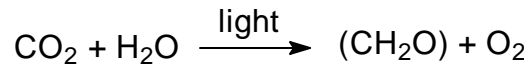


Chapter 18 - Photosynthesis

Introduction

- The capture of solar energy by photosynthetic organisms is the ultimate source of nearly all biological energy. Solar energy is stored as both ATP and NADPH (reducing power).

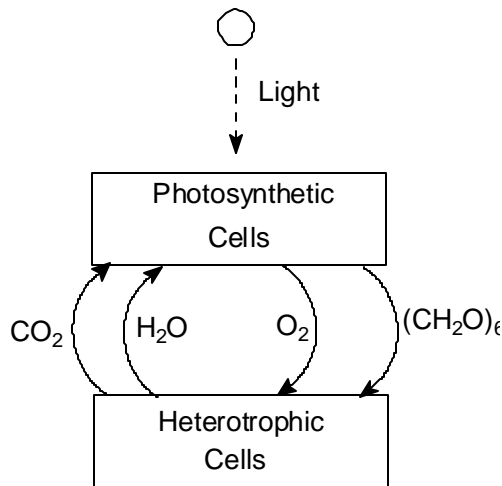
- Plants are thus able to reverse the spontaneous flow of electrons we have been studying in metabolism:



This process is known as carbon fixing. It is estimated that photosynthesis annually “fixes” about 10^{11} tons of carbon, representing an energy storage of over 10^{18} kJ of energy.

Notes:

- Since the above, overall reaction of photosynthesis, is the reversal of the complete catabolism of glucose by heterotrophic organisms, there is an important symbiotic relationship which exists between photosynthetic and heterotrophic organisms:



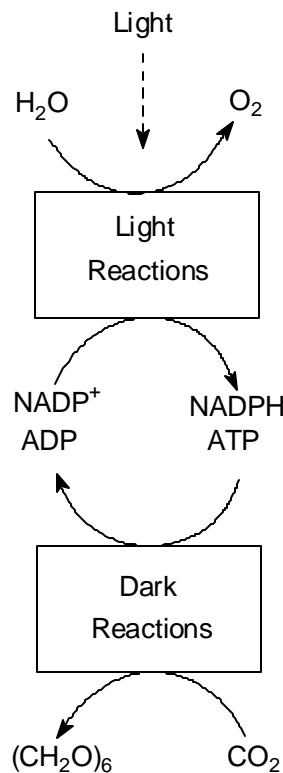
- In the above reaction, water serves as the reducing agent, or the source of electrons. Other electron donors are utilized in nature, including H₂S (green sulfur bacteria) or organic molecules such as lactate.

- This is the reverse of the catabolism of glucose, which includes glycolysis, CAC and electron transport. This implies a very important symbiotic relationship between photosynthetic organisms and heterotrophic organisms, such as ourselves (Our byproducts are the starting materials for photosynthetic organisms, and vice versa).

- In this reaction carbon is reduced (+4 as CO_2 to -1 - +1 as carbohydrate). The reducing agent is H_2O . As in catabolism, electrons are not transferred directly between CO_2 and H_2O ; rather an intermediate redox carrier, NADPH, is used.

- Photosynthesis takes place in two stages, the **light** and **dark** reactions:

1. Specialized pigments absorb light energy in the light reactions, thereby enabling electrons to be removed from H_2O . These electrons are transferred to NADP^+ , and in the process ATP is created, in much the same manner it is created during oxidative phosphorylation. O_2 is generated as water is oxidized.
2. The dark reactions (which also occur in the presence of light) use the ATP and NADPH created in the light reactions to reduce CO_2 to carbohydrate.



Light Reactions :

- Photosynthesis takes place in specialized organelles called **chloroplasts** in eukaryotes.

Chloroplasts resemble mitochondria in many ways, one of which is that they both contain a **double membrane**. The inner membrane of both is highly selective regarding permeability. The **stroma** of a chloroplast is analogous to the matrix of a mitochondrion. Unlike a mitochondrion, a chloroplast contains a membranous third compartment, the **thylakoid** (see Figure 18-1). The thylakoid consists of stacks of disklike sacs, called **grana**. The thylakoid membranes contains the light-harvesting apparatus, the ATP-synthesizing apparatus (very similar to that in oxidative phosphorylation), and an electron transport system also very similar to that in mitochondria. The interior of the thylakoids is the **lumen**

- The light-absorbing pigments of the chloroplasts include **chlorophylls**, which contain four substituted pyrrole rings similar to the heme group of globins and cytochromes (which contain protoporphrin IX). In chlorophylls, the central metal ion is Mg^{++} , rather than Fe^{++} and there is a third, cyclopentanone ring, which fuses one of the pyrrole rings. Chlorophylls also differ from heme in the degree of unsaturation in the pyrrole rings and in the substituents. In plants, the major chlorophylls are chlorophyll a (Chl a) and Chl b. See page 532.

- The chlorophylls absorb light between 400 - 500 nm. Accessory pigments, including **carotenoids** (linear polyenes) such as β -carotene, and in water-dwelling photosynthetic organisms, linear tetrapyrroles known as **phycobilins**, such as **phycoerythrin** and **phycocyanin**, absorb further to the red and serve to “fill in” the absorption spectra (See Figure 18-3 for the absorption spectra of these pigments and p 535 for structures). Carotenoids are largely responsible for the brilliant fall colors of deciduous trees. Phycoerythrin and phycocyanin absorb between 500 - 600 nm because water absorbs light of lower wavelength.

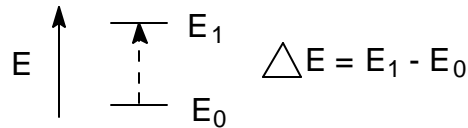
- These light-absorbing pigments are contained within the thylakoid as membrane-bound arrays, or photosynthetic assemblies, at the center of which are the actual transducing pigments called **photosynthetic reaction centers**. These are responsible for converting light energy into chemical energy. They consist of a protein complex and several chlorophylls, as well as several tightly-bound quinones. Surrounding the reaction centers are other pigments which act as antenna, forming a **light-harvesting complex (LHC)**, which passes light energy from molecule to molecule until it reaches a

reaction center.

- Einstein was the first to state that light energy, previously thought of as an electromagnetic wave, is propagated as discrete quanta, the energy of which is given as

$$E = h\nu = hc/\lambda$$

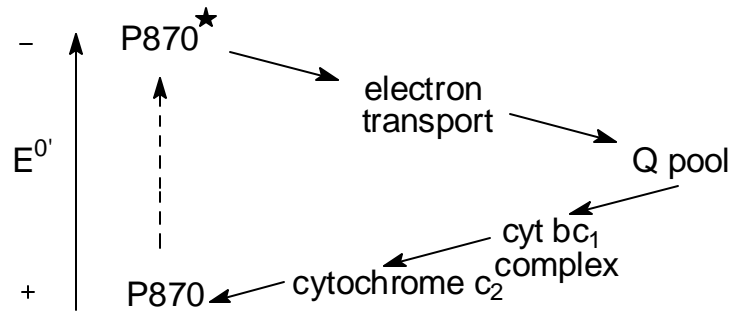
since $\nu\lambda = c$. h = Planck's constant = 6.63×10^{-34} Joule-seconds. Molecules will absorb incident EM radiation when the frequency corresponds to the energy difference, ΔE , between quantized energy levels.



Then $\Delta E = hc/\lambda$, or $\lambda = hc/\Delta E$. Sunlight has wavelengths (400 - 700 nm) that correspond to separations in electronic energy levels of chlorophylls and the accessory pigments. Typically, a photon of sunlight is absorbed to an excited vibrational state within an excited electronic state. Some of this energy is inevitably lost during **internal conversion**, a radiationless transition to a lower vibrational level within the first excited electronic state. A very small percentage of this energy may be lost as **fluorescence** to the ground state. A large percentage of the energy is conserved in the antenna as energy is passed from one molecule to another via **exciton transfer**, in which light energy is funnelled to the reaction centers. The transduction mechanism in the reaction centers is a **photooxidation**, in which an excited pigment molecule donates an electron into an electron transport system similar to that in mitochondria (see Figure 18-6).

- Notice that, whereas electron transport in mitochondria is an exergonic process in which electrons are transferred from a good donor, NADH, to a good acceptor, O_2 , electron transfer in photosynthetic organisms is from a poor donor, H_2O , to a poor acceptor, $NADP^+$. Due to the excitation of an electron in a reaction center, followed by photooxidation, electrons are extracted from the water, which constitutes the transduction process in which light energy is coupled to an otherwise endergonic electron transport process, rendering the overall process exergonic.

- In purple photosynthetic bacteria, electron transport is a cyclic process in which the reaction center, known as **P870** in one species (*Rhodospseudomonas viridis*), serves both as electron acceptor as well as donor:



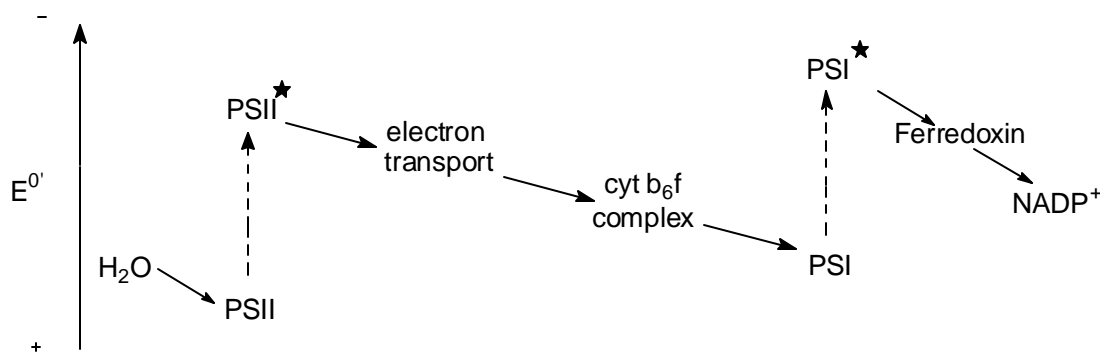
- Note the ordinate, arranged with negative E^0 values at the top, positive at the bottom, such that exergonic electron transfers are depicted as being downhill.

- Note that in this scheme the cytochrome bc_1 complex and cytochrome c_2 are analogous to system III and cytochrome c , respectively, in mitochondria.

- Note also that since P870 is the donor as well as acceptor, no NADPH is formed during this cyclic process.

- Electron transport in this membrane-bound process is accompanied by a translocation of H^+ across the membrane, the dissipation of which drives a chloroplast version of an ATPase in reverse, resulting in ATP synthesis.

- In plants and cyanobacteria (formerly called blue-green algae) photosynthesis is a noncyclic process in which the final electron acceptor is $NADP^+$, resulting in NADPH production as well as ATP synthesis:



- This is known as the **Z scheme**.

- The photo-absorbing center of PSII is named P680, and that of PSI is named P700 after their wavelengths of maximal light absorption.

- The electron transport system of plants consists of **FeS centers**, **cytochromes** and **quinones** which are similar to those in mitochondria quinones. The **cytochrome b₆f** complex is analogous to system III in mitochondria. This complex passes electrons to a mobile protein, **plastocyanin**, which is analogous to cytochrome c.

- PSII contains an **oxygen-evolving center (OEC)**, which extracts electrons from H₂O and donates them to PSII. The OEC contains 4 protein-bound Mn ions, as well as 2 or 3 Ca⁺⁺ ions and 4 or 5 Cl⁻ ions. The Mn ions, with their unfilled d orbitals, can exist in several oxidation states, which is useful in their role in accepting electrons from water.

- ATP is synthesized as a proton gradient created during electron transport is dissipated through a chlorophyll version of the mitochondrial ATPase.

- Each O₂ molecule requires 4 electrons from 2 H₂O molecules. In this way 4 H⁺ are translocated, as well as 8 more pumped through the cyt b₆f complex. These 4 electrons are donated to 2 NADP⁺ to form 2 NADPH, which as we've seen are "worth" 6 ATP as this reducing power drives electrons through a mitochondrial electron transport system (in the cytoplasm of plants).

Experimentally, it is found that 1 ATP is synthesized per 3 H⁺, and 2 photons must be absorbed per 1 electron donated from H₂O. The number of ATP molecules synthesized per photon absorbed can thus be determined:

$$(4 + 8) \text{ H}^+ / 4 \text{ electrons} \times 1 (\text{ATP}/3 \text{ H}^+) = 4 \text{ ATP}/4 \text{ e}$$

via the Z scheme shown above. Added to this are 6 ATP (via the 2 NADPH), or 10 ATP per O₂.

Thus

$$(10 \text{ ATP}/\text{O}_2)(1 \text{ O}_2/4 \text{ e}^-)(1 \text{ e}^-/\text{photon}) = 1.25 \text{ ATP}/\text{photon}$$

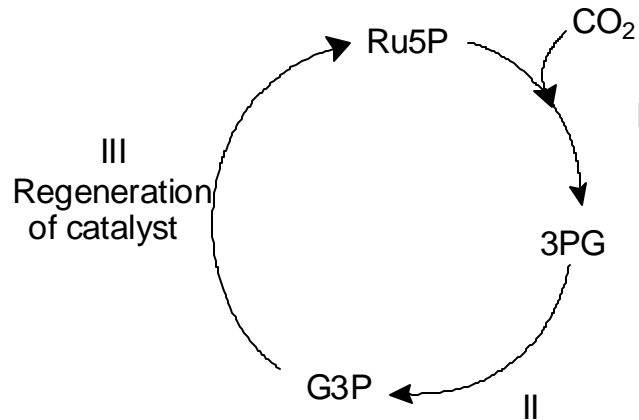
Dark Reactions

- The dark reactions actually take place predominantly during light hours, but do not directly require light. It is in the dark reactions that CO₂ is incorporated into organic molecules.

- Calvin (early '50's), by adding radiolabeled CO₂ to green algae, determined that the label initially wound up as a glycolytic intermediate, **3-phosphoglycerate (3PG)**. A search for a 2-Carbon precursor was unsuccessful. Eventually it was determined that CO₂ incorporation involves a cyclic,

catalytic process, and that the catalyst is a 5-Carbon molecules familiar to us from the nonoxidative branch of the pentose phosphate pathway, ribulose **5-phosphate (ru5P)**.

- The catalytic process can be thought of as occurring in three stages, I - III, all of which occur in the stroma of the chloroplast.

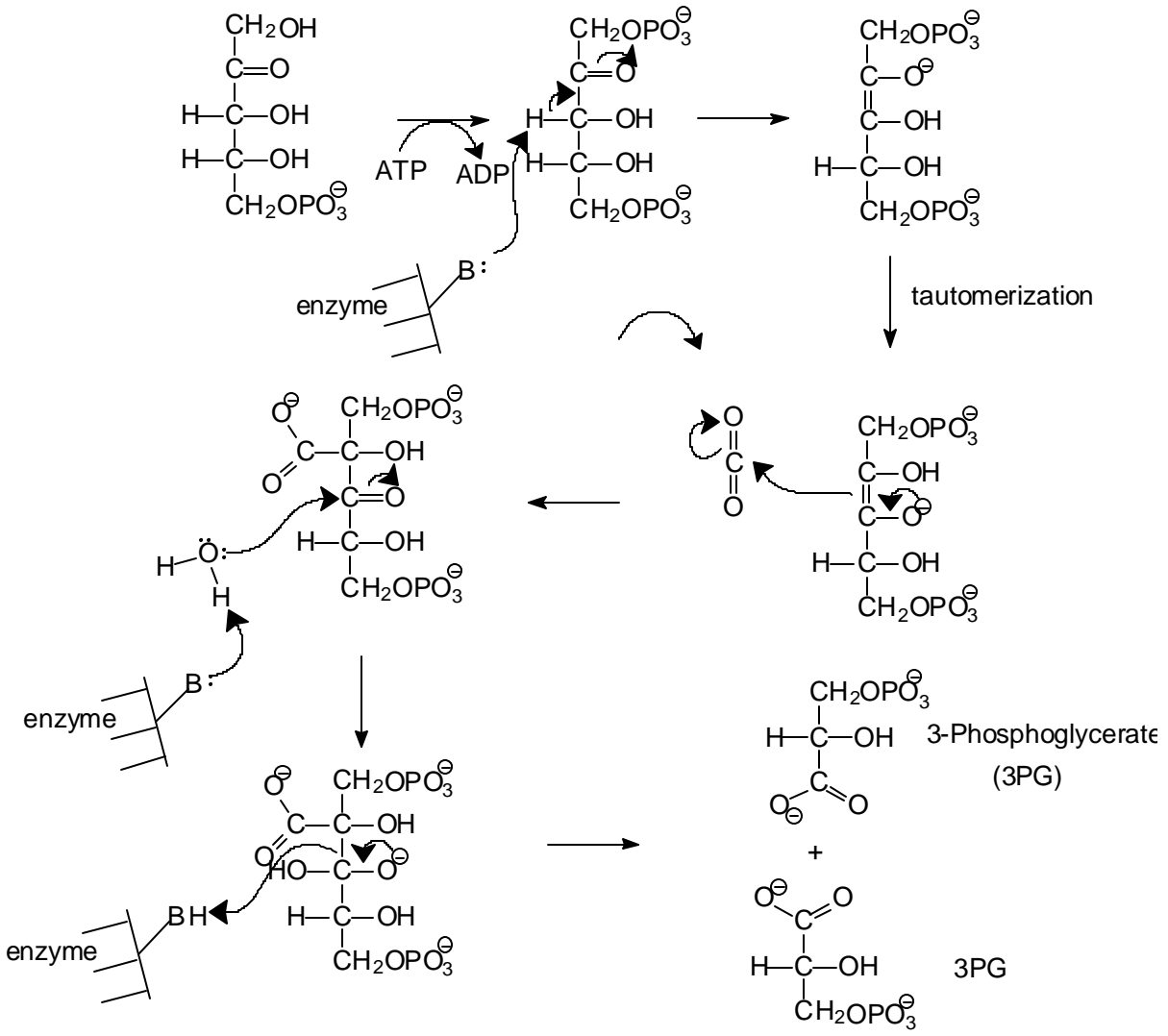


- Stage I corresponds to carbon fixation, Stage II to reduction of 3PG to glyceraldehyde 3-phosphate (G3P) in a series of reactions identical to the portions of gluconeogenesis, and Stage III, the regeneration of the catalyst, Ru5P. (Note that your text describes the dark reactions, combining stages I and II).

Stage I

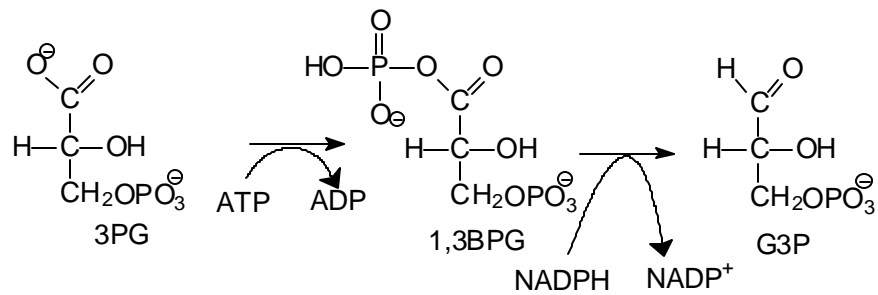
- CO₂ fixation actually involves ribulose-1,5-bisphosphate (RuBP). The enzyme involved in incorporating CO₂ into RuBP is RuBP carboxylase, one of the world's most important enzymes. Approximately 10¹¹ tons of carbon are incorporated into biomolecules in this way annually.

- Beginning with the catalyst, Ru5P, stage I proceeds as follows:



Stage II

- This reductive stage consists of several reactions identical to those in gluconeogenesis:

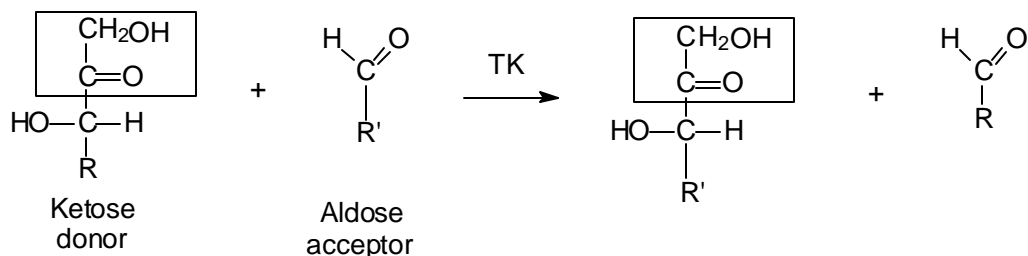


- The glyceraldehyde 3-phosphate thus formed has various possible fates. Keep in mind that each “round” of the Calvin Cycle incorporates one carbon atom. Thus three rounds are necessary to produce one net three-carbon G3P from CO₂ (although 3 G3P are actually formed, each of which incorporates one carbon from CO₂). Since each round of the cycle results in two molecules of G3P, three rounds will produce six G3P, five of which (containing 5 x 3 = 15 carbons) must be used to regenerate the three molecules of catalyst necessary for the three rounds of the cycle. In other words, every three rounds of the cycle results in production of one additional G3P. The other five must enter stage III, regeneration of catalyst. The sixth, additional, G3P can be converted to fructose 6-phosphate via gluconeogenesis-related reactions in the stroma, then to starch. Alternatively, the additional G3P can be converted to dihydroxyacetone phosphate (DHAP), which can leave the chloroplast and undergo glycolysis to generate energy via glycolysis in the cytosol. Some of the cytosolic DHAP is converted to sucrose.

Stage III

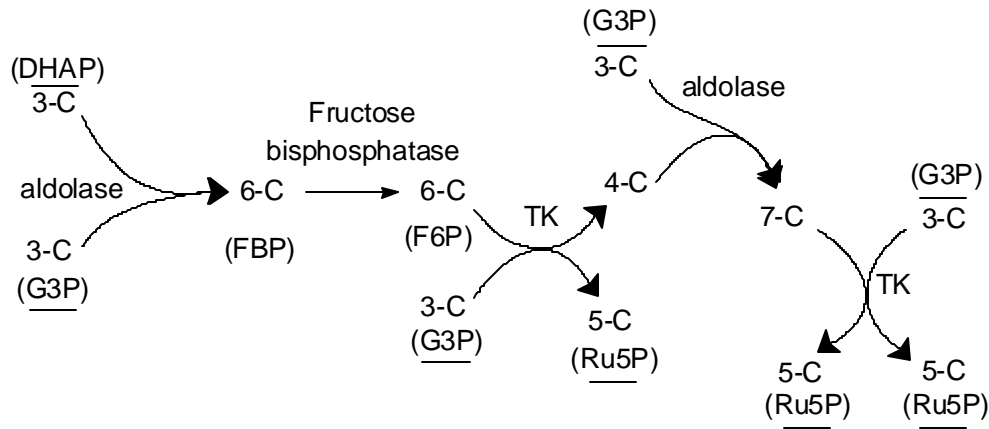
- This recovery stage is very similar to reactions in the nonoxidative branch of the pentose phosphate pathway. Recall that in this pathway, the five carbons of ribose 5-phosphate are re-routed into the three- and six-carbon intermediates of glycolysis. This was accomplished by transaldolase (TA) and transketolase (TK), which transfer two- and three-carbon fragments, respectively.

Transketolase is the only one of these enzymes required in the Calvin Cycle:



- Transketolase, as well as various enzymes of glycolysis (aldolase) and gluconeogenesis (fructose biphosphatase) are used in this recovery stage of the Calvin Cycle to convert the fifteen carbons of three molecules of G3P to to fifteen carbons of three molecules of Ru5P. Only one enzyme not seen in pathways previously covered is necessary (sedoheptulose biphosphatase). Keep in mind that these five molecules of G3P are produced during stages I and I of three rounds of the cycle, the sixth molecule of G3P having been siphoned off into carbohydrate biosynthesis.

- The essence of this process, outlined in more detail in Figure 18-20, is shown below:



The three-carbon G3P (and DHAP - precursors) and five-carbon Ru5P (products) are underlined.

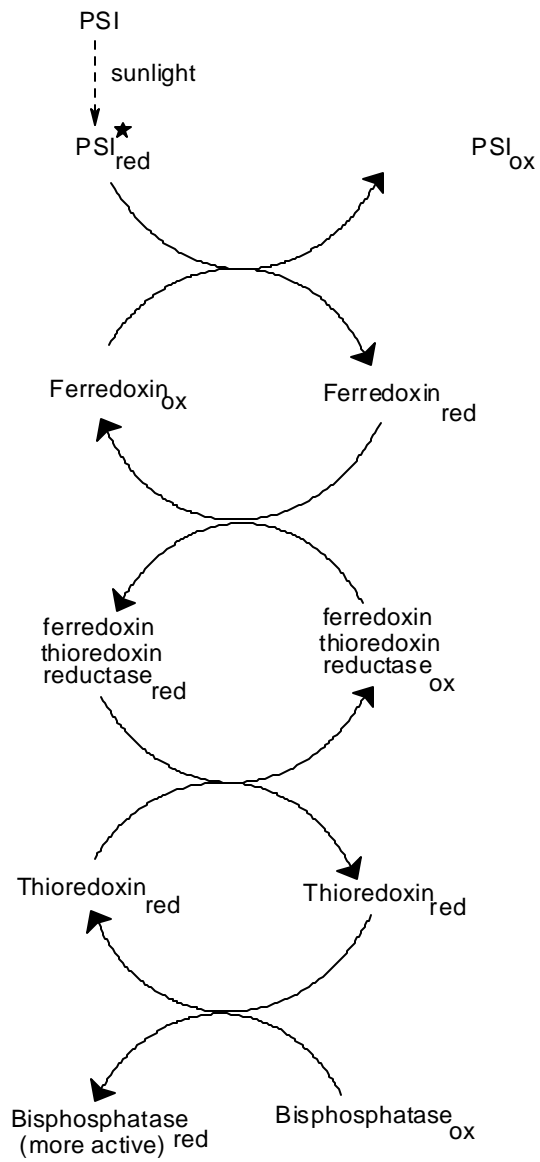
Regulation

- The Calvin Cycle is energy-requiring. The only detail you need to know from Figure 18-3 is that three ATP and two NADPH are required per CO₂ incorporated into G3P. Glycolysis, on the other hand is energy-yielding. Thus, if both the Calvin Cycle and glycolysis were both active at the same time, a wasteful futile cycling would result. Hence the need for regulation. The light and dark reactions are active during daylight; at night glycolysis, and also the pentose phosphate pathway, must supply ATP and the reducing power of NADPH.

- The light reactions result in both a pH gradient across the thylakoid membrane separating stroma from lumen (as protons are pumped into the lumen via photo-electron transport, the pH in the stroma increases as it becomes increasingly basic. Ru5P carboxylase has a pH optimum of about 8. Thus the activity of this enzyme increases during daylight hours.

- As H⁺ are pumped into the lumen, Mg⁺⁺ are pumped out (antiport). Mg⁺⁺ stimulates Ru5P carboxylase.

- Sunlight also results in photooxidation, thus producing strong reducing agents, in this case Photosystem I (PSI & PSI*). PSI* indirectly reduces a disulfide linkage in both fructose bisphosphatase and sedoheptulose bisphosphatase, thereby increasing their activity.



Problems: 2, 4, 6, 8