Chapter

8 Photosynthesis: Carbon Reactions

IN CHAPTER 5 WE DISCUSSED plants' requirements for mineral nutrients and light in order to grow and complete their life cycle. Because living organisms interact with one another and their environment, mineral nutrients cycle through the biosphere. These cycles involve complex interactions, and each cycle is critical in its own right. Because the amount of matter in the biosphere remains constant, energy must be supplied to keep the cycles operational. Otherwise increasing entropy dictates that the flow of matter would ultimately stop.

Autotrophic organisms have the ability to convert physical and chemical sources of energy into carbohydrates in the absence of organic substrates. Most of the external energy is consumed in transforming CO_2 to a reduced state that is compatible with the needs of the cell (—CHOH—).

Recent estimates indicate that about 200 billion tons of CO_2 are converted to biomass each year. About 40% of this mass originates from the activities of marine phytoplankton. The bulk of the carbon is incorporated into organic compounds by the carbon reduction reactions associated with photosynthesis.

In Chapter 7 we saw how the photochemical oxidation of water to molecular oxygen is coupled to the generation of ATP and reduced pyridine nucleotide (NADPH) by reactions taking place in the chloroplast thylakoid membrane. The reactions catalyzing the reduction of CO_2 to carbohydrate are coupled to the consumption of NADPH and ATP by enzymes found in the stroma, the soluble phase of chloroplasts.

These stroma reactions were long thought to be independent of light and, as a consequence, were referred to as the *dark reactions*. However, because these stroma-localized reactions depend on the products of the photochemical processes, and are also directly regulated by light, they are more properly referred to as the *carbon reactions of photosynthesis*.

In this chapter we will examine the cyclic reactions that accomplish fixation and reduction of CO_2, then consider how the phenomenon of photorespiration catalyzed by the carboxylating enzyme alters the effi-

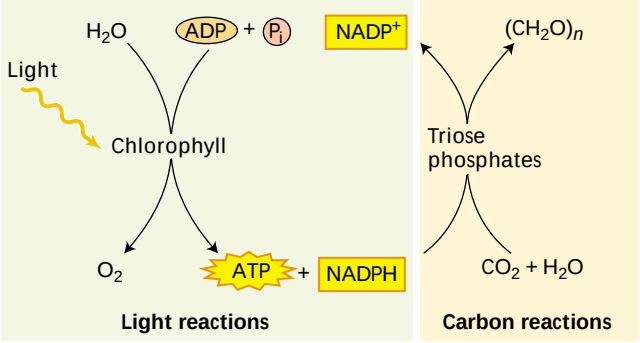


FIGURE 8.1 The light and carbon reactions of photosynthesis. Light is required for the generation of ATP and NADPH. The ATP and NADPH are consumed by the carbon reactions, which reduce CO_2 to carbohydrate (triose phosphates).

ciency of photosynthesis. This chapter will also describe biochemical mechanisms for concentrating carbon dioxide that allow plants to mitigate the impact of photorespiration: CO_2 pumps, C_4 metabolism, and crassulacean acid metabolism (CAM). We will close the chapter with a consideration of the synthesis of sucrose and starch.

THE CALVIN CYCLE

All photosynthetic eukaryotes, from the most primitive alga to the most advanced angiosperm, reduce CO_2 to carbohydrate via the same basic mechanism: the photosynthetic carbon reduction cycle originally described for C_3 species (the **Calvin cycle**, or **reductive pentose phosphate [RPP] cycle**). Other metabolic pathways associated with the photosynthetic fixation of CO_2, such as the C_4 photosynthetic carbon assimilation cycle and the photorespiratory carbon oxidation cycle, are either auxiliary to or dependent on the basic Calvin cycle.

In this section we will examine how CO_2 is fixed by the Calvin cycle through the use of ATP and NADPH generated by the light reactions (Figure 8.1), and how the Calvin cycle is regulated.

The Calvin Cycle Has Three Stages: Carboxylation, Reduction, and Regeneration

The Calvin cycle was elucidated as a result of a series of elegant experiments by Melvin Calvin and his colleagues in the 1950s, for which a Nobel Prize was awarded in 1961 (see **Web Topic 8.1**). In the Calvin cycle, CO_2 and water from the environment are enzymatically combined with a five-carbon acceptor molecule to generate two molecules of a three-carbon intermediate. This intermediate (3-phosphoglycerate) is reduced to carbohydrate by use of the ATP and NADPH generated photochemically. The cycle is completed by regeneration of the five-carbon acceptor (ribulose-1,5-bisphosphate, abbreviated RuBP).

The Calvin cycle proceeds in three stages (Figure 8.2):

1. *Carboxylation* of the CO_2 acceptor ribulose-1,5-bisphosphate, forming two molecules of 3-phosphoglycerate, the first stable intermediate of the Calvin cycle
2. *Reduction* of 3-phosphoglycerate, forming gyceraldehyde-3-phosphate, a carbohydrate
3. *Regeneration* of the CO_2 acceptor ribulose-1,5-bisphosphate from glyceraldehyde-3-phosphate

The carbon in CO_2 is the most oxidized form found in nature (+4). The carbon of the first stable intermediate, 3-phosphoglycerate, is more reduced (+3), and it is further reduced in the glyceraldehyde-3-phosphate product (+1). Overall, the early reactions of the Calvin cycle complete the reduction of atmospheric carbon and, in so doing, facilitate its incorporation into organic compounds.

The Carboxylation of Ribulose Bisphosphate Is Catalyzed by the Enzyme Rubisco

CO_2 enters the Calvin cycle by reacting with ribulose-1,5-bisphosphate to yield two molecules of 3-phosphoglycerate (Figure 8.3 and Table 8.1), a reaction catalyzed by the chloroplast enzyme ribulose bisphosphate carboxylase/oxygenase, referred to as **rubisco** (see **Web Topic 8.2**). As indi-

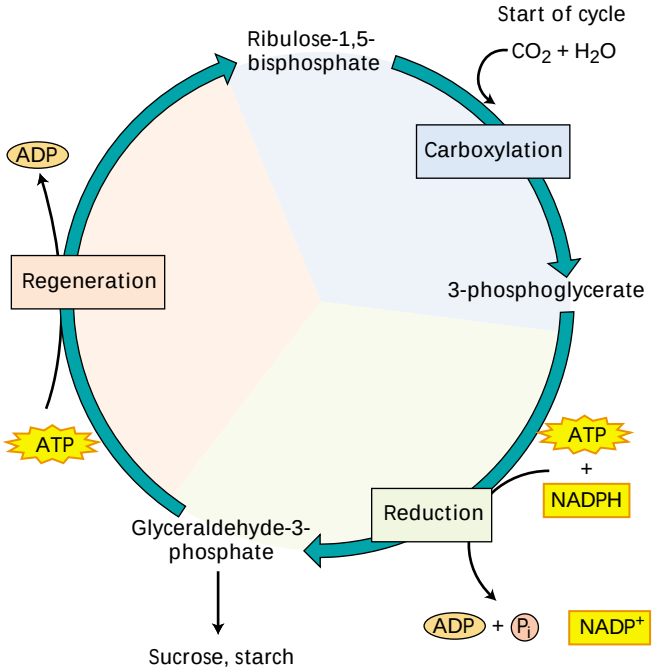


FIGURE 8.2 The Calvin cycle proceeds in three stages: (1) carboxylation, during which CO_2 is covalently linked to a carbon skeleton; (2) reduction, during which carbohydrate is formed at the expense of the photochemically derived ATP and reducing equivalents in the form of NADPH; and (3) regeneration, during which the CO_2 acceptor ribulose-1,5-bisphosphate re-forms.

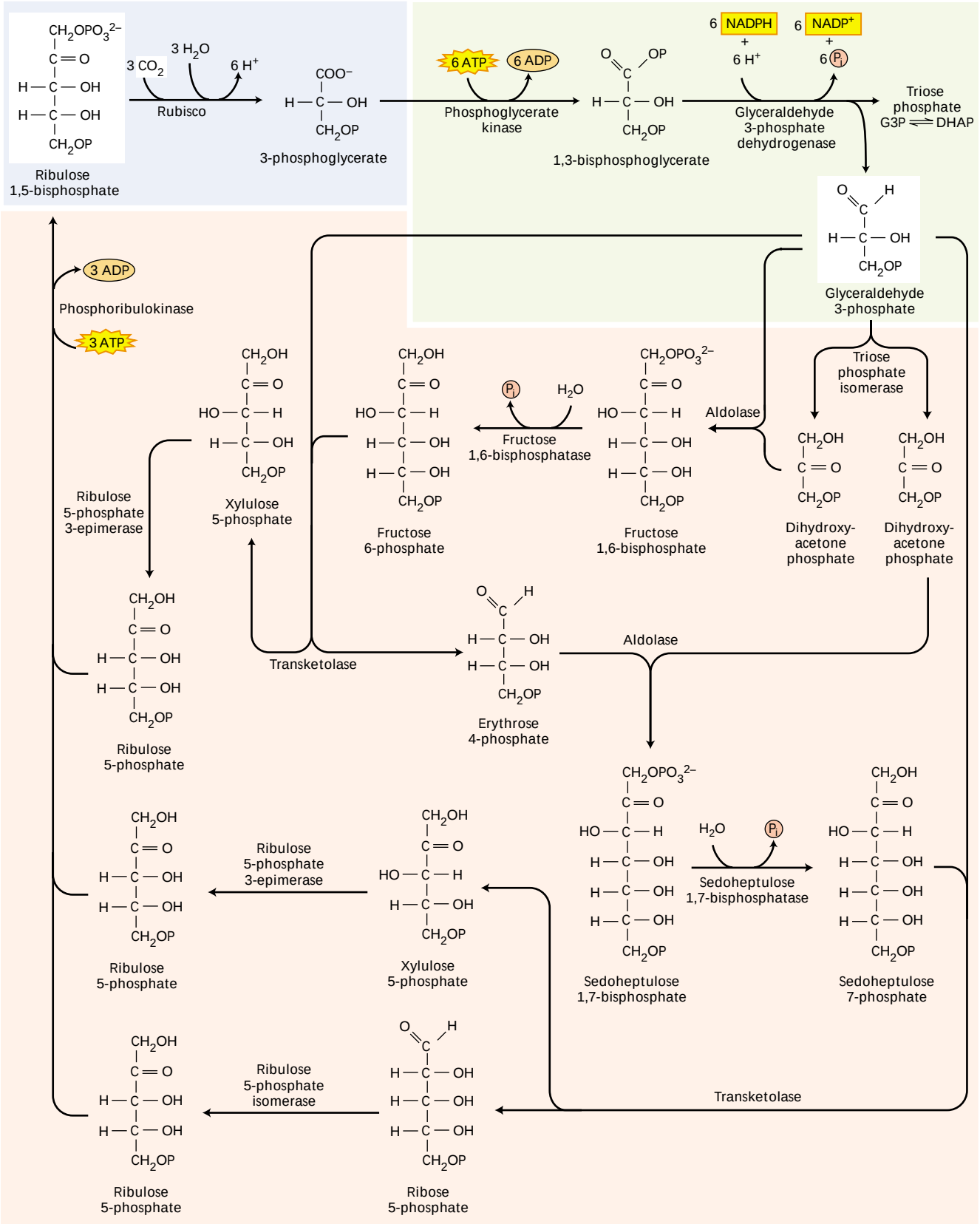


FIGURE 8.3 The Calvin cycle. The carboxylation of three molecules of ribulose-1,5-bisphosphate leads to the *net* synthesis of one molecule of glyceraldehyde-3-phosphate and the regeneration of the three molecules of starting material. This process starts and ends with three molecules of ribulose-1,5-bisphosphate, reflecting the cyclic nature of the pathway.

TABLE 8.1
Reactions of the Calvin cycle

Enzyme	Reaction
1. Ribulose-1,5-bisphosphate carboxylase/oxygenase	6 Ribulose-1,5-bisphosphate + 6 CO_2 + 6 H_2O → 12 (3-phosphoglycerate) + 12 H^+
2. 3-Phosphoglycerate kinase	12 (3-Phosphoglycerate) + 12 ATP → 12 (1,3-bisphosphoglycerate) + 12 ADP
3. NADP:glyceraldehyde-3-phosphate dehydrogenase	12 (1,3-Bisphosphoglycerate) + 12 NADPH + 12 H^+ → 12 glyceraldehye-3-phosphate + 12 $NADP^+$ + 12 P_i
4. Triose phosphate isomerase	5 Glyceraldehyde-3-phosphate → 5 dihydroxyacetone-3-phosphate
5. Aldolase	3 Glyceraldehyde-3-phosphate + 3 dihydroxyacetone-3-phosphate → 3 fructose-1,6-bisphosphate
6. Fructose-1,6-bisphosphatase	3 Fructose-1,6-bisphosphate + 3 H_2O → 3 fructose-6-phosphate + 3 P_i
7. Transketolase	2 Fructose-6-phosphate + 2 glyceraldehyde-3-phosphate → 2 erythrose-4-phosphate + 2 xylulose-5-phosphate
8. Aldolase	2 Erythrose-4-phosphate + 2 dihydroxyacetone-3-phosphate → 2 sedoheptulose-1,7-bisphosphate
9. Sedoheptulose-1,7,bisphosphatase	2 Sedoheptulose-1,7-bisphosphate + 2 H_2O → 2 sedoheptulose-7-phosphate + 2 P_i
10. Transketolase	2 Sedoheptulose-7-phosphate + 2 glyceraldehyde-3-phosphate → 2 ribose-5-phosphate + 2 xylulose-5-phosphate
11a. Ribulose-5-phosphate epimerase	4 Xylulose-5-phosphate → 4 ribulose-5-phosphate
11b. Ribose-5-phosphate isomerase	2 Ribose-5-phosphate → 2 ribulose-5-phosphate
12. Ribulose-5-phosphate kinase	6 Ribulose-5-phosphate + 6 ATP → 6 ribulose-1,5-bisphosphate + 6 ADP + 6 H^+
Net: 6 CO_2 + 11 H_2O + 12 NADPH + 18 ATP → Fructose-6-phosphate + 12 $NADP^+$ + 6 H^+ + 18 ADP + 17 P_i	

Note: P_i stands for inorganic phosphate.

cated by the full name, the enzyme also has an oxygenase activity in which O_2 competes with CO_2 for the common substrate ribulose-1,5-bisphosphate (Lorimer 1983). As we will discuss later, this property limits net CO_2 fixation.

As shown in Figure 8.4, CO_2 is added to carbon 2 of ribulose-1,5-bisphosphate, yielding an unstable, enzyme-bound intermediate, which is hydrolyzed to yield two molecules of the stable product 3-phosphoglycerate (see Table 8.1, reaction 1). The two molecules of 3-phosphoglycerate—labeled "upper" and "lower" on the figure—are distinguished by the fact that the upper molecule contains the newly incorporated carbon dioxide, designated here as $*CO_2$.

Two properties of the carboxylase reaction are especially important:

1. The negative change in free energy (see Chapter 2 on the web site for a discussion of free energy) associated with the carboxylation of ribulose-1,5-bisphosphate is large; thus the forward reaction is strongly favored.
2. The affinity of rubisco for CO_2 is sufficiently high to ensure rapid carboxylation at the low concentrations of CO_2 found in photosynthetic cells.

