxyacetone phosphate is converted to 3-phosphoglycerate using the glycolytic enzymes triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglycerate kinase. These reactions generate NADH and ATP in the cytoplasm. The 3-phosphoglycerate produced then re-enters the chloroplast using the phosphate/triose phosphate antiport, and is reconverted to dihydroxyacetone phosphate using the enzymes of the carbon assimilation pathway.



The shuttle system can therefore be used to move ATP and reducing equivalents, without using carbon compounds; alternatively, the dihydroxyacetone phosphate can leave the chloroplast, with only phosphate returning.

3) Sucrose synthesis: Sucrose is a non-reducing (and therefore chemically less reactive) disaccharide of fructose and glucose used to transport energy from photosynthetic apparatus to non-photosynthetic cells. Production of sucrose occurs in the cytoplasm.

As with starch production, sucrose synthesis is regulated by availability of triose phosphates. The relative levels of starch and sucrose production are tightly regulated to prevent the photosynthetic cell from depleting its energy stores.



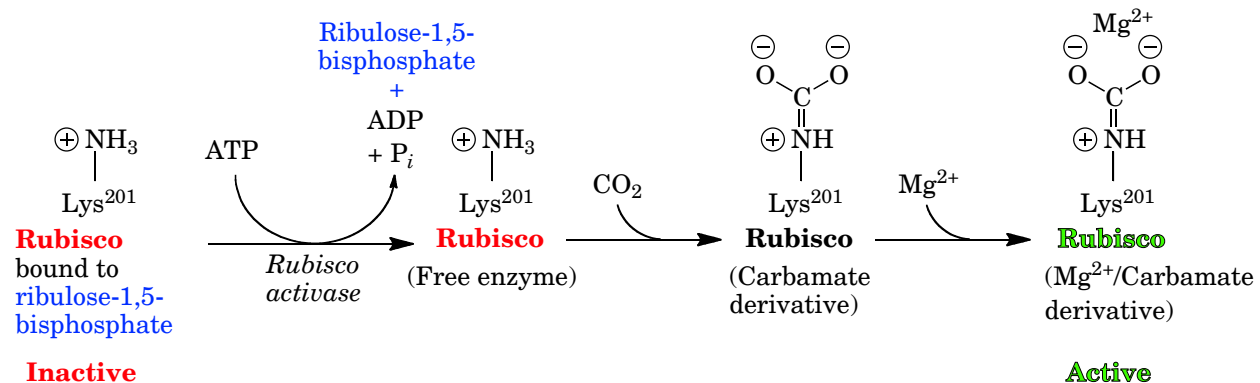
4) **Glycolysis:** Plants, like all eukaryotic cells, contain mitochondria; some of the triose phosphates generated during photosynthesis (or at night during starch breakdown) are used in the cytoplasm and mitochondria for generation of energy and biosynthetic intermediates. The glycolytic and TCA cycle pathways of plants are similar to those of animals.

Regulation of carbon assimilation

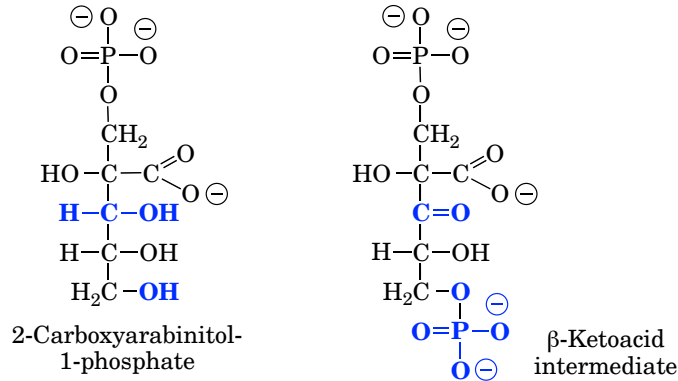
Unless ATP and NADPH generated by the light reactions is available, the rubisco reaction and the other carbon assimilation reactions merely waste energy to no useful purpose. Therefore, several of the Calvin cycle enzymes are tightly regulated to prevent the cycle in the absence of light. (In other words the so-called “dark reactions” do **not** occur in the dark.)

The regulation of rubisco occurs at several steps. The free rubisco enzyme has a high affinity for ribulose-1,5-bisphosphate but is inactive. In order to become activated, carbon dioxide must bind to a critical lysine residue (Lys²⁰¹ of the large subunit); the presence of ribulose-1,5-bisphosphate in the binding site prevents this from happening. Removal of the ribulose-1,5-bisphosphate requires an ATP-hydrolysis dependent enzyme, **rubisco activase**, which catalyzes the release of ribulose-1,5-bisphosphate and the covalent modification of Lys²⁰¹.

The carbamate derivative of rubisco requires Mg²⁺ for activity (it is thought that the cation is required to stabilize the charges that develop on some of the reaction intermediates). The concentration of Mg²⁺ in the stroma increases during the light reactions, because the thylakoid lumen releases Mg²⁺ when the internal proton concentration is high (*i.e.* when protons are pumped into the lumen during the light reactions). Proton pumping also increases pH of the stroma to ~8; rubisco is most active at this pH.



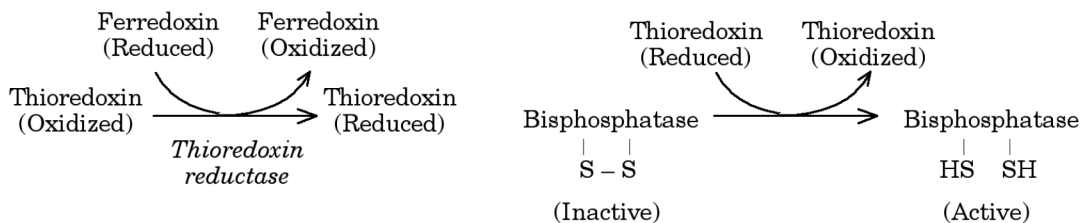
Although the carbamate derivative of rubisco is potentially active, it is also regulated by an endogenous inhibitor. 2-Carboxyarabinitol 1-phosphate is related to the β -ketoacid intermediate of the rubisco reaction, and binds to rubisco with high affinity. 2-Carboxyarabinitol 1-phosphate is synthesized in the dark and broken down in response to light by other enzymes.



Thus, rubisco is **inactive**: 1) when bound to an inhibitor, 2) when bound to its ribulose-1,5-bisphosphate substrate if the enzyme is not covalently modified by carbon dioxide, and 3) in the absence of Mg^{2+} .

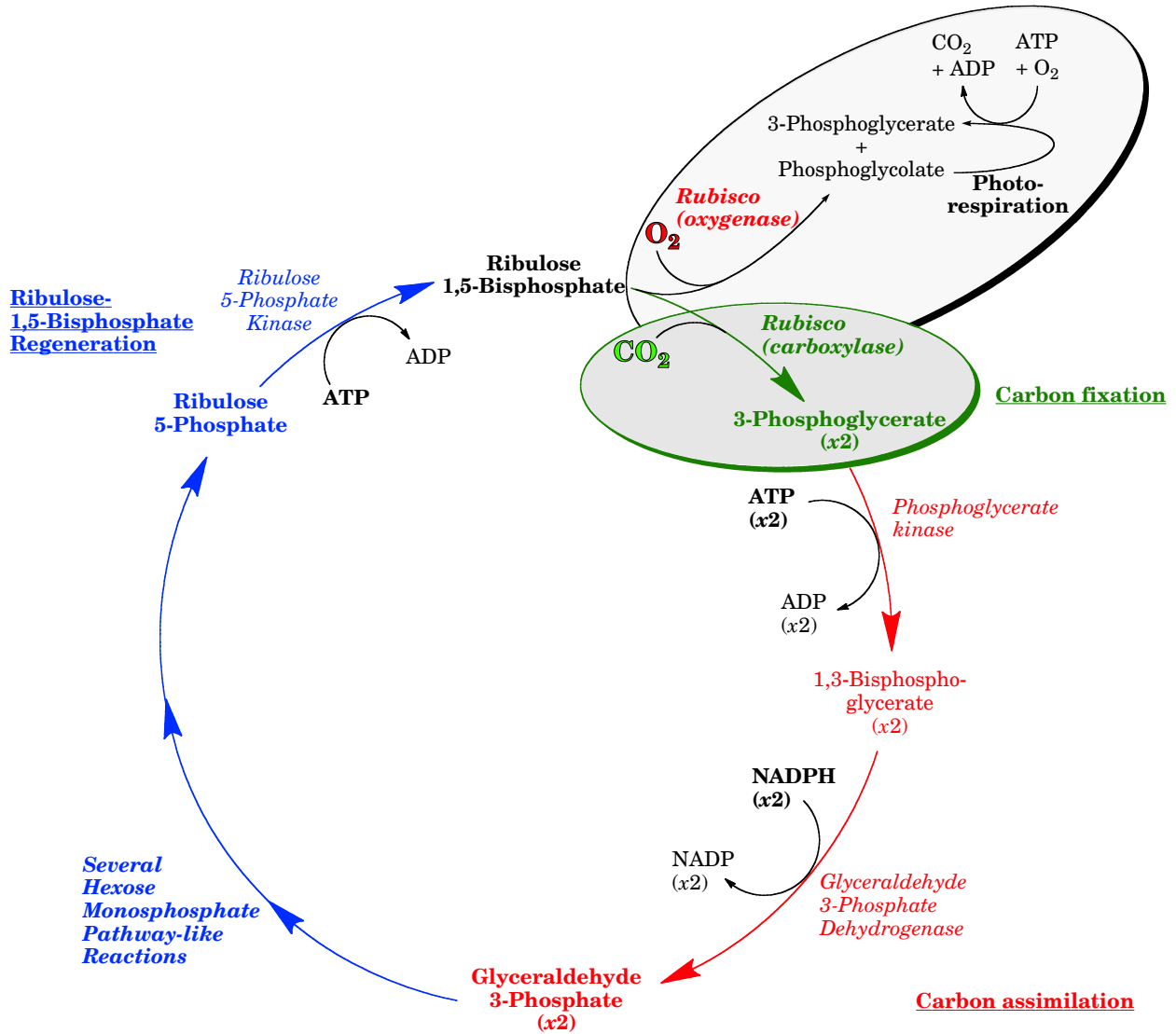
Rubisco is **active**: 1) when Lys²⁰¹ is carbamoylated, 2) when bound to Mg^{2+} , 3) when 2-Carboxyarabinitol 1-phosphate has been degraded by light-dependent enzymes, and 4) under the conditions of elevated pH that occur when the chloroplast is exposed to light.

Fructose biphosphatase and **sedoheptulose biphosphatase**, two of the irreversible enzymes of the regeneration pathway are also stimulated by elevated pH and elevated Mg^{2+} concentration. In addition, these enzymes contain a pair of cysteine residues that are critical for activity. In the absence of light, the enzymes become oxidized, and form a disulfide bond between the cysteine side-chains. Illumination of the chloroplast results in reduction of the soluble ferredoxin (a protein found in the electron transport chain involved in NADPH production). Reduced ferredoxin can donate electrons to thioredoxin (a process catalyzed by thioredoxin reductase). The reduced thioredoxin then reduces the disulfide bonds of fructose biphosphatase and sedoheptulose biphosphatase to free sulfhydryl groups in order to activate these enzymes. These enzymes are therefore activated indirectly by light.

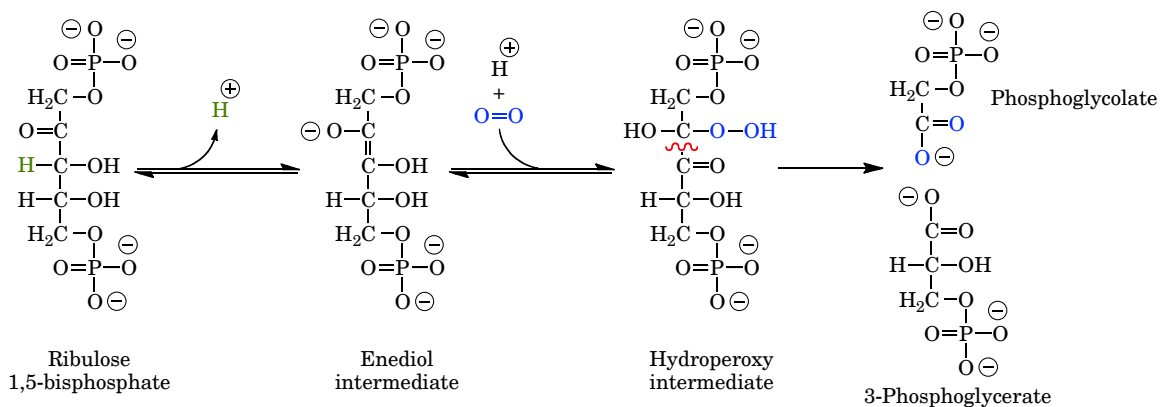


Photorespiration

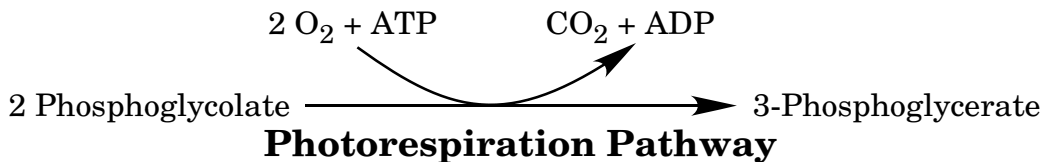
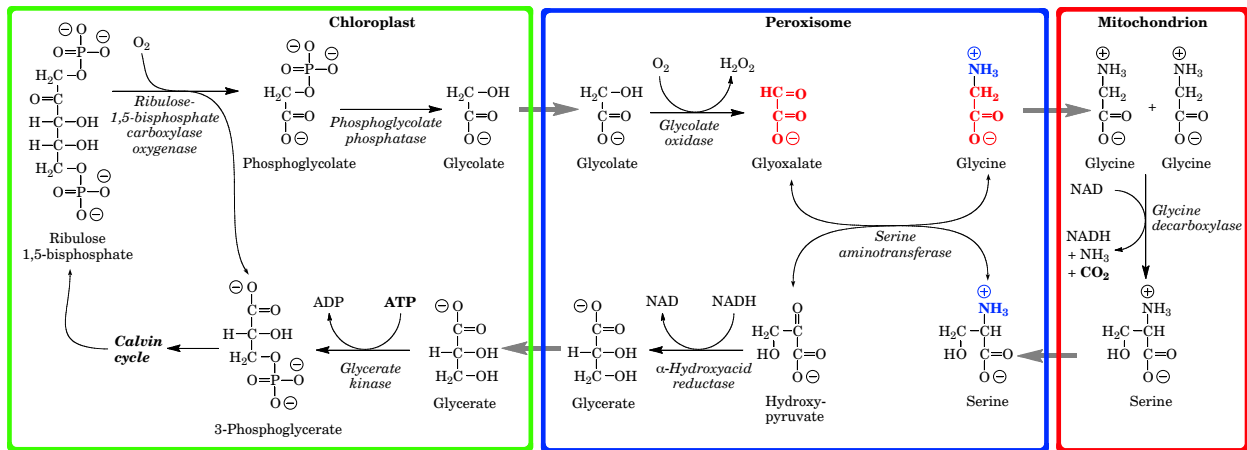
Rubisco, in addition to its critically important carboxylase activity, is capable of using oxygen as a substrate in place of carbon dioxide. This results in oxygenase activity (hence the “o” in the word “rubisco”). The product of the oxygenase activity must then be converted back to Calvin cycle intermediates by the poorly named “photorespiration pathway”.



The rubisco oxygenase activity results in the formation of a molecule of the two-carbon compound phosphoglycolate and a molecule of 3-phosphoglycerate.



The phosphoglycolate produced must be converted to 3-phosphoglycerate by the photorespiration pathway. The process is called photorespiration because it results in oxygen consumption and carbon dioxide production; in effect it uses energy to run the rubisco reaction in reverse. Photorespiration uses several different compartments, and requires glycine as a pseudo-coenzyme (the glycine carbon effectively comes from phosphoglycolate, but the amino group nitrogen is lost). In the overall process, two molecules of phosphoglycolate are converted to one molecule of 3-phosphoglycerate, with loss of nitrogen as ammonia, loss of carbon dioxide, and the utilization of a molecule of ATP



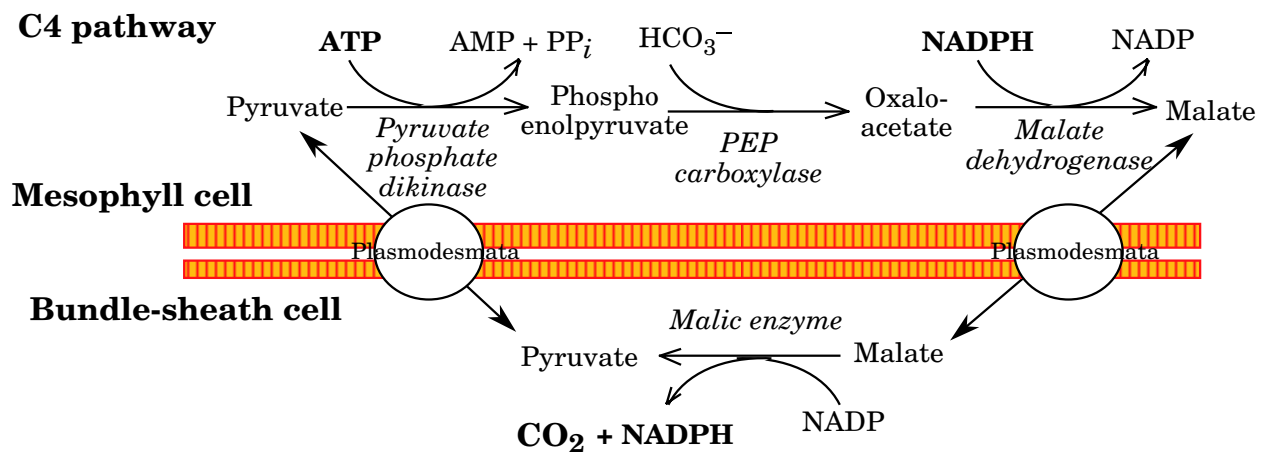
Photorespiration is a remarkably wasteful process; both conversion of phosphoglycolate to 3-phosphoglycerate and regeneration of ribulose-1,5-phosphate require ATP. The energy lost in this process has been estimated to decrease plant growth by as much as 50%.

The purpose of the oxygenase activity of rubisco is not known. However, no organisms have evolved a rubisco that lacks this oxygenase activity. It is possible that the oxygenase may act as protection from damaging photooxidation; light in absence of CO₂ appears to result in photooxidation damage to the cell. Alternatively, the limited specificity of the enzyme may be a vestige of the historical time period when free oxygen was rare.

The mechanistic reason for the oxygenase activity is clear. The rubisco K_m for CO₂ is $\sim 9 \mu\text{M}$, a value that increases with temperature. The rubisco K_m for O₂ is $\sim 350 \mu\text{M}$. Atmospheric partial pressures of oxygen and carbon dioxide result in dissolved concentrations in water for carbon dioxide of $\sim 11 \mu\text{M}$ and for oxygen of $\sim 250 \mu\text{M}$. Under normal conditions, the ratio of carbon dioxide to oxygen utilization is roughly 3 to 1. At higher temperatures, the K_m for carbon dioxide rises, and the result is

that the oxygenase activity becomes an even larger fraction of the total activity of rubisco.

Plants that use the pathways discussed above are called C3 plants, to differentiate them from the rarer C4 types that use a modified pathway intended to decrease photorespiration. The C4 plants temporarily fix carbon dioxide using phosphoenolpyruvate carboxylase (an enzyme that does not react with oxygen) in mesophyll cells. The phosphoenolpyruvate carboxylase reaction converts phosphoenolpyruvate to oxaloacetate. The oxaloacetate is then converted to malate by malate dehydrogenase. The malate moves to the bundle-sheath cells by passing through plasmodesmata (structures that function like gap junctions), where malic enzyme releases the temporarily fixed carbon dioxide. The result is a much higher carbon dioxide concentration in the local environment near the rubisco.



The pyruvate must be converted back to phosphoenolpyruvate using an unusual enzyme, pyruvate phosphate dikinase, which uses ATP to phosphorylate both the pyruvate and an inorganic phosphate (the pyrophosphate produced is then hydrolyzed to help drive the reaction). The C4 pathway thus requires the additional energy of two ATP equivalents per carbon fixed. However, the reduction in wasted energy due to lower oxygenase activity (and therefore lower photorespiration) allows C4 plants to outgrow C3 plants under conditions of high temperature with intense sunlight.

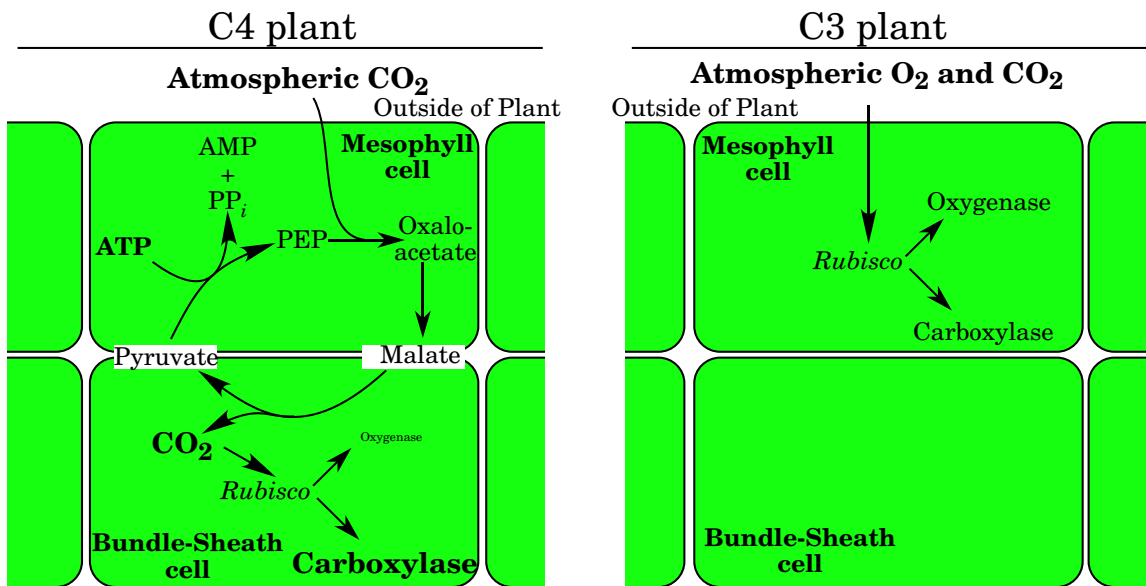
C3 plants perform both photorespiration and the normal carbon-fixing Calvin cycle. C4 plants **also** perform both photorespiration and an **identical** carbon-fixing Calvin cycle. However, by adding an additional (energy-dependent) pathway, and by creating a spatial separation between the outside of the plant and the carbon fixation machinery, C4 plants perform much less oxygenase activity than do C3 plants, and therefore are more energy efficient under some conditions.

In **C3 plants**, most photosynthesis occurs in the mesophyll cells. These cells are exposed to atmospheric oxygen and carbon dioxide. As a result, rubisco can use either carbon dioxide or oxygen as substrate, and therefore performs both carboxylase and oxygenase reactions. At high temperatures, rubisco has reduced affinity for carbon dioxide. Because the affinity for oxygen does not change

significantly, the reduced affinity for carbon dioxide means that the oxygenase activity occurs more frequently at high temperatures.

In **C4 plants**, the mesophyll cell fixes carbon dioxide temporarily by converting the three-carbon phosphoenolpyruvate to four-carbon oxaloacetate (C4 = four-carbon). This carbon is then transported to the bundle-sheath cell further inside the plant, where carbon dioxide is released.

Because carbon dioxide concentration inside the bundle-sheath cell is higher, and because oxygen concentration is lower than in the mesophyll cell, the rubisco performs far fewer oxygenase reactions and more carboxylase reactions. This becomes important at high temperatures.



If the rubisco performs only carboxylase activity C4 plants require more energy per carbon fixed. However, if the C3 plant oxygenase/carboxylase ratio is high (e.g., at high temperatures), the energy saved by not performing the oxygenase pathway in C4 plants means that C4 plants may grow faster than C3 plants.

Summary

The light reactions of photosynthesis store energy in the form of ATP and NADPH. The reactions of the Calvin cycle (sometimes referred to by the historical but inaccurate title of the dark reactions) use ATP and NADPH to fix and assimilate carbon dioxide.

The initial reaction is catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) which incorporates one molecule of carbon dioxide into the pentose and releases two 3-phosphoglycerate molecules. The 3-phosphoglycerate molecules are converted to glyceraldehyde-3-phosphate using chloroplast isozymes of the glycolytic enzymes phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase. Some of the glyceraldehyde-3-phosphate is diverted to other processes (such as starch synthesis, sucrose synthesis, or other forms of metabolism); the remainder is converted back to ribulose-1,5-bisphosphate by a series of reactions similar to those in the hexose monophosphate pathway.

The Calvin cycle is stimulated by light, and is turned off in the dark, because light is required to generate the energy required for the carbon assimilation process.

The rubisco enzyme catalyzes a wasteful side reaction using oxygen instead of carbon dioxide, resulting in the photorespiration pathway to regenerate the ribulose-1,5-bisphosphate substrate. C₄ plants have evolved a somewhat more expensive variant of the Calvin cycle that reduces the oxygenase activity of rubisco.

The overall process of photosynthesis uses the energy in light to synthesize carbohydrates, and, in plants, to release oxygen. These processes are required, directly or indirectly, for the survival of the vast majority of known life forms.