Rubisco is very abundant, representing up to 40% of the total soluble protein of most leaves. The concentration of rubisco active sites within the chloroplast stroma is calculated to be about 4 m*M*, or about 500 times greater than the concentration of its CO_2 substrate (see **Web Topic 8.3**).

Triose Phosphates Are Formed in the Reduction Step of the Calvin Cycle

Next in the Calvin cycle (Figure 8.3 and Table 8.1), the 3-phosphoglycerate formed in the carboxylation stage undergoes two modifications:

1. It is first phosphorylated via 3-phosphoglycerate kinase to 1,3-bisphosphoglycerate through use of the ATP generated in the light reactions (Table 8.1, reaction 2).
2. Then it is reduced to glyceraldehyde-3-phosphate through use of the NADPH generated by the light reactions (Table 8.1, reaction 3). The chloroplast enzyme NADP:glyceraldehyde-3-phosphate dehydrogenase catalyzes this step. Note that the enzyme is similar to that of glycolysis (which will be dis-

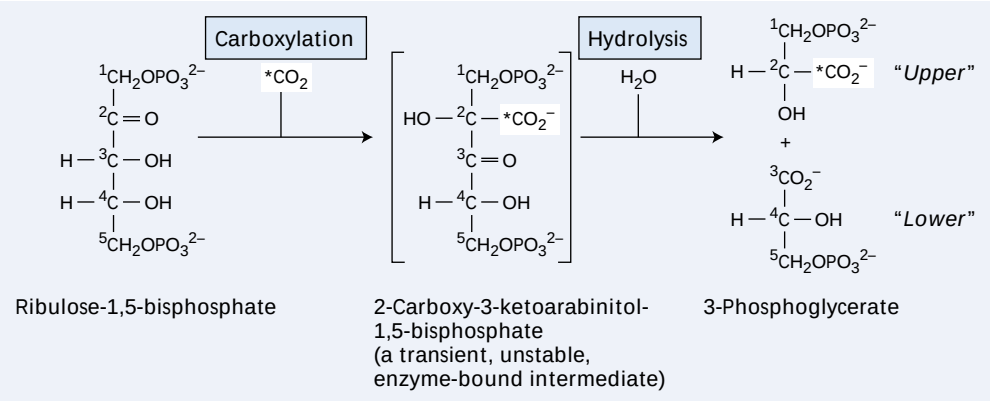


FIGURE 8.4 The carboxylation of ribulose-1,5-bisphosphate by rubisco.

cussed in Chapter 11), except that NADP rather than NAD is the coenzyme. An NADP-linked form of the enzyme is synthesized during chloroplast development (greening), and this form is preferentially used in biosynthetic reactions.

Operation of the Calvin Cycle Requires the Regeneration of Ribulose-1,5-Bisphosphate

The continued uptake of CO_2 requires that the CO_2 acceptor, ribulose-1,5-bisphosphate, be constantly regenerated. To prevent depletion of Calvin cycle intermediates, three molecules of ribulose-1,5-bisphosphate (15 carbons total) are formed by reactions that reshuffle the carbons from the five molecules of triose phosphate ($5 \times 3 = 15$ carbons). This reshuffling consists of reactions 4 through 12 in Table 8.1 (see also Figure 8.3):

1. One molecule of glyceraldehyde-3-phosphate is converted via triose phosphate isomerase to dihydroxyacetone-3-phosphate in an isomerization reaction (reaction 4).
2. Dihydroxyacetone-3-phosphate then undergoes aldol condensation with a second molecule of glyceraldehyde-3-phosphate, a reaction catalyzed by aldolase to give fructose-1,6-bisphosphate (reaction 5).
3. Fructose-1,6-bisphosphate occupies a key position in the cycle and is hydrolyzed to fructose-6-phosphate (reaction 6), which then reacts with the enzyme transketolase.
4. A two-carbon unit (C-1 and C-2 of fructose-6-phosphate) is transferred via transketolase to a third molecule of glyceraldehyde-3-phosphate to give erythrose-4-phosphate (from C-3 to C-6 of the fructose) and xylulose-5-phosphate (from C-2 of the fructose and the glyceraldehyde-3-phosphate) (reaction 7).
5. Erythrose-4-phosphate then combines via aldolase with a fourth molecule of triose phosphate (dihydroxyacetone-3-phosphate) to yield the seven-carbon sugar sedoheptulose-1,7-bisphosphate (reaction 8).
6. This seven-carbon bisphosphate is then hydrolyzed by way of a specific phosphatase to give sedoheptulose-7-phosphate (reaction 9).
7. Sedoheptulose-7-phosphate donates a two-carbon unit to the fifth (and last) molecule of glyceraldehyde-3-phosphate via transketolase and produces ribose-5-phosphate (from C-3 to C-7 of sedoheptulose) and xylulose-5-phosphate (from C-2 of the sedoheptulose and the glyceraldehyde-3-phosphate) (reaction 10).
8. The two molecules of xylulose-5-phosphate are converted to two molecules of ribulose-5-phosphate sugars by a ribulose-5-phosphate epimerase (reaction 11a). The third molecule of ribulose-5-phosphate is formed from ribose-5-phosphate by ribose-5-phosphate isomerase (reaction 11b).
9. Finally, ribulose-5-phosphate kinase catalyzes the phosphorylation of ribulose-5-phosphate with ATP, thus regenerating the three needed molecules of the initial CO_2 acceptor, ribulose-1,5-bisphosphate (reaction 12).

The Calvin Cycle Regenerates Its Own Biochemical Components

The Calvin cycle reactions regenerate the biochemical intermediates that are necessary to maintain the operation of the cycle. But more importantly, the rate of operation of the Calvin cycle can be enhanced by increases in the concentration of its intermediates; that is, the cycle is **autocatalytic**. As a consequence, the Calvin cycle has the metabolically desirable feature of producing more substrate than is consumed, as long as triose phosphate is not being diverted elsewhere:

$$5\ RuBP^{4-} + 5\ CO_2 + 9\ H_2O + 16\ ATP^{4-} + 10\ NADPH \rightarrow 6\ RuBP^{4-} + 14\ P_i + 6\ H^+ + 16\ ADP^{3-} + 10\ NADP^+$$

The importance of this autocatalytic property is shown by experiments in which previously darkened leaves or isolated chloroplasts are illuminated. In such experiments, CO_2 fixation starts only after a lag, called the *induction period*, and the rate of photosynthesis increases with time in the first few minutes after the onset of illumination. The

increase in the rate of photosynthesis during the induction period is due in part to the activation of enzymes by light (discussed later), and in part to an increase in the concentration of intermediates of the Calvin cycle.

Calvin Cycle Stoichiometry Shows That Only One-Sixth of the Triose Phosphate Is Used for Sucrose or Starch

The synthesis of carbohydrates (starch, sucrose) provides a sink ensuring an adequate flow of carbon atoms through the Calvin cycle under conditions of continuous CO_2 uptake. An important feature of the cycle is its overall stoichiometry. At the onset of illumination, most of the triose phosphates are drawn back into the cycle to facilitate the buildup of an adequate concentration of metabolites. When photosynthesis reaches a steady state, however, five-sixths of the triose phosphate contributes to regeneration of the ribulose-1,5-bisphosphate, and one-sixth is exported to the cytosol for the synthesis of sucrose or other metabolites that are converted to starch in the chloroplast.

An input of energy, provided by ATP and NADPH, is required in order to keep the cycle functioning in the fixation of CO_2. The calculation at the end of Table 8.1 shows that in order to synthesize the equivalent of 1 molecule of hexose, 6 molecules of CO_2 are fixed at the expense of 18 ATP and 12 NADPH. In other words, the Calvin cycle consumes two molecules of NADPH and three molecules of ATP for every molecule of CO_2 fixed into carbohydrate.

We can compute the maximal overall thermodynamic efficiency of photosynthesis if we know the energy content of the light, the minimum quantum requirement (moles of quanta absorbed per mole of CO_2 fixed; see Chapter 7), and the energy stored in a mole of carbohydrate (hexose).

Red light at 680 nm contains 175 kJ (42 kcal) per quantum mole of photons. The minimum quantum requirement is usually calculated to be 8 photons per molecule of CO_2 fixed, although the number obtained experimentally is 9 to 10 (see Chapter 7). Therefore, the minimum light energy needed to reduce 6 moles of CO_2 to a mole of hexose is approximately $6 \times 8 \times 175$ kJ = 8400 kJ (2016 kcal). However, a mole of a hexose such as fructose yields only 2804 kJ (673 kcal) when totally oxidized.

Comparing 8400 and 2804 kJ, we see that the maximum overall thermodynamic efficiency of photosynthesis is about 33%. However, most of the unused light energy is lost in the generation of ATP and NADPH by the light reactions (see Chapter 7) rather than during operation of the Calvin cycle.

We can calculate the efficiency of the Calvin cycle more directly by computing the changes in free energy associated with the hydrolysis of ATP and the oxidation of NADPH, which are 29 and 217 kJ (7 and 52 kcal) per mole, respectively. We saw in the list summarizing the Calvin cycle reactions that the synthesis of 1 molecule of fructose-6-phosphate from 6 molecules of CO_2 uses 12 NADPH and 18 ATP molecules. Therefore the Calvin cycle consumes $(12 \times 217) + (18 \times 29) = 3126$ kJ (750 kcal) in the form of NADPH and ATP, resulting in a thermodynamic efficiency close to 90%.

An examination of these calculations shows that the bulk of the energy required for the conversion of CO_2 to carbohydrate comes from NADPH. That is, 2 mol NADPH $\times$ 52 kcal mol^{-1} = 104 kcal, but 3 mol ATP $\times$ 7 kcal mol^{-1} = 21 kcal. Thus, 83% (104 of 125 kcal) of the energy stored comes from the reductant NADPH.

The Calvin cycle does not occur in all autotrophic cells. Some anaerobic bacteria use other pathways for autotrophic growth:

- The ferredoxin-mediated synthesis of organic acids from acetyl– and succinyl– CoA derivatives via a reversal of the citric acid cycle (the reductive carboxylic acid cycle of green sulfur bacteria)
- The glyoxylate-producing cycle (the hydroxypropionate pathway of green nonsulfur bacteria)
- The linear route (acetyl-CoA pathway) of acetogenic, methanogenic bacteria

Thus although the Calvin cycle is quantitatively the most important pathway of autotrophic CO_2 fixation, others have been described.

REGULATION OF THE CALVIN CYCLE

The high energy efficiency of the Calvin cycle indicates that some form of regulation ensures that all intermediates in the cycle are present at adequate concentrations and that the cycle is turned off when it is not needed in the dark. In general, variation in the concentration or in the specific activity of enzymes modulates catalytic rates, thereby adjusting the level of metabolites in the cycle.

Changes in gene expression and protein biosynthesis regulate enzyme concentration. Posttranslational modification of proteins contributes to the regulation of enzyme activity. At the genetic level the amount of each enzyme present in the chloroplast stroma is regulated by mechanisms that control expression of the nuclear and chloroplast genomes (Maier et al. 1995; Purton 1995).

Short-term regulation of the Calvin cycle is achieved by several mechanisms that optimize the concentration of intermediates. These mechanisms minimize reactions operating in opposing directions, which would waste resources (Wolosiuk et al. 1993). Two general mechanisms can change the kinetic properties of enzymes:

1. The transformation of covalent bonds such as the reduction of disulfides and the carbamylation of amino groups, which generate a chemically modified enzyme.
2. The modification of noncovalent interactions, such as the binding of metabolites or changes in the composi-

tion of the cellular milieu (e.g., pH). In addition, the binding of the enzymes to the thylakoid membranes enhances the efficiency of the Calvin cycle, thereby achieving a higher level of organization that favors the channeling and protection of substrates.

Light-Dependent Enzyme Activation Regulates the Calvin Cycle

Five light-regulated enzymes operate in the Calvin cycle:

1. Rubisco
2. NADP:glyceraldehyde-3-phosphate dehydrogenase
3. Fructose-1,6-bisphosphatase
4. Sedoheptulose-1,7-bisphosphatase
5. Ribulose-5-phosphate kinase

The last four enzymes contain one or more disulfide (—S—S—) groups. Light controls the activity of these four enzymes via the **ferredoxin–thioredoxin system**, a covalent thiol-based oxidation–reduction mechanism identified by Bob Buchanan and colleagues (Buchanan 1980; Wolosiuk et al. 1993; Besse and Buchanan 1997; Schürmann and Jacquot 2000). In the dark these residues exist in the oxidized state (—S—S—), which renders the enzyme inactive or subactive. In the light the —S—S— group is reduced to the sulfhydryl state (—SH HS—). This redox change leads to activation of the enzyme (Figure 8.5). The resolution of the crystal structure of each member of the ferredoxin–thioredoxin system and of the target enzymes fructose-1,6-bisphosphatase and NADP:malate dehydrogenase (Dai et al. 2000) have provided valuable information about the mechanisms involved.

This sulfhydryl (also called dithiol) signal of the regulatory protein thioredoxin is transmitted to specific target enzymes, resulting in their activation (see **Web Topic 8.4**). In some cases (such as fructose-1,6-bisphosphatase), the thioredoxin-linked activation is enhanced by an effector (e.g., fructose-1,6-bisphosphate substrate).

Inactivation of the target enzymes observed upon darkening appears to take place by a reversal of the reduction (activation) pathway. That is, oxygen converts the thioredoxin and target enzyme from the reduced state (—SH HS—) to the oxidized state (—S—S—) and, in so doing, leads to inactivation of the enzyme (see Figure 8.5; see also **Web Topic 8.4**). The last four of the enzymes listed here are regulated directly by thioredoxin; the first, rubisco, is regulated indirectly by a thioredoxin accessory enzyme, rubisco activase (see the next section).

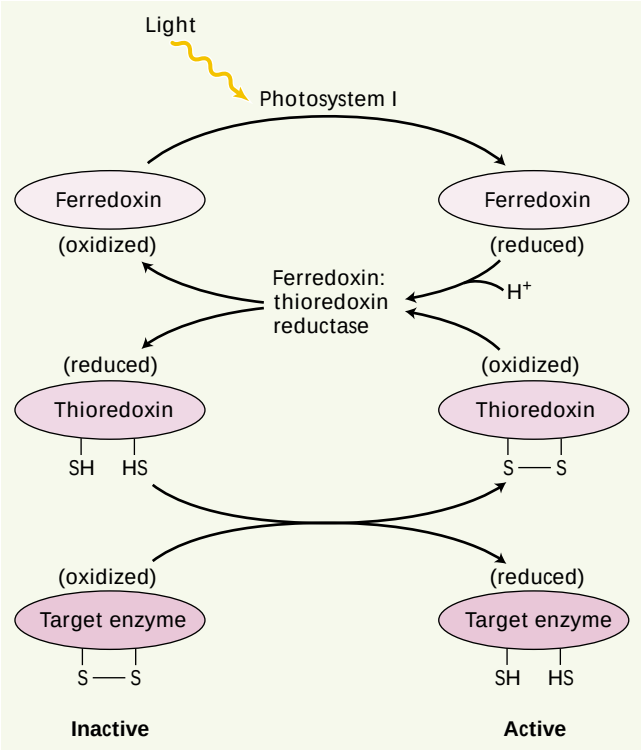


FIGURE 8.5 The ferredoxin–thioredoxin system reduces specific enzymes in the light. Upon reduction, biosynthetic enzymes are converted from an inactive to an active state. The activation process starts in the light by a reduction of ferredoxin by photosystem I (see Chapter 7). The reduced ferredoxin plus two protons are used to reduce a catalytically active disulfide (—S—S—) group of the iron–sulfur enzyme ferredoxin:thioredoxin reductase, which in turn reduces the highly specific disulfide (—S—S—) bond of the small regulatory protein thioredoxin (see Web Topic 8.4 for details). The reduced form (—SH HS—) of thioredoxin then reduces the critical disulfide bond (converts —S—S— to —SH HS—) of a target enzyme and thereby leads to activation of that enzyme. The light signal is thus converted to a sulfhydryl, or —SH, signal via ferredoxin and the enzyme ferredoxin:thioredoxin reductase.

Rubisco Activity Increases in the Light

The activity of rubisco is also regulated by light, but the enzyme itself does not respond to thioredoxin. George Lorimer and colleagues found that rubisco is activated when activator CO_2 (a different molecule from the substrate CO_2 that becomes fixed) reacts slowly with an uncharged ε-NH_2 group of lysine within the active site of the enzyme. The resulting carbamate derivative (a new anionic site) then rapidly binds Mg^{2+} to yield the activated complex (Figure 8.6).

Two protons are released during the formation of the ternary complex rubisco–CO_2–Mg^{2+}, so activation is promoted by an increase in both pH and Mg^{2+} concentration. Thus, light-dependent stromal changes in pH and Mg^{2+} (see the next section) appear to facilitate the observed activation of rubisco by light.

In the active state, rubisco binds another molecule of CO_2, which reacts with the 2,3-enediol form of ribulose-1,5-bisphosphate (P—O—CH_2—COH═COH—CHOH—CH_2O—P) yielding 2-carboxy-3-ketoribitol 1,5-bisphos-

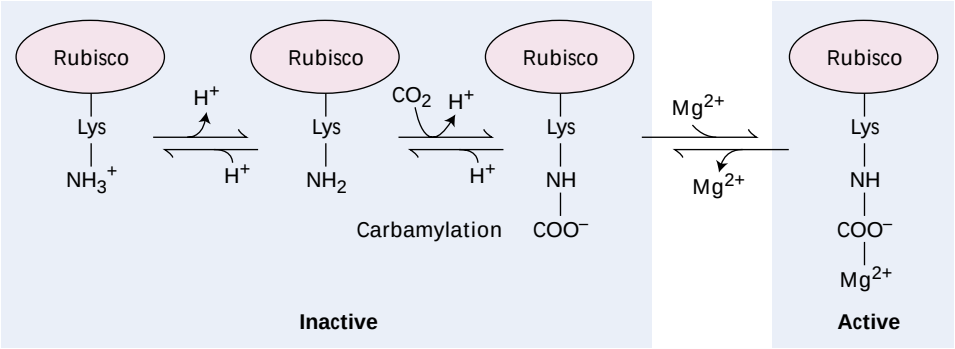


FIGURE 8.6 One way in which rubisco is activated involves the formation of a carbamate–Mg^{2+} complex on the ε-amino group of a lysine within the active site of the enzyme. Two protons are released. Activation is enhanced by the increase in Mg^{2+} concentration and higher pH (low H^+ concentration) that result from illumination. The CO_2 involved in the carbamate–Mg^{2+} reaction is not the same as the CO_2 involved in the carboxylation of ribulose-1,5-bisphosphate.

phate. The extreme instability of the latter intermediate leads to the cleavage of the bond that links carbons 2 and 3 of ribulose-1,5-bisphosphate, and as a consequence, rubisco releases two molecules of 3-phosphoglycerate.

The binding of sugar phosphates, such as ribulose-1,5-bisphosphate, to rubisco prevents carbamylation. The sugar phosphates can be removed by the enzyme rubisco activase, in a reaction that requires ATP. The primary role of rubisco activase is to accelerate the release of bound sugar phosphates, thus preparing rubisco for carbamylation (Salvucci and Ogren 1996, see also **Web Topic 8.5**).

Rubisco is also regulated by a natural sugar phosphate, carboxyarabinitol-1-phosphate, that closely resembles the six-carbon transition intermediate of the carboxylation reaction. This inhibitor is present at low concentrations in leaves of many species and at high concentrations in leaves of legumes such as soybean and bean. Carboxyarabinitol-1-phosphate binds to rubisco at night, and it is removed by the action of rubisco activase in the morning, when photon flux density increases.

Recent work has shown that in some plants rubisco activase is regulated by the ferredoxin–thioredoxin system (Zhang and Portis 1999). In addition to connecting thioredoxin to all five regulatory enzymes of the Calvin cycle, this finding provides a new mechanism for linking light to the regulation of enzyme activity.

Light-Dependent Ion Movements Regulate Calvin Cycle Enzymes

Light causes reversible ion changes in the stroma that influence the activity of rubisco and other chloroplast enzymes. Upon illumination, protons are pumped from the stroma into the lumen of the thylakoids. The proton efflux is coupled to Mg^{2+} uptake into the stroma. These ion fluxes decrease the stromal concentration of H^+ (pH 7 → 8) and increase that of Mg^{2+}. These changes in the ionic composition of the chloroplast stroma are reversed upon darkening.

Several Calvin cycle enzymes (rubisco, fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, and ribulose-5-phosphate kinase) are more active at pH 8 than at pH 7 and require Mg^{2+} as a cofactor for catalysis. Hence these light-dependent ion fluxes enhance the activity of key enzymes of the Calvin cycle (Heldt 1979).

Light-Dependent Membrane Transport Regulates the Calvin Cycle

The rate at which carbon is exported from the chloroplast plays a role in regulation of the Calvin cycle. Carbon is exported as triose phosphates in exchange for orthophosphate via the phosphate translocator in the inner membrane of the chloroplast envelope (Flügge and Heldt 1991). To ensure continued operation of the Calvin cycle, at least five-sixths of the triose phosphate must be recycled (see Table 8.1 and Figure 8.3). Thus, at most one-sixth can be exported for sucrose synthesis in the cytosol or diverted to starch synthesis within the chloroplast. The regulation of this aspect of photosynthetic carbon metabolism will be discussed further when the syntheses of sucrose and starch are considered in detail later in this chapter.

THE C_2 OXIDATIVE PHOTOSYNTHETIC CARBON CYCLE

An important property of rubisco is its ability to catalyze both the carboxylation and the oxygenation of RuBP. Oxygenation is the primary reaction in a process known as **photorespiration**. Because photosynthesis and photorespiration work in diametrically opposite directions, photorespiration results in loss of CO_2 from cells that are simultaneously fixing CO_2 by the Calvin cycle (Ogren 1984; Leegood et al. 1995).

In this section we will describe the C_2 oxidative photosynthetic carbon cycle—the reactions that result in the partial recovery of carbon lost through oxidation.

Photosynthetic CO_2 Fixation and Photorespiratory Oxygenation Are Competing Reactions

The incorporation of one molecule of O_2 into the 2,3-enediol isomer of ribulose-1,5-bisphosphate generates an unstable intermediate that rapidly splits into 2-phosphoglycolate and 3-phosphoglycerate (Figure 8.7 and Table 8.2, reaction 1). The ability to catalyze the oxygenation of ribulose-1,5-bisphosphate is a property of all rubiscos, regard-

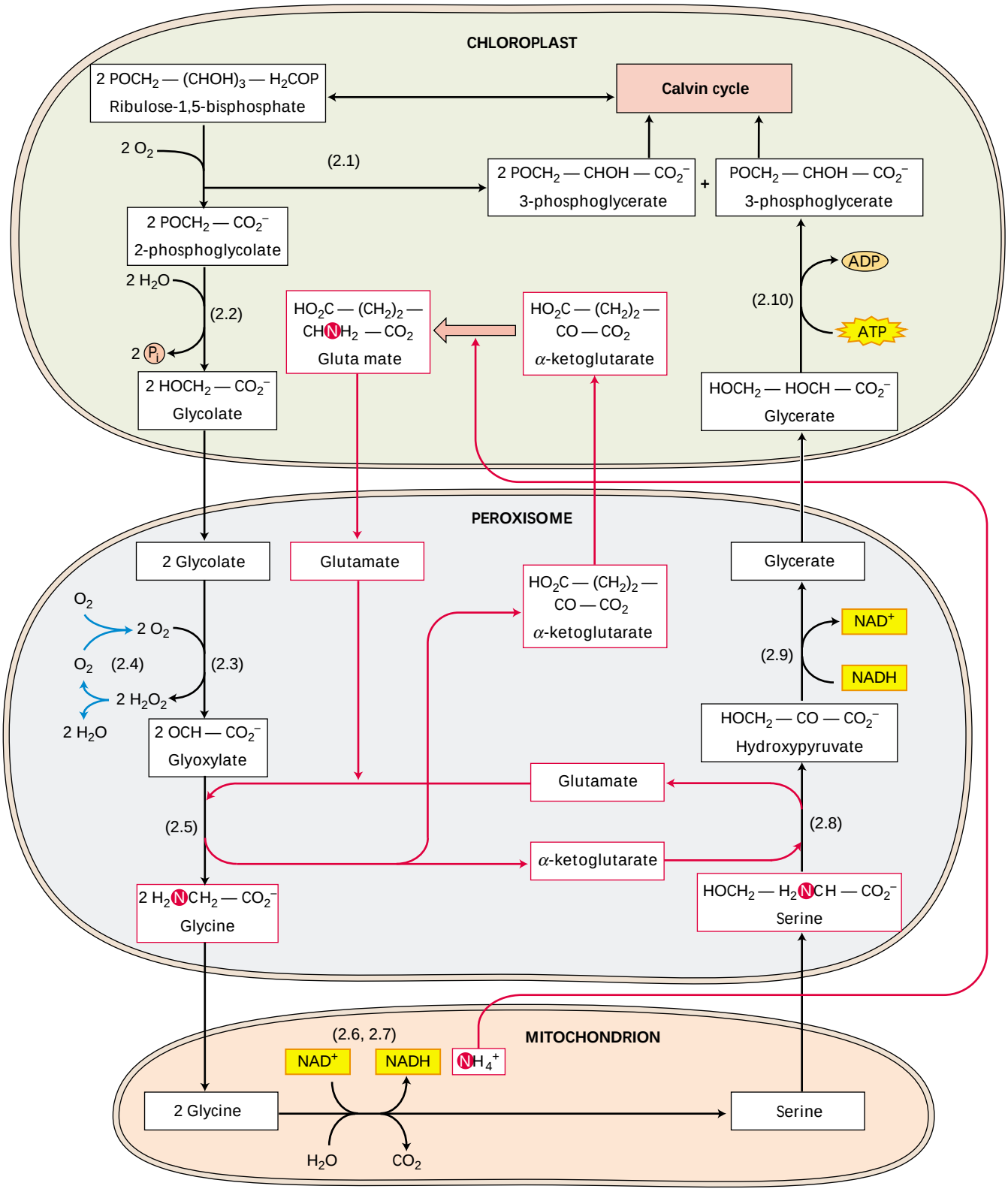


FIGURE 8.7 The main reactions of the photorespiratory cycle. Operation of the C_2 oxidative photosynthetic cycle involves the cooperative interaction among three organelles: chloroplasts, mitochondria, and peroxisomes. Two molecules of glycolate (four carbons) transported from the chloroplast into the peroxisome are converted to glycine, which in turn is exported to the mitochondrion and transformed to serine (three carbons) with the concurrent release of carbon dioxide (one carbon). Serine is transported to the peroxisome and transformed to glycerate. The latter flows to the chloroplast where it is phosphorylated to 3-phosphoglycerate and incorporated into the Calvin cycle. Inorganic nitrogen (ammonia) released by the mitochondrion is captured by the chloroplast for the incorporation into amino acids by using appropiate skeletons (α-ketoglutarate). The heavy arrow in red marks the assimilation of ammonia into glutamate catalyzed by glutamine synthetase. In addition, the uptake of oxygen in the peroxisome supports a short oxygen cycle coupled to oxidative reactions. The flow of carbon, nitrogen and oxygen are indicated in black, red and blue, respectively. See Table 8.2 for a description of each numbered reaction.

TABLE 8.2
Reactions of the C_2 oxidative photosynthetic carbon cycle

Enzyme	Reaction
1. Ribulose-1,5-bisphosphate carboxylase/oxygenase (chloroplast)	2 Ribulose-1,5-bisphosphate + 2 O_2 → 2 phosphoglycolate + 2 3-phosphoglycerate + 4 H^+
2. Phosphoglycolate phosphatase (chloroplast)	2 Phosphoglycolate + 2 H_2O → 2 glycolate + 2 P_i
3. Glycolate oxidase (peroxisome)	2 Glycolate + 2 O_2 → 2 glyoxylate + 2 H_2O_2
4. Catalase (peroxisome)	2 H_2O_2 → 2 H_2O + O_2
5. Glyoxylate:glutamate aminotransferase (peroxisome)	2 Glyoxylate + 2 glutamate → 2 glycine + 2 α-ketoglutarate
6. Glycine decarboxylase (mitochondrion)	Glycine + NAD^+ + H^+ + H_4-folate → NADH + CO_2 + NH_4^+ + methylene-H_4-folate
7. Serine hydroxymethyltransferase (mitochondrion)	Methylene-H_4-folate + H_2O + glycine → serine + H_4-folate
8. Serine aminotransferase (peroxisome)	Serine + α-ketoglutarate → hydroxypyruvate + glutamate
9. Hydroxypyruvate reductase (peroxisome)	Hydroxypyruvate + NADH + H^+ → glycerate + NAD^+
10. Glycerate kinase (chloroplast)	Glycerate + ATP → 3-phosphoglycerate + ADP + H^+

Note: Upon the release of glycolate from the chloroplast (reactions 2 → 3), the interplay of this organelle with the peroxisome and the mitochondrion drives the following overall reaction:

2 Glycolate + glutamate + O_2 → glycerate + α-ketoglutarate + NH_4^+ + CO_2 + H_2O

The 3-phosphoglycerate formed in the chloroplast (reaction 10) is converted to ribulose-1,5-bisphosphate via the reductive and regenerative reactions of the Calvin cycle. The ammonia and α-ketoglutarate are converted to glutamate in the chloroplast by ferrodoxin-linked glutamate synthase (GOGAT).

P_i stands for inorganic phosphate.

less of taxonomic origin. Even the rubisco from anaerobic, autotrophic bacteria catalyzes the oxygenase reaction when exposed to oxygen.

As alternative substrates for rubisco, CO_2 and O_2 compete for reaction with ribulose-1,5-bisphosphate because carboxylation and oxygenation occur within the same active site of the enzyme. Offered equal concentrations of CO_2 and O_2 in a test tube, angiosperm rubiscos fix CO_2 about 80 times faster than they oxygenate. However, an aqueous solution in equilibrium with air at 25°C has a CO_2:O_2 ratio of 0.0416 (see **Web Topics 8.2 and 8.3**). At these concentrations, carboxylation in air outruns oxygenation by a scant three to one.

The C_2 oxidative photosynthetic carbon cycle acts as a scavenger operation to recover fixed carbon lost during photorespiration by the oxygenase reaction of rubisco (**Web Topic 8.6**). The 2-phosphoglycolate formed in the chloroplast by oxygenation of ribulose-1,5-bisphosphate is rapidly hydrolyzed to glycolate by a specific chloroplast phosphatase (Figure 8.7 and Table 8.2, reaction 2). Subsequent metabolism of the glycolate involves the cooperation of two other organelles: peroxisomes and mitochondria (see Chapter 1) (Tolbert 1981).

Glycolate leaves the chloroplast via a specific transporter protein in the envelope membrane and diffuses to the peroxisome. There it is oxidized to glyoxylate and hydrogen peroxide (H_2O_2) by a flavin mononucleotide-dependent oxidase: glycolate oxidase (Figure 8.7 and Table 8.2, reaction 3). The vast amounts of hydrogen peroxide released in the peroxisome are destroyed by the action of catalase (Table 8.2, reaction 4) while the glyoxylate undergoes transamination (reaction 5). The amino donor for this transamination is probably glutamate, and the product is the amino acid glycine.

Glycine leaves the peroxisome and enters the mitochondrion (see Figure 8.7). There the glycine decarboxylase multienzyme complex catalyzes the conversion of two molecules of glycine and one of NAD^+ to one molecule each of serine, NADH, NH_4^+ and CO_2 (Table 8.2, reactions 6 and 7). This multienzyme complex, present in large concentrations in the matrix of plant mitochondria, comprises four proteins, named H-protein (a lipoamide-containing polypeptide), P-protein (a 200 kDa, homodimer, pyridoxal phosphate-containing protein), T-protein (a folate-dependent protein), and L-protein (a flavin adenine nucleotide–containing protein).

The ammonia formed in the oxidation of glycine diffuses rapidly from the matrix of mitochondria to chloroplasts, where glutamine synthetase combines it with carbon skeletons to form amino acids. The newly formed serine leaves the mitochondria and enters the peroxisome, where it is converted first by transamination to hydroxypyruvate (Table 8.2, reaction 8) and then by an NADH-dependent reduction to glycerate (reaction 9).

A malate-oxaloacetate shuttle transfers NADH from the cytoplasm into the peroxisome, thus maintaining an adequate concentration of NADH for this reaction. Finally, glycerate reenters the chloroplast, where it is phosphorylated to yield 3-phosphoglycerate (Table 8.2, reaction 10).

In photorespiration, various compounds are circulated in concert through two cycles. In one of the cycles, carbon exits the chloroplast in two molecules of glycolate and returns in one molecule of glycerate. In the other cycle, nitrogen exits the chloroplast in one molecule of glutamate and returns in one molecule of ammonia (together with one molecule of α-ketoglutarate) (see Figure 8.7).

Thus overall, two molecules of phosphoglycolate (four carbon atoms), lost from the Calvin cycle by the oxygenation of RuBP, are converted into one molecule of 3-phosphoglycerate (three carbon atoms) and one CO_2. In other words, 75% of the carbon lost by the oxygenation of ribulose-1,5-bisphosphate is recovered by the C_2 oxidative photosynthetic carbon cycle and returned to the Calvin cycle (Lorimer 1981).

On the other hand, the total organic nitrogen remains unchanged because the formation of inorganic nitrogen (NH_4^+) in the mitochondrion is balanced by the synthesis of glutamine in the chloroplast. Similarly, the use of NADH in the peroxisome (by hydroxypyruvate reductase) is balanced by the reduction of NAD^+ in the mitochondrion (by glycine decarboxylase).

Competition between Carboxylation and Oxygenation Decreases the Efficiency of Photosynthesis

Because photorespiration is concurrent with photosynthesis, it is difficult to measure the rate of photorespiration in intact cells. Two molecules of 2-phosphoglycolate (four carbon atoms) are needed to make one molecule of 3-phosphoglycerate, with the release of one molecule of CO_2; so theoretically one-fourth of the carbon entering the C_2 oxidative photosynthetic carbon cycle is released as CO_2.

Measurements of CO_2 release by sunflower leaves support this calculated value. This result indicates that the actual rate of photosynthesis is approximately 120 to 125% of the measured rate. The ratio of carboxylation to oxygenation in air at 25°C is computed to be between 2.5 and 3. Further calculations indicate that photorespiration lowers the efficiency of photosynthetic carbon fixation from 90% to approximately 50%.

This decreased efficiency can be measured as an increase in the quantum requirement for CO_2 fixation under photorespiratory conditions (air with high O_2 and low CO_2) as opposed to nonphotorespiratory conditions (low O_2 and high CO_2).

Carboxylation and Oxygenation Are Closely Interlocked in the Intact Leaf

Photosynthetic carbon metabolism in the intact leaf reflects the integrated balance between two mutually opposing and interlocking cycles (Figure 8.8). The Calvin cycle can operate independently, but the C_2 oxidative photosynthetic carbon cycle depends on the Calvin cycle for a supply of ribulose-1,5-bisphosphate. The balance between the two cycles is determined by three factors: the kinetic properties of rubisco, the concentrations of the substrates CO_2 and O_2, and temperature.

As the temperature increases, the concentration of CO_2 in a solution in equilibrium with air decreases more than the concentration of O_2 does (see **Web Topic 8.3**). Consequently, the concentration ratio of CO_2 to O_2 decreases as the temperature rises. As a result of this property, photorespiration (oxygenation) increases relative to photosynthesis (carboxylation) as the temperature rises. This effect is enhanced by the kinetic properties of rubisco, which also result in a relative increase in oxygenation at higher temperatures (Ku and Edwards 1978). Overall, then, increasing temperatures progressively tilt the balance away from the Calvin cycle and toward the oxidative photosynthetic carbon cycle (see Chapter 9).

The Biological Function of Photorespiration Is Unknown

Although the C_2 oxidative photosynthetic carbon cycle recovers 75% of the carbon originally lost from the Calvin cycle as 2-phosphoglycolate, why does 2-phosphoglycolate form at all? One possible explanation is that the formation

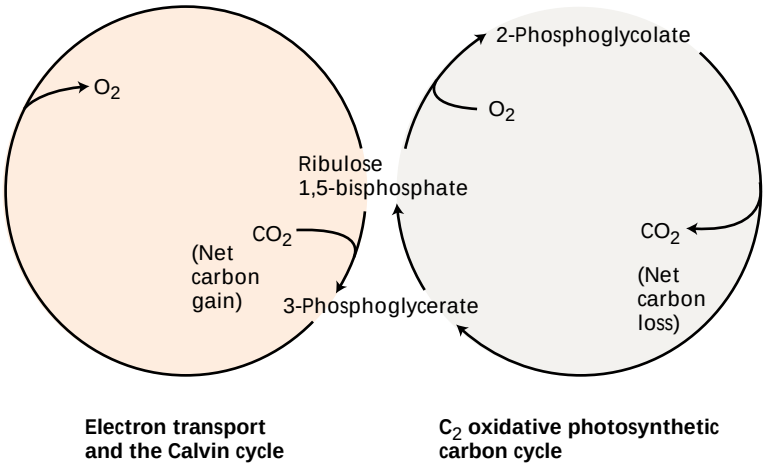


FIGURE 8.8 The flow of carbon in the leaf is determined by the balance between two mutually opposing cycles. Whereas the Calvin cycle is capable of independent operation in the presence of adequate substrates generated by photosynthetic electron transport, the C_2 oxidative photosynthetic carbon cycle requires continued operation of the Calvin cycle to regenerate its starting material, ribulose-1,5-bisphosphate.

of 2-phosphoglycolate is a consequence of the chemistry of the carboxylation reaction, which requires an intermediate that can react with both CO_2 and O_2.

Such a reaction would have had little consequence in early evolutionary times if the ratio of CO_2 to O_2 in air were higher than it is today. However, the low CO_2:O_2 ratios prevalent in modern times are conducive to photorespiration, with no other function than the recovery of some of the carbon present in 2-phosphoglycolate.

Another possible explanation is that photorespiration is important, especially under conditions of high light intensity and low intercellular CO_2 concentration (e.g., when stomata are closed because of water stress), to dissipate excess ATP and reducing power from the light reactions, thus preventing damage to the photosynthetic apparatus. *Arabidopsis* mutants that are unable to photorespire grow normally under 2% CO_2, but they die rapidly if transferred to normal air. There is evidence from work with transgenic plants that photorespiration protects C_3 plants from photooxidation and photoinhibition (Kozaki and Takeba 1996). Further work is needed to improve our understanding of the function of photorespiration.

CO_2-CONCENTRATING MECHANISMS I: ALGAL AND CYANOBACTERIAL PUMPS

Many plants either do not photorespire at all, or they do so to only a limited extent. These plants have normal rubiscos, and their lack of photorespiration is a consequence of mechanisms that concentrate CO_2 in the rubisco environment and thereby suppress the oxygenation reaction.

In this and the two following sections we will discuss three mechanisms for concentrating CO_2 at the site of carboxylation:

1. C_4 photosynthetic carbon fixation (C_4)
2. Crassulacean acid metabolism (CAM)
3. CO_2 pumps at the plasma membrane

The first two of these CO_2-concentrating mechanisms are found in some angiosperms and involve "add-ons" to the Calvin cycle. Plants with C_4 metabolism are often found in hot environments; CAM plants are typical of desert environments. We will examine each of these two systems after we consider the third mechanism: a CO_2 pump found in aquatic plants that has been studied extensively in unicellular cyanobacteria and algae.

When algal and cyanobacterial cells are grown in air enriched with 5% CO_2 and then transferred to a low-CO_2 medium, they display symptoms typical of photorespiration (O_2 inhibition of photosynthesis at low concentration of CO_2). But if the cells are grown in air containing 0.03% CO_2, they rapidly develop the ability to concentrate inorganic carbon (CO_2 plus HCO_3^-) internally. Under these low-CO_2 conditions, the cells no longer photorespire.

At the concentrations of CO_2 found in aquatic environments, rubisco operates far below its maximal specific activity. Marine and freshwater organisms overcome this drawback by accumulating inorganic carbon by the use of CO_2 and HCO_3^- pumps at the plasma membrane. ATP derived from the light reactions provides the energy necessary for the active uptake of CO_2 and HCO_3^-. Total inorganic carbon inside some cyanobacterial cells can reach concentrations of 50 m*M* (Ogawa and Kaplan 1987). Recent work indicates that a single gene encoding a transcription factor can regulate the expression of genes that encode the components of the CO_2-concentrating mechanism in algae (Xiang et al. 2001).

The proteins that function as CO_2–HCO_3^- pumps are not present in cells grown in high concentrations of CO_2 but are induced upon exposure to low concentrations of CO_2. The accumulated HCO_3^- is converted to CO_2 by the enzyme carbonic anhydrase, and the CO_2 enters the Calvin cycle.

The metabolic consequence of this CO_2 enrichment is suppression of the oxygenation of ribulose bisphosphate and hence also suppression of photorespiration. The energetic cost of this adaptation is the additional ATP needed for concentrating the CO_2.

CO_2-CONCENTRATING MECHANISMS II: THE C_4 CARBON CYCLE

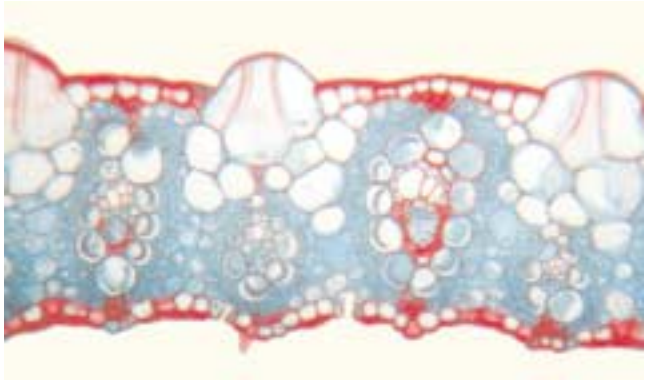
There are differences in leaf anatomy between plants that have a C_4 carbon cycle (called *C_4 plants*) and those that photosynthesize solely via the Calvin photosynthetic cycle (*C_3 plants*). A cross section of a typical C_3 leaf reveals one major cell type that has chloroplasts, the **mesophyll**. In contrast, a typical C_4 leaf has two distinct chloroplast-containing cell types: mesophyll and **bundle sheath** (or *Kranz*, German for "wreath") cells (Figure 8.9).

There is considerable anatomic variation in the arrangement of the bundle sheath cells with respect to the mesophyll and vascular tissue. In all cases, however, operation of the C_4 cycle requires the cooperative effort of both cell types. No mesophyll cell of a C_4 plant is more than two or three cells away from the nearest bundle sheath cell (see Figure 8.9A). In addition, an extensive network of plasmodesmata (see Figure 1.27) connects mesophyll and bundle sheath cells, thus providing a pathway for the flow of metabolites between the cell types.

Malate and Aspartate Are Carboxylation Products of the C_4 Cycle

Early labeling of C_4 acids was first observed in $^{14}CO_2$ labeling studies of sugarcane by H. P. Kortschack and colleagues and of maize by Y. Karpilov and coworkers. When leaves were exposed for a few seconds to $^{14}CO_2$ in the light, 70 to 80% of the label was found in the C_4 acids malate and aspartate—a pattern very different from the one observed in leaves that photosynthesize solely via the Calvin cycle.

(A)



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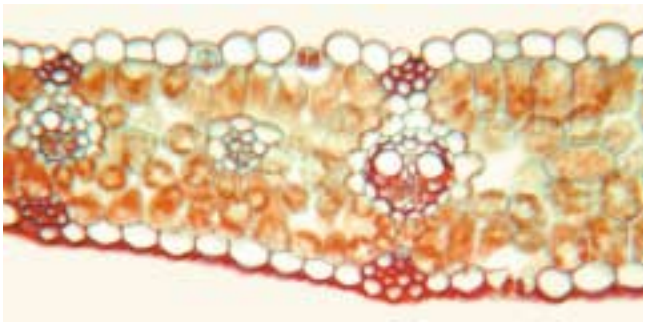

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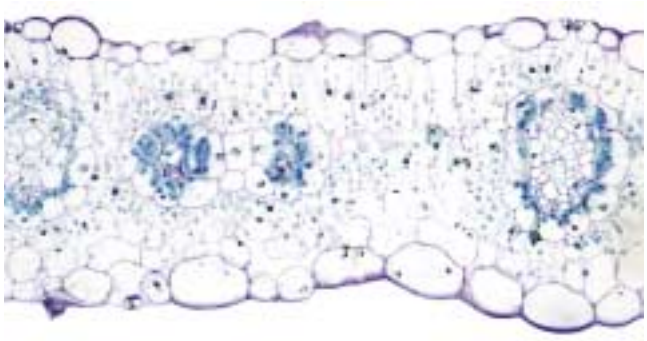

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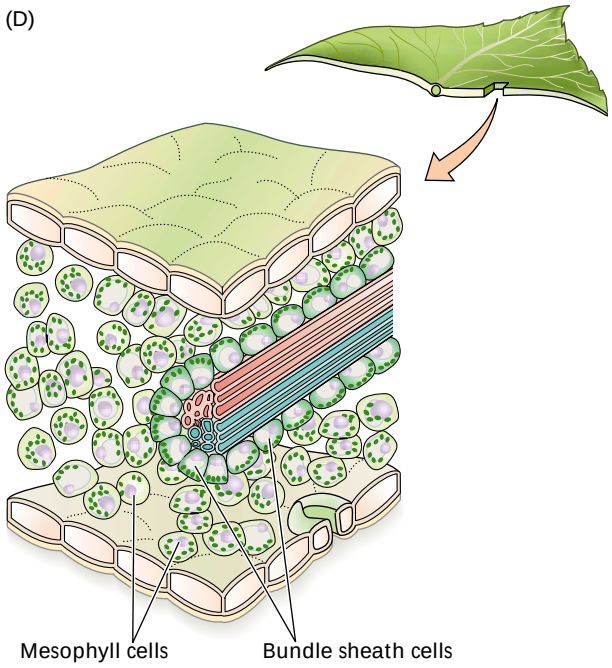

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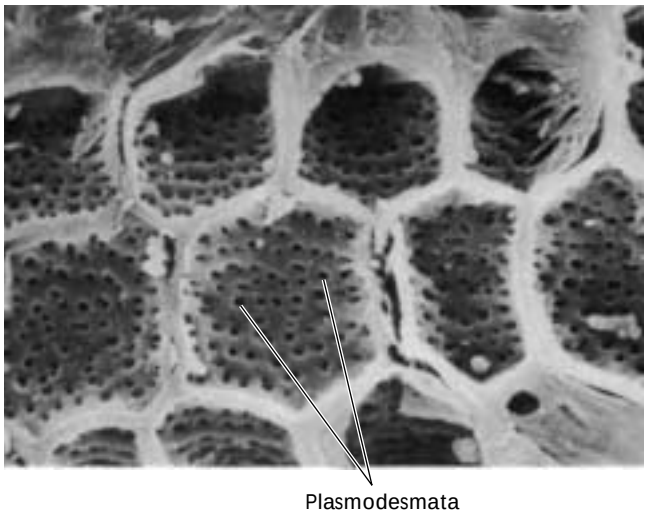


FIGURE 8.9 Cross-sections of leaves, showing the anatomic differences between C_3 and C_4 plants. (A) A C_4 monocot, *saccharum officinarum* (sugarcane). (135×) (B) A C_3 monocot, Poa sp. (a grass). (240×) (C) A C_4 dicot, *Flaveria australasica* (Asteraceae). (740×) The bundle sheath cells are large in C_4 leaves (A and C), and no mesophyll cell is more than two or three cells away from the nearest bundle sheath cell. These anatomic features are absent in the C_3 leaf (B). (D) Three-dimensional model of a C_4 leaf. (A and B © David Webb; C courtesy of Athena McKown; D after Lüttge and Higinbotham; E from Craig and Goodchild 1977.)

In pursuing these initial observations, M. D. Hatch and C. R. Slack elucidated what is now known as the C_4 photosynthetic carbon cycle (C_4 cycle) (Figure 8.10). They established that the C_4 acids malate and aspartate are the first stable, detectable intermediates of photosynthesis in leaves of sugarcane and that carbon atom 4 of malate subsequently becomes carbon atom 1 of 3-phosphoglycerate (Hatch and Slack 1966). The primary carboxylation in these leaves is catalyzed not by rubisco, but by PEP (phosphoenylpyruvate) carboxylase (Chollet et al. 1996).

The manner in which carbon is transferred from carbon atom 4 of malate to carbon atom 1 of 3-phosphoglycerate became clear when the involvement of mesophyll and bundle sheath cells was elucidated. The participating enzymes occur in one of the two cell types: PEP carboxylase and pyruvate–orthophosphate dikinase are restricted to mesophyll cells; the decarboxylases and the enzymes of the complete Calvin cycle are confined to the bundle sheath cells. With this knowledge, Hatch and Slack were able to formulate the basic model of the cycle (Figure 8.11 and Table 8.3).

The C_4 Cycle Concentrates CO_2 in Bundle Sheath Cells

The basic C_4 cycle consists of four stages:

1. Fixation of CO_2 by the carboxylation of phosphoenolpyruvate in the mesophyll cells to form a C_4 acid (malate and/or aspartate)
2. Transport of the C_4 acids to the bundle sheath cells
3. Decarboxylation of the C_4 acids within the bundle sheath cells and generation of CO_2, which is then reduced to carbohydrate via the Calvin cycle

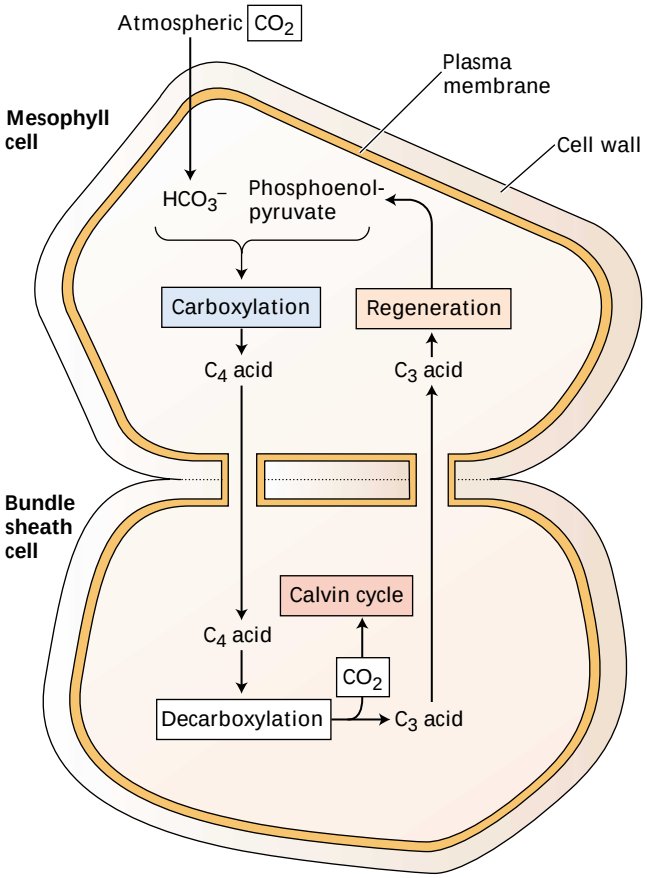


FIGURE 8.10 The basic C_4 photosynthetic carbon cycle involves four stages in two different cell types: (1) Fixation of CO_2 into a four-carbon acid in a mesophyll cell; (2) Transport of the four-carbon acid from the mesophyll cell to a bundle sheath cell; (3) Decarboxylation of the four-carbon acid, and the generation of a high CO_2 concentration in the bundle sheath cell. The CO_2 released is fixed by rubisco and converted to carbohydrate by the Calvin cycle.(4) Transport of the residual three-carbon acid back to the mesophyll cell, where the original CO_2 acceptor, phosphoenolpyruvate, is regenerated.

TABLE 8.3
Reactions of the C_4 photosynthetic carbon cycle

Enzyme	Reaction
1. Phosphoenolpyruvate (PEP) carboxylase	Phosphoenolpyruvate + $HCO_3^- \rightarrow$ oxaloacetate + P_i
2. NADP:malate dehydrogenase	Oxaloacetate + NADPH + $H^+ \rightarrow$ malate + $NADP^+$
3. Aspartate aminotransferase	Oxaloacetate + glutamate $\rightarrow$ aspartate + α-ketoglutarate
4. NAD(P) malic enzyme	Malate + $NAD(P)^+ \rightarrow$ pyruvate + CO_2 + NAD(P)H + H^+
5. Phosphoenolpyruvate carboxykinase	Oxaloacetate + ATP $\rightarrow$ phosphoenolpyruvate + CO_2 + ADP
6. Alanine aminotransferase	Pyruvate + glutamate $\leftrightarrow$ alanine + α-ketoglutarate
7. Adenylate kinase	AMP + ATP $\rightarrow$ 2 ADP
8. Pyruvate–orthophosphate dikinase	Pyruvate + P_i + ATP $\rightarrow$ phosphoenolpyruvate + AMP + PP_i
9. Pyrophosphatase	$PP_i + H_2O \rightarrow 2\ P_i$

Note: P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively.

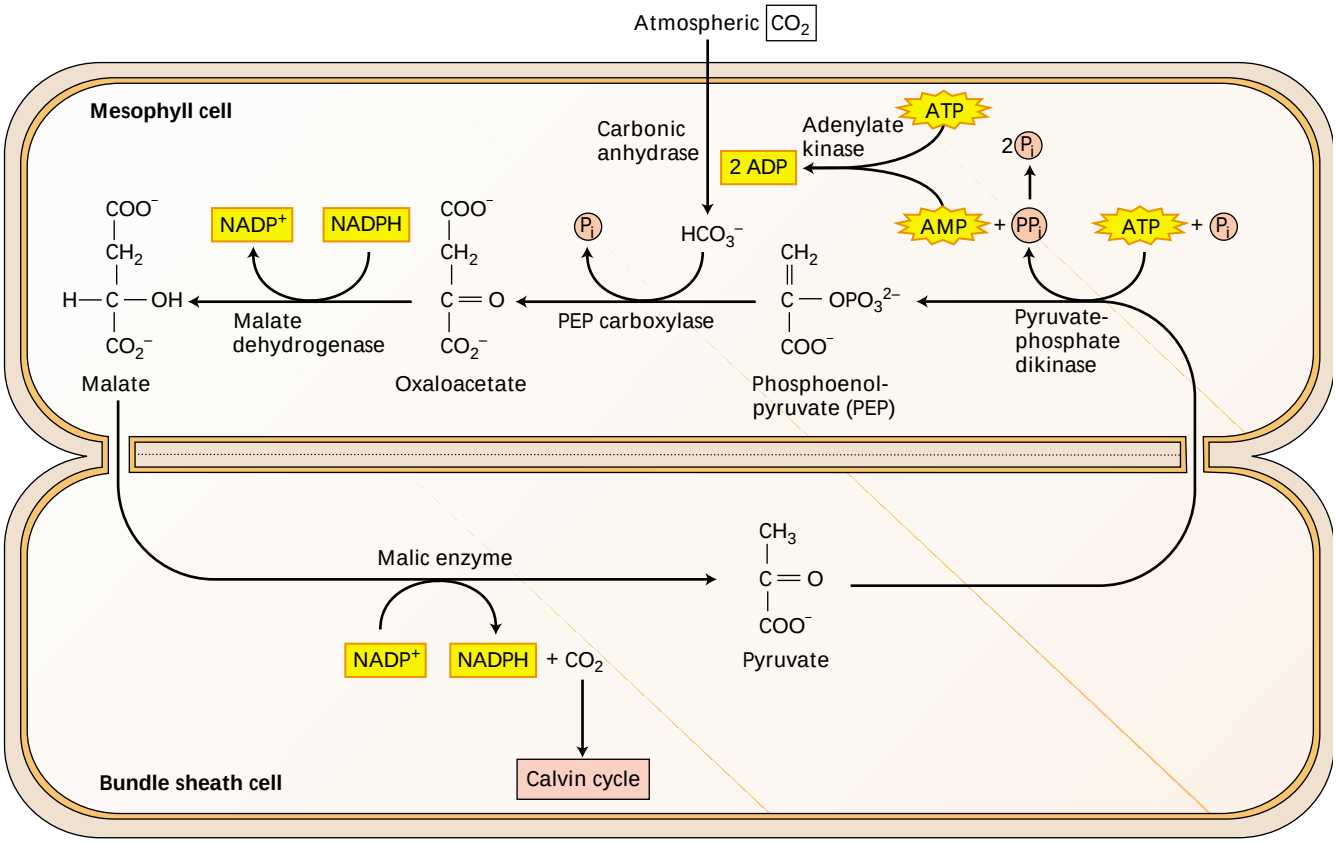


FIGURE 8.11 The C_4 photosynthetic pathway. The hydrolysis of two ATP drives the cycle in the direction of the arrows, thus pumping CO_2 from the atmosphere to the Calvin cycle of the chloroplasts from bundle sheath cells.

4. Transport of the C_3 acid (pyruvate or alanine) that is formed by the decarboxylation step back to the mesophyll cell and regeneration of the CO_2 acceptor phosphoenolpyruvate

One interesting feature of the cycle is that regeneration of the primary acceptor—phosphoenolpyruvate—consumes two "high-energy" phosphate bonds: one in the reaction catalyzed by pyruvate–orthophosphate dikinase (Table 8.3, reaction 8) and another in the conversion of PP_i to $2P_i$ catalyzed by pyrophosphatase (reaction 9; see also Figure 8.11).

Shuttling of metabolites between mesophyll and bundle sheath cells is driven by diffusion gradients along numerous plasmodesmata, and transport within the cells is regulated by concentration gradients and the operation of specialized translocators at the chloroplast envelope. The cycle thus effectively shuttles CO_2 from the atmosphere into the bundle sheath cells. This transport process generates a much higher concentration of CO_2 in the bundle sheath cells than would occur in equilibrium with the external atmosphere. This elevated concentration of CO_2 at the carboxylation site of rubisco results in suppression of the oxygenation of ribulose-1,5-bisphosphate and hence of photorespiration.

Discovered in the tropical grasses, sugarcane, and maize, the C_4 cycle is now known to occur in 16 families of both monocotyledons and dicotyledons, and it is particularly prominent in Gramineae (corn, millet, sorghum, sugarcane), Chenopodiaceae (*Atriplex*), and Cyperaceae (sedges). About 1% of all known species have C_4 metabolism (Edwards and Walker 1983).

There are three variations of the basic C_4 pathway that occur in different species (see **Web Topic 8.7**). The variations differ principally in the C_4 acid (malate or aspartate) transported into the bundle sheath cells and in the manner of decarboxylation.

The Concentration of CO_2 in Bundle Sheath Cells Has an Energy Cost

The net effect of the C_4 cycle is to convert a dilute solution of CO_2 in the mesophyll cells into a concentrated CO_2 solution in cells of the bundle sheath. Studies of a PEP carboxylase–deficient mutant of *Amaranthus edulis* clearly showed that the lack of an effective mechanism for concentrating CO_2 in the bundle sheath markedly enhances photorespiration in a C_4 plant (Dever et al. 1996).

Thermodynamics tells us that work must be done to establish and maintain the CO_2 concentration gradient in the bundle sheath (for a detailed discussion of theomodynamics, see Chapter 2 on the web site). This principle also applies to the operation of the C_4 cycle. From a summation

TABLE 8.4
Energetics of the C_4 photosynthetic carbon cycle

Phosphoenolpyruvate + H_2O + NADPH + CO_2 (mesophyll)	→	malate + $NADP^+$ + P_i (mesophyll)
Malate + $NADP^+$	→	pyruvate + NADPH + CO_2 (bundle sheath)
Pyruvate + P_i + ATP	→	phosphoenolpyruvate + AMP + PP_i (mesophyll)
PP_i + H_2O	→	2 P_i (mesophyll)
AMP + ATP	→	2ADP
Net: CO_2 (mesophyll) + ATP + 2 H_2O	→	CO_2 (bundle sheath) + 2ADP + 2 P_i
Cost of concentrating CO_2 within the bundle sheath cell = 2 ATP per CO_2		

Note: As shown in reaction 1 of Table 8.3, the H_2O and CO_2 shown in the first line of this table actually react with phosphoenolpyruvate as HCO_3^-.
P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively.

of the reactions involved, we can calculate the energy cost to the plant (Table 8.4). The calculation shows that the CO_2-concentrating process consumes two ATP equivalents (2 "high-energy" bonds) per CO_2 molecule transported. Thus the total energy requirement for fixing CO_2 by the combined C_4 and Calvin cycles (calculated in Tables 8.4 and 8.1, respectively) is five ATP plus two NADPH per CO_2 fixed.

Because of this higher energy demand, C_4 plants photosynthesizing under nonphotorespiratory conditions (high CO_2 and low O_2) require more quanta of light per CO_2 than C_3 leaves do. In normal air, the quantum requirement of C_3 plants changes with factors that affect the balance between photosynthesis and photorespiration, such as temperature. By contrast, owing to the mechanisms built in to avoid photorespiration, the quantum requirement of C_4 plants remains relatively constant under different environmental conditions (see Figure 9.23).

Light Regulates the Activity of Key C_4 Enzymes

Light is essential for the operation of the C_4 cycle because it regulates several specific enzymes. For example, the activities of PEP carboxylase, NADP:malate dehydrogenase, and pyruvate–orthophosphate dikinase (see Table 8.3) are regulated in response to variations in photon flux density by two different processes: reduction–oxidation of thiol groups and phosphorylation–dephosphorylation.

NADP:malate dehydrogenase is regulated via the thioredoxin system of the chloroplast (see Figure 8.5). The enzyme is reduced (activated) upon illumination of leaves and is oxidized (inactivated) upon darkening. PEP carboxylase is activated by a light-dependent phosphorylation–dephosphorylation mechanism yet to be characterized.

The third regulatory member of the C_4 pathway, pyruvate–orthophosphate dikinase, is rapidly inactivated by an unusual ADP-dependent phosphorylation of the enzyme when the photon flux density drops (Burnell and Hatch 1985). Activation is accomplished by phosphorolytic cleavage of this phosphate group. Both reactions, phosphorylation and dephosphorylation, appear to be catalyzed by a single regulatory protein.

In Hot, Dry Climates, the C_4 Cycle Reduces Photorespiration and Water Loss

Two features of the C_4 cycle in C_4 plants overcome the deleterious effects of higher temperature on photosynthesis that were noted earlier. First, the affinity of PEP carboxylase for its substrate, HCO_3^-, is sufficiently high that the enzyme is saturated by HCO_3^- in equlibrium with air levels of CO_2. Furthermore, because the substrate is HCO_3^-, oxygen is not a competitor in the reaction. This high activity of PEP carboxylase enables C_4 plants to reduce the stomatal aperture and thereby conserve water while fixing CO_2 at rates equal to or greater than those of C_3 plants. The second beneficial feature is the suppression of photorespiration resulting from the concentration of CO_2 in bundle sheath cells (Marocco et al. 1998).

These features enable C_4 plants to photosynthesize more efficiently at high temperatures than C_3 plants, and they are probably the reason for the relative abundance of C_4 plants in drier, hotter climates. Depending on their natural environment, some plants show properties intermediate between strictly C_3 and C_4 species.

CO_2-CONCENTRATING MECHANISMS III: CRASSULACEAN ACID METABOLISM

A third mechanism for concentrating CO_2 at the site of rubisco is found in crassulacean acid metabolism (CAM). Despite its name, CAM is not restricted to the family Crassulaceae (*Crassula*, *Kalanchoe*, *Sedum*); it is found in numerous angiosperm families. Cacti and euphorbias are CAM plants, as well as pineapple, vanilla, and agave.

The CAM mechanism enables plants to improve water use efficiency. Typically, a CAM plant loses 50 to 100 g of water for every gram of CO_2 gained, compared with values of 250 to 300 g and 400 to 500 g for C_4 and C_3 plants,

respectively (see Chapter 4). Thus, CAM plants have a competitive advantage in dry environments.

The CAM mechanism is similar in many respects to the C_4 cycle. In C_4 plants, formation of the C_4 acids in the mesophyll is spatially separated from decarboxylation of the C_4 acids and from refixation of the resulting CO_2 by the Calvin cycle in the bundle sheath. In CAM plants, formation of the C_4 acids is both temporally and spatially separated. At night, CO_2 is captured by PEP carboxylase in the cytosol, and the malate that forms from the oxaloacetate product is stored in the vacuole (Figure 8.12). During the day, the stored malate is transported to the chloroplast and decarboxylated by NADP-malic enzyme, the released CO_2 is fixed by the Calvin cycle, and the NADPH is used for converting the decarboxylated triose phosphate product to starch.

The Stomata of CAM Plants Open at Night and Close during the Day

CAM plants such as cacti achieve their high water use efficiency by opening their stomata during the cool, desert nights and closing them during the hot, dry days. Closing the stomata during the day minimizes water loss, but because H_2O and CO_2 share the same diffusion pathway, CO_2 must then be taken up at night.

CO_2 is incorporated via carboxylation of phosphoenolpyruvate to oxaloacetate, which is then reduced to malate. The malate accumulates and is stored in the large vacuoles that are a typical, but not obligatory, anatomic feature of the leaf cells of CAM plants (see Figure 8.12). The accumulation of substantial amounts of malic acid, equivalent to the amount of CO_2 assimilated at night, has long been recognized as a nocturnal acidification of the leaf (Bonner and Bonner 1948).

With the onset of day, the stomata close, preventing loss of water and further uptake of CO_2. The leaf cells deacidify as the reserves of vacuolar malic acid are consumed. Decarboxylation is usually achieved by the action of NADP-malic enzyme on malate (Drincovich et al. 2001). Because the stomata are closed, the internally released CO_2 cannot escape from the leaf and instead is fixed and converted to carbohydrate by the Calvin cycle.

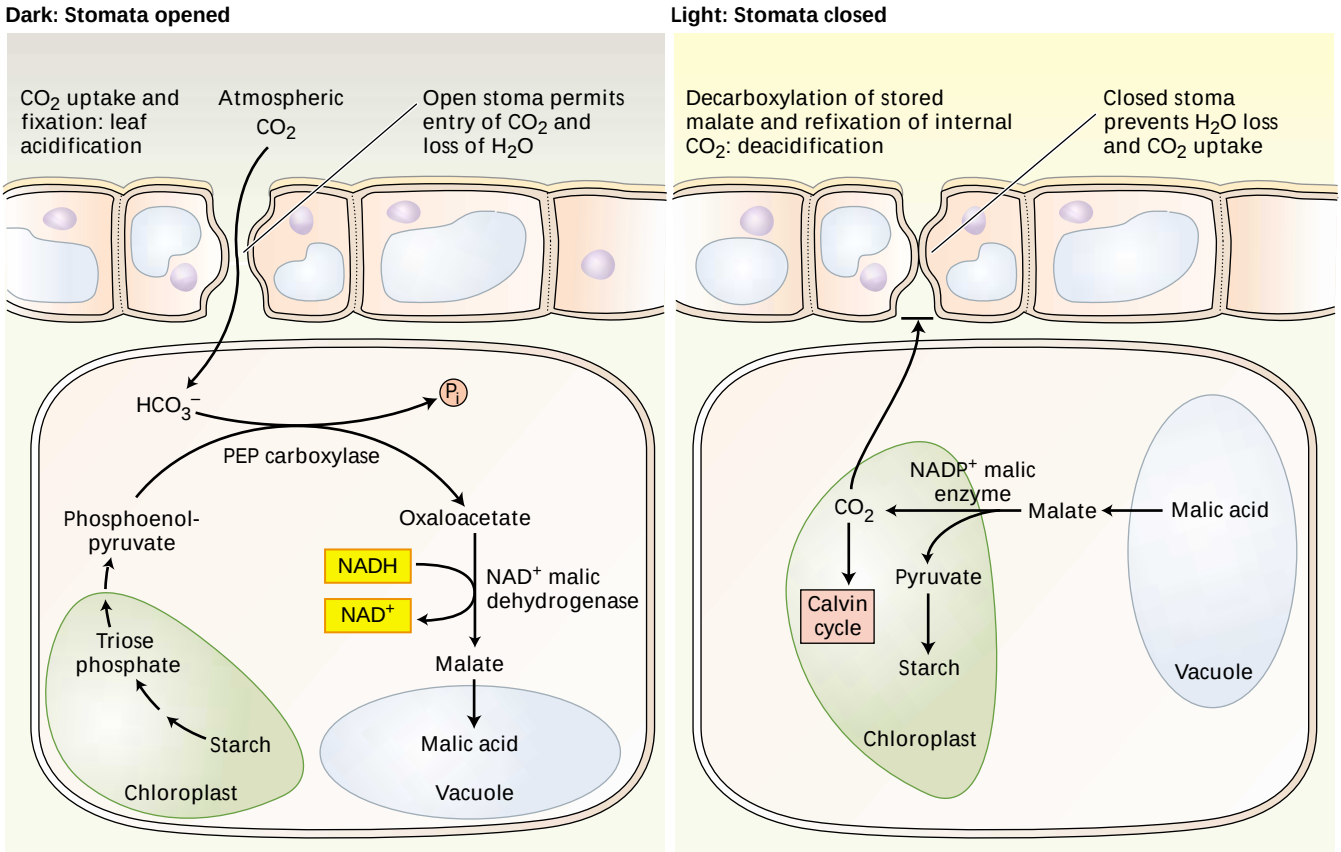


FIGURE 8.12 Crassulacean acid metabolism (CAM). Temporal separation of CO_2 uptake from photosynthetic reactions: CO_2 uptake and fixation take place at night, and decarboxylation and refixation of the internally released CO_2 occur during the day. The adaptive advantage of CAM is the reduction of water loss by transpiration, achieved by the stomatal opening during the night.

The elevated internal concentration of CO_2 effectively suppresses the photorespiratory oxygenation of ribulose bisphosphate and favors carboxylation. The C_3 acid resulting from the decarboxylation is thought to be converted first to triose phosphate and then to starch or sucrose, thus regenerating the source of the original carbon acceptor.

Phosphorylation Regulates the Activity of PEP Carboxylase in C_4 and CAM Plants

The CAM mechanism that we have outlined in this discussion requires separation of the initial carboxylation from the subsequent decarboxylation, to avoid a futile cycle. In addition to the spatial and temporal separation exhibited by C_4 and CAM plants, respectively, a futile cycle is avoided by the regulation of PEP carboxylase (Figure 8.13). In C_4 plants the carboxylase is "switched on," or active, during the day and in CAM plants during the night. In both C_4 and CAM plants, PEP carboxylase is inhibited by malate and activated by glucose-6-phosphate (see **Web Essay 8.1** for a detailed discussion of the regulation of PEP carboxylase).

Phosphorylation of a single serine residue of the CAM enzyme diminishes the malate inhibition and enhances the action of glucose-6-phosphate so that the enzyme becomes catalytically more active (Chollet et al. 1996; Vidal and Chollet 1997) (see Figure 8.13). The phosphorylation is catalyzed by a PEP carboxylase-kinase. The synthesis of this kinase is stimulated by the efflux of Ca^{2+} from the vacuole to the cytosol and the resulting activation of a Ca^{2+}/calmodulin protein kinase (Giglioli-Guivarc'h et al. 1996; Coursol et al. 2000; Nimmo 2000; Bakrim et al. 2001).

Some Plants Adjust Their Pattern of CO_2 Uptake to Environmental Conditions

Plants have many mechanisms that maximize water and CO_2 supply during development and reproduction. C_3 plants regulate the stomatal aperture of their leaves during the day, and stomata close during the night. C_4 and CAM plants utilize PEP carboxylase to fix CO_2, and they separate that enzyme from rubisco either spatially (C_4 plants) or temporally (CAM plants).

Some CAM plants show longer-term regulation and are able to adjust their pattern of CO_2 uptake to environmental conditions. Facultative CAM plants such as the ice plant (*Mesembryanthemum crystallinum*) carry on C_3 metabolism under unstressed conditions, and they shift to CAM in response to heat, water, or salt stress. This form of regulation requires the expression of numerous CAM genes in response to stress signals (Adams et al. 1998; Cushman 2001).

In aquatic environments, cyanobacteria and green algae have abundant water but find low CO_2 concentrations in their surroundings and actively concentrate inorganic CO_2 intracellularly. In diatoms, which abound in the phytoplankton, a CO_2-concentrating mechanism operates simultaneously with a C_4 pathway (Reinfelder et al. 2000). Diatoms are a fine example of photosynthetic organisms that have the capacity to use different CO_2-concentrating mechanisms in response to environmental fluctuations.

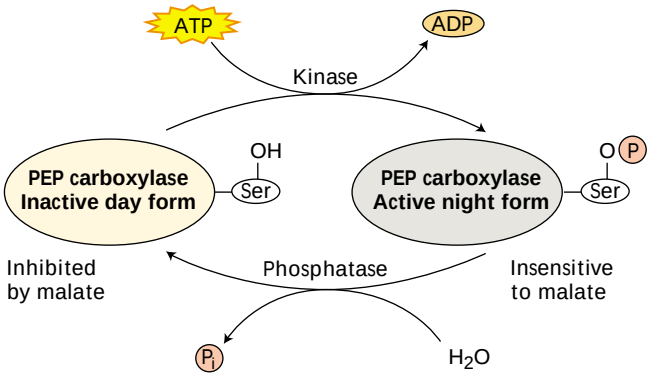


FIGURE 8.13 Diurnal regulation of CAM phosphoenolpyruvate (PEP) carboxylase. Phosphorylation of the serine residue (Ser-OP) yields a form of the enzyme which is active during the night and relatively insensitive to malate. During the day, dephosphorylation of the serine (Ser-OH) gives a form of the enzyme which is inhibited by malate.

SYNTHESIS OF STARCH AND SUCROSE

In most species, sucrose is the principal form of carbohydrate translocated throughout the plant by the phloem. Starch is an insoluble stable carbohydrate reserve that is present in almost all plants. Both starch and sucrose are synthesized from the triose phosphate that is generated by the Calvin cycle (see Table 8.1) (Beck and Ziegler 1989). The pathways for the synthesis of starch and sucrose are shown in Figure 8.14.

Starch Is Synthesized in the Chloroplast

Electron micrographs showing prominent starch deposits, as well as enzyme localization studies, leave no doubt that the chloroplast is the site of starch synthesis in leaves (Figure 8.15). Starch is synthesized from triose phosphate via fructose-1,6-bisphosphate (Table 8.5 and Figure 8.14). The glucose-1-phosphate intermediate is converted to ADP-glucose via ADP-glucose pyrophosphorylase (Figure 8.14 and Table 8.5, reaction 5) in a reaction that requires ATP and generates pyrophosphate (PP_i, or $H_2P_2O_7^{2-}$).

As in many biosynthetic reactions, the pyrophosphate is hydrolyzed via a specific inorganic pyrophosphatase to two orthophosphate (P_i) molecules (Table 8.5, reaction 6), thereby driving reaction 5 toward ADP-glucose synthesis. Finally, the glucose moiety of ADP-glucose is transferred to the nonreducing end (carbon 4) of the terminal glucose of a growing starch chain (Table 8.5, reaction 7), thus completing the reaction sequence.

Sucrose Is Synthesized in the Cytosol

The site of sucrose synthesis has been studied by cell fractionation, in which the organelles are isolated and separated from one another. Enzyme analyses have shown that sucrose is synthesized in the cytosol from triose phosphates

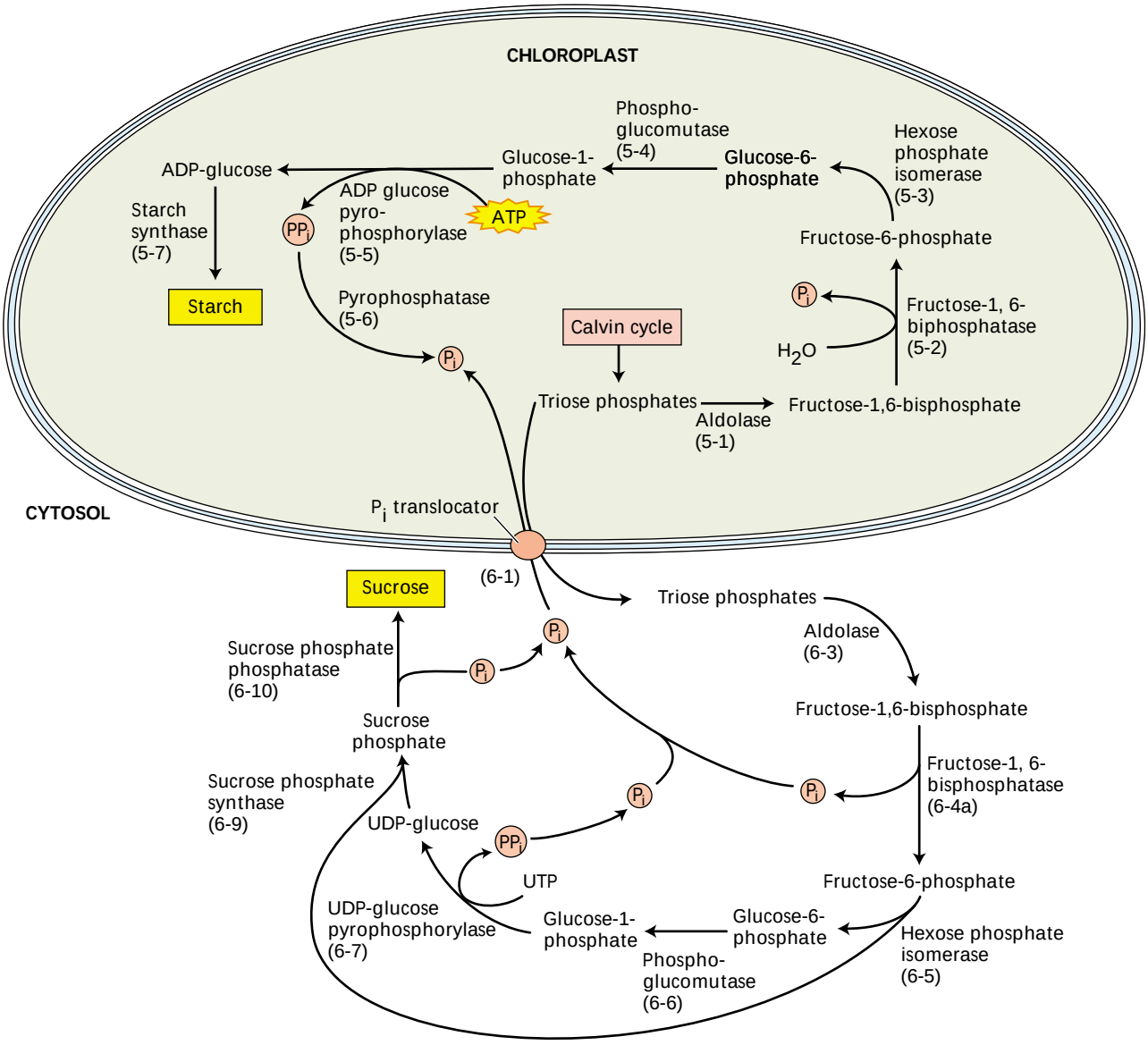


FIGURE 8.14 The syntheses of starch and sucrose are competing processes that occur in the chloroplast and the cytosol, respectively. When the cytosolic P_i concentration is high, chloroplast triose phosphate is exported to the cytosol via the P_i in exchange for P_i, and sucrose is synthesized. When the cytosolic P_i concentration is low, triose phosphate is retained within the chloroplast, and starch is synthesized. The numbers facing the arrows are keyed to Tables 8.5 and 8.6.

by a pathway similar to that of starch—that is, by way of fructose-1,6-bisphosphate and glucose-1-phosphate (Figure 8.14 and Table 8.6, reactions 2–6).

In sucrose synthesis, the glucose-1-phosphate is converted to UDP-glucose via a specific UDP-glucose pyrophosphorylase (Table 8.6, reaction 7) that is analogous to the ADP-glucose pyrophosphorylase of chloroplasts. At this stage, two consecutive reactions complete the synthesis of sucrose (Huber and Huber 1996). First, sucrose-6-phosphate synthase catalyzes the reaction of UDP-glucose with fructose-6-phosphate to yield sucrose-6-phosphate and UDP (Table 8.6, reaction 9). Second, the sucrose-6-phosphate phosphatase (phosphohydrolase) cleaves the phosphate from sucrose-6-phosphate, yielding sucrose (Table 8.6, reaction 10). The latter reaction, which is essentially irreversible, pulls the former in the direction of sucrose synthesis.

As in starch synthesis, the pyrophosphate formed in the reaction catalyzed by UDP-glucose pyrophosphorylase (Table 8.6, reaction 7) is hydrolyzed, but not immediately as in the chloroplasts. Because of the absence of an inorganic pyrophosphatase, the pyrophosphate can be used by other enzymes, in transphosphorylation reactions. One example is fructose-6-phosphate phosphotransferase, an enzyme that catalyzes a reaction like the one catalyzed by phosphofructokinase (Table 8.6, reaction 4a) except that pyrophosphate replaces ATP as the phosphoryl donor.

A comparison of the reactions in Tables 8.5 and 8.6 (as illustrated in Figure 8.14) reveals that the conversion of triose phosphates to glucose-1-phosphate in the pathways

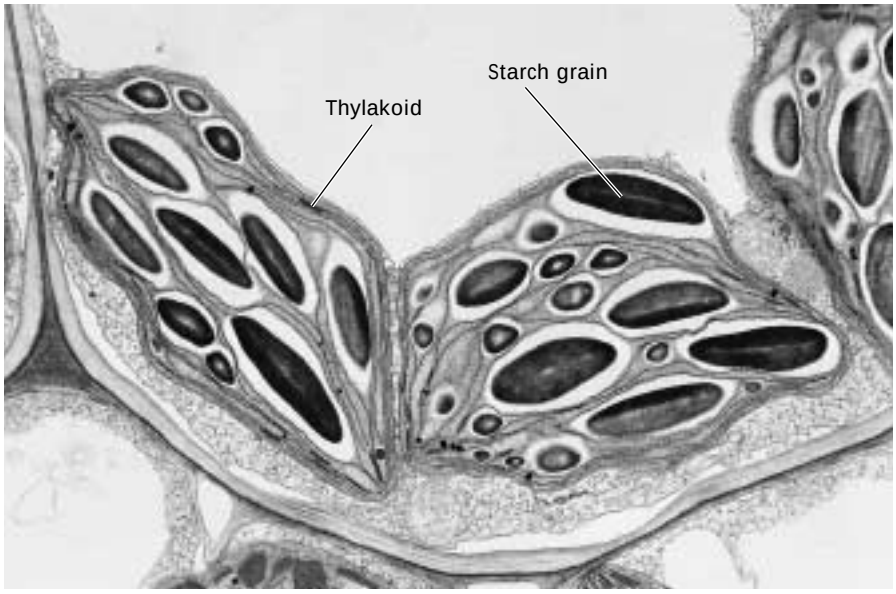


FIGURE 8.15 Electron micrograph of a bundle sheath cell from maize, showing the starch grains in the chloroplasts. (15,800×) (Photo by S. E. Frederick, courtesy of E. H. Newcomb.)

leading to the synthesis of starch and sucrose have several steps in common. However, these pathways utilize isozymes (different forms of enzymes catalyzing the same reaction) that are unique to the chloroplast or cytosol.

The isozymes show markedly different properties. For example, the chloroplastic fructose-1,6-bisphosphatase is regulated by the thioredoxin system but not by fructose-2,6-bisphosphate and AMP. Conversely, the cytosolic form of the enzyme is regulated by fructose-2,6-bisphosphate (see the next section), is sensitive to AMP especially in the presence of fructose-2,6-bisphosphate, and is unaffected by thioredoxin.

Aside from the cytosolic fructose-1,6-bisphosphatase, sucrose synthesis is regulated at the level of sucrose phosphate synthase, an allosteric enzyme that is activated by glucose-6-phosphate and inhibited by orthophosphate. The enzyme is inactivated in the dark by phosphorylation of a specific serine residue via a protein kinase and activated in the light by dephosphorylation via a protein phosphatase. Glucose-6-phosphate inhibits the kinase, and P_i inhibits the phosphatase.

The recent purification and cloning of sucrose-6-phosphate phosphatase from rice leaves (Lund et al. 2000) is providing new information on the molecular and functional properties of this enzyme. These studies indicate that sucrose-6-phosphate synthase and sucrose-6-phosphatase exist as a supramolecular complex showing an enzymatic activity that is higher than that of the isolated constituent enzymes (Salerno et al. 1996). This noncovalent interaction of the two enzymes involved in the last two steps of sucrose synthesis points to a novel regulatory feature of carbohydrate metabolism in plants.

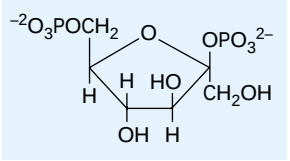
The Syntheses of Sucrose and Starch Are Competing Reactions

The relative concentrations of orthophosphate and triose phosphate are major factors that control whether photosynthetically fixed carbon is partitioned as starch in the chloroplast or as sucrose in the cytosol. The two compartments communicate with one another via the phosphate / triose phosphate translocator, also called the phosphate translocator (see Table 8.6, reaction 1), a strict stoichiometric antiporter.

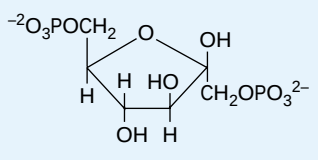
The phosphate translocator catalyzes the movement of orthophosphate and triose phosphate in opposite directions between chloroplast and cytosol. A low concentration of orthophosphate in the cytosol limits the export of triose phosphate from the chloroplast through the translocator, thereby promoting the synthesis of starch. Conversely, an abundance of orthophosphate in the cytosol inhibits starch synthesis within the chloroplast and promotes the export of triose phosphate into the cytosol, where it is converted to sucrose.

Orthophosphate and triose phosphate control the activity of several regulatory enzymes in the sucrose and starch biosynthetic pathways. The chloroplast enzyme ADP-glucose pyrophosphorylase (see Table 8.5, reaction 5) is the key enzyme that regulates the synthesis of starch from glucose-1-phosphate. This enzyme is stimulated by 3-phosphoglycerate and inhibited by orthophosphate. A high concentration ratio of 3-phosphoglycerate to orthophosphate is typically found in illuminated chloroplasts that are actively synthesizing starch. Reciprocal conditions prevail in the dark.

Fructose-2,6-bisphosphate is a key control molecule that allows increased synthesis of sucrose in the light and decreased synthesis in the dark. It is found in the cytosol in minute concentrations, and it exerts a regulatory effect on the cytosolic interconversion of fructose-1,6-bisphosphate and fructose-6-phosphate (Huber 1986; Stitt 1990):



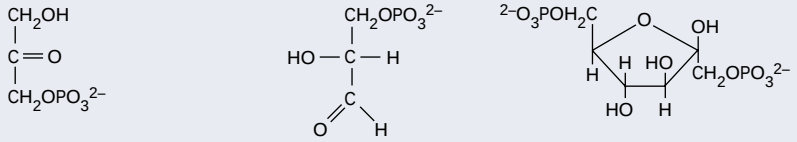
Fructose-2,6-bisphosphate
(a regulatory metabolite)



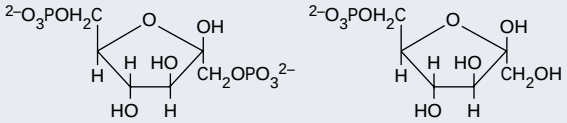
Fructose-1,6-bisphosphate
(an intermediary metabolite)

TABLE 8.5
Reactions of starch synthesis from triose phosphate in chloroplasts

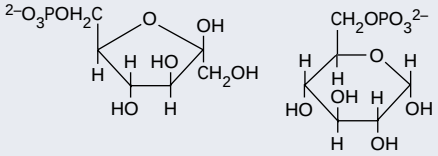
1. *Fructose-1,6,bisphosphate aldolase*
   Dihydroxyacetone-3-phosphate + glyceraldehyde-3-phosphate→ fructose-1,6-bisphosphate



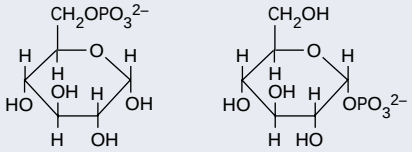
2. *Fructose-1,6-bisphosphatase*
   Fructose-1,6-bisphosphate + $H_2O \rightarrow$ fructose-6-phosphate + P_i



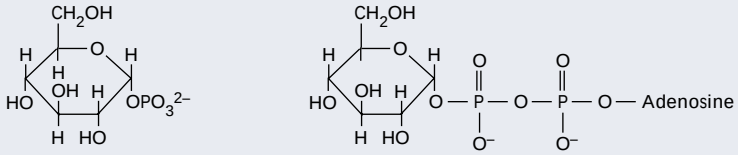
3. *Hexose phosphate isomerase*
   Fructose-6-phosphate → glucose-6-phosphate



4. *Phosphoglucomutase*
   Glucose-6-phosphate → glucose-1-phosphate



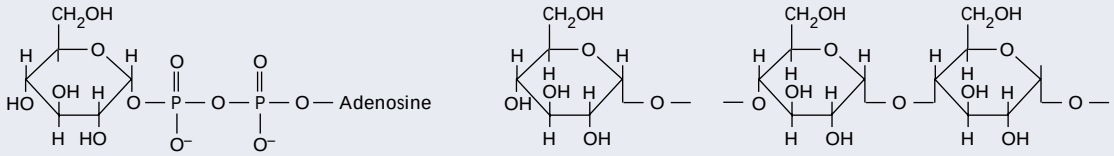
5. *ADP-glucose pyrophosphorylase*
   Glucose-1-phosphate + ATP → ADP-glucose + PP_i



6. *Pyrophosphatase*

   $PP_i + H_2O \rightarrow 2\ P_i + 2H^+$

7. *Starch synthase*
   ADP-glucose + $(1,4\text{-}\alpha\text{-D-glucosyl})_n \rightarrow$ ADP + $(1,4\text{-}\alpha\text{-D-glucosyl})_{n+1}$

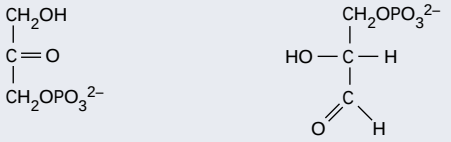


Note: Reaction 6 is irreversible and "pulls" the preceding reaction to the right.
P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively.

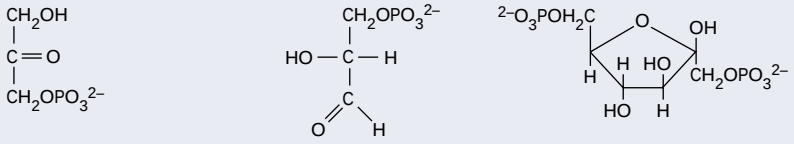
TABLE 8.6
Reactions of sucrose synthesis from triose phosphate in the cytosol

1. *Phosphate/triose phosphate translocator*
   Triose phosphate (chloroplast) + P_i (cytosol) → triose phosphate (cytosol) + P_i (chloroplast)

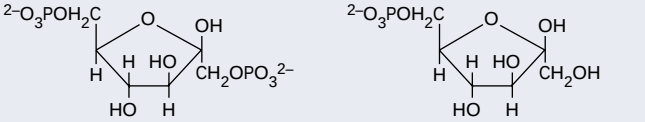
2. *Triose phosphate isomerase*
   Dihydroxyacetone-3-phosphate → glyceraldehyde-3-phosphate



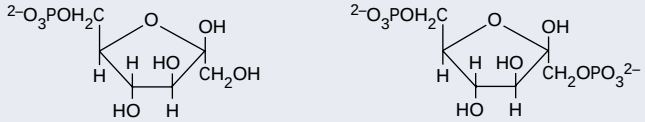
3. *Fructose-1,6-bisphosphate aldolase*
   Dihydroxyacetone-3-phosphate + glyceraldehyde-3-phosphate → fructose-1,6-bisphosphate



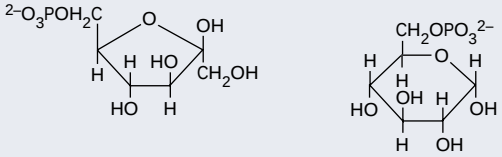
4a. *Fructose-1,6-phosphatase*
   Fructose-1,6-bisphosphate + H_2O → fructose-6-phosphate + P_i



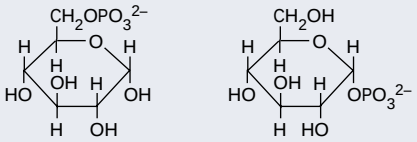
4b. *PP_i-linked phosphofructokinase*
   Fructose-6-phosphate + PP_i → fructose-1,6-bisphosphate + P_i



5. *Hexose phosphate isomerase*
   Fructose-6-phosphate → glucose-6-phosphate



6. *Phosphoglucomutase*
   Glucose-6-phosphate → glucose-1-phosphate



7. *UDP-glucose pyrophosphorylase*
   Glucose-1-phosphate + UTP → UDP-glucose + PP_i

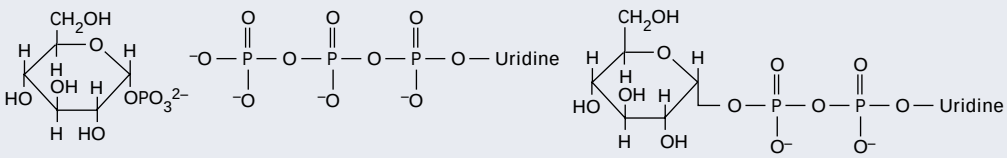
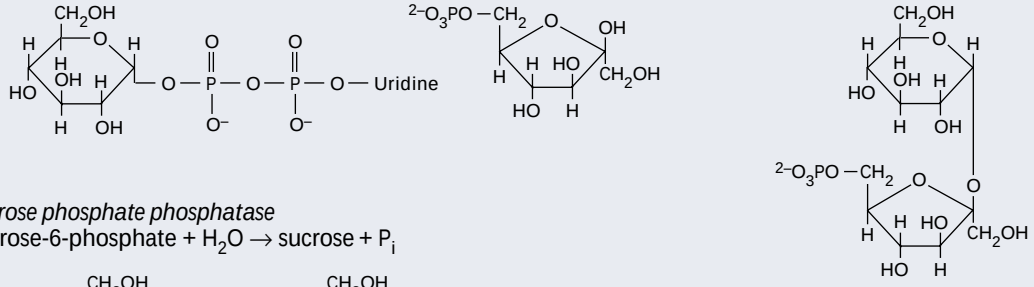


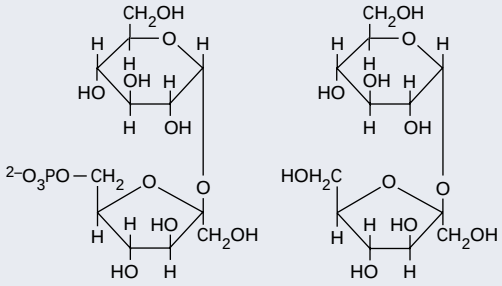
TABLE 8.6 (continued)
Reactions of sucrose synthesis from triose phosphate in the cytosol

8. *Pyrophosphatase*
$PP_i + H_2O \rightarrow 2\ P_i + 2\ H^+$

9. *Sucrose phosphate synthase*
UDP-glucose + fructose-6-phosphate → UDP + sucrose-6-phosphate



10. *Sucrose phosphate phosphatase*
Sucrose-6-phosphate + $H_2O \rightarrow$ sucrose + P_i



Note: Reaction 1 takes place on the chloroplast inner envelope membrane. Reactions 2 through 10 take place in the cytosol. Reaction 8 is irreversible and "pulls" the preceding reaction to the right.
P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively .

Increased cytosolic fructose-2,6-bisphosphate is associated with decreased rates of sucrose synthesis because fructose-2,6-bisphosphate is a powerful inhibitor of cytosolic fructose-1,6-bisphosphatase (see Table 8.6, reaction 4a) and an activator of the pryophosphate-dependent (PP_i-linked) phosphofructokinase (reaction 4b). But what, in turn, controls the cytosolic concentration of fructose-2,6-bisphosphate?

Fructose-2,6-bisphosphate is synthesized from fructose-6-phosphate by a special fructose-6-phosphate 2-kinase (not to be confused with the fructose-6-phosphate 1-kinase of glycolysis) and is degraded specifically by fructose-2,6-bisphosphatase (not to be confused with fructose-1,6-bisphosphatase of the Calvin cycle). Recent evidence suggests that, as in animal cells, both plant activities reside on a single polypeptide chain.

The kinase and phosphatase activities are controlled by orthophosphate and triose phosphate. Orthophosphate stimulates fructose-6-phosphate 2-kinase and inhibits fructose-2,6-bisphosphatase; triose phosphate inhibits the 2-kinase (Figure 8.16). Consequently, a low cytosolic ratio of triose phosphate to orthophosphate promotes the formation of fructose-2,6-bisphosphate, which in turn inhibits the hydrolysis of cytosolic fructose-1,6-bisphosphate and slows the rate of sucrose synthesis. A high cytosolic ratio of triose phosphate to orthophosphate has the opposite effect.

Light regulates the concentration of these activators and inhibitors through the reactions associated with photosynthesis and thereby controls the concentration of fructose-2,6-bisphosphate in the cytosol. The glycolytic enzyme phosphofructokinase also functions in the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, but in plants it is not appreciably affected by fructose-2,6-bisphosphate.

The activity of phosphofructokinase in plants appears to be regulated by the relative concentrations of ATP, ADP, and AMP. The remarkable plasticity of plants was once again illustrated by recent gene deletion experiments with transformed tobacco plants. This experiment shows that the transformed plants can grow without a functional pyrophosphate-dependent fructose-6-phosphate kinase enzyme. In this case the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate is apparently catalyzed exclusively by phosphofructokinase (Paul et al. 1995).

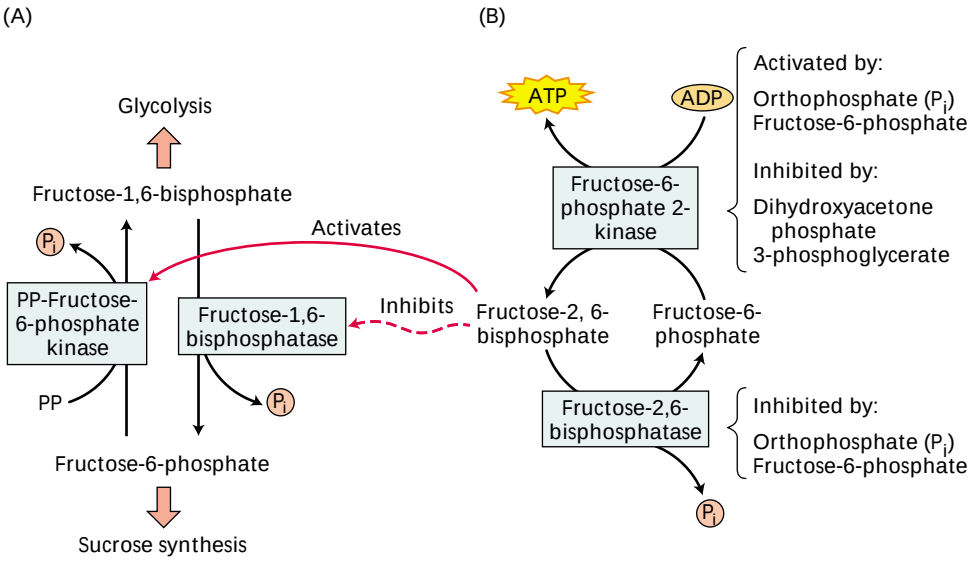


FIGURE 8.16 Regulation of the cytosolic interconversion of fructose-6-phosphate and fructose-1,6-bisphosphate. (A) The key metabolites in the allocation between glycolysis and sucrose synthesis. The regulatory metabolite fructose 2,6-bisphosphate regulates the interconversion by inhibiting the phosphatase and activating the kinase, as shown. (B) The synthesis of fructose-2,6-bisphosphate itself is under strict regulation by the activators and inhibitors shown in the figure.

SUMMARY

The reduction of CO_2 to carbohydrate via the carbon-linked reactions of photosynthesis is coupled to the consumption of NADPH and ATP synthesized by the light reactions of thylakoid membranes. Photosynthetic eukaryotes reduce CO_2 via the Calvin cycle that takes place in the stroma, or soluble phase, of chloroplasts. Here, CO_2 and water are combined with ribulose-1,5-bisphosphate to form two molecules of 3-phosphoglycerate, which are reduced and converted to carbohydrate. The continued operation of the cycle is ensured by the regeneration of ribulose-1,5-bisphosphate. The Calvin cycle consumes two molecules of NADPH and three molecules of ATP for every CO_2 fixed and, provided these substrates, has a thermodynamic efficiency close to 90%.

Several light-dependent systems act jointly to regulate the Calvin cycle: changes in ions (Mg^{2+} and H^+), effector metabolites (enzyme substrates), and protein-mediated systems (rubisco activase, ferredoxin–thioredoxin system).

The ferredoxin–thioredoxin control system plays a versatile role by linking light to the regulation of other chloroplast processes, such as carbohydrate breakdown, photophosphorylation, fatty acid biosynthesis, and mRNA translation. Control of these reactions by light separates opposing biosynthetic from degradative processes and thereby minimizes the waste of resources that would occur if the processes operated concurrently.

Rubisco, the enzyme that catalyzes the carboxylation of ribulose-1,5-bisphosphate, also acts as an oxygenase. In both cases the enzyme must be carbamylated to be fully active. The carboxylation and oxygenation reactions take place at the active site of rubisco. When reacting with oxygen, rubisco produces 2-phosphoglycolate and 3-phosphoglycerate from ribulose-1,5-bisphosphate rather than two 3-phosphoglycerates as with CO_2, thereby decreasing the efficiency of photosynthesis.

The C_2 oxidative photosynthetic carbon cycle rescues the carbon lost as 2-phosphoglycolate by rubisco oxygenase activity. The dissipative effects of photorespiration are avoided in some plants by mechanisms that concentrate CO_2 at the carboxylation sites in the chloroplast. These mechanisms include a C_4 photosynthetic carbon cycle, CAM metabolism, and "CO_2 pumps" of algae and cyanobacteria.

The carbohydrates synthesized by the Calvin cycle are converted into storage forms of energy and carbon: sucrose and starch. Sucrose, the transportable form of carbon and energy in most plants, is synthesized in the cytosol, and its synthesis is regulated by phosphorylation of sucrose phosphate synthase. Starch is synthesized in the chloroplast. The balance between the biosynthetic pathways for sucrose and starch is determined by the relative concentrations of metabolite effectors (orthophosphate, fructose-6-phosphate, 3-phosphoglycerate, and dihydroxyacetone phosphate).

These metabolite effectors function in the cytosol by way of the enzymes synthesizing and degrading fructose-2,6-bisphosphate, the regulatory metabolite that plays a primary role in controlling the partitioning of photosynthetically fixed carbon between sucrose and starch. Two of these effectors, 3-phosphoglycerate and orthophosphate, also act on

starch synthesis in the chloroplast by allosterically regulating the activity of ADP-glucose pyrophosphorylase. In this way the synthesis of starch from triose phosphates during the day can be separated from its breakdown, which is required to provide energy to the plant at night.

Web Material

Web Topics

8.1 How the Calvin Cycle Was Elucidated

Experiments carried out in the 1950s led to the discovery of the path of CO_2 fixation.

8.2 Rubisco: A Model Enzyme for Studying Structure and Function

As the most abundant enzyme on Earth, rubisco was obtained in quantities sufficient for elucidating its structure and catalytic properties.

8.3 Carbon Dioxide: Some Important Physicochemical Properties

Plants have adapted to the properties of CO_2 by altering the reactions catalyzing its fixation.

8.4 Thioredoxins

First found to regulate chloroplast enzymes, thioredoxins are now known to play a regulatory role in all types of cells.

8.5 Rubisco Activase

Rubisco is unique among Calvin cycle enzymes in its regulation by a specific protein, rubisco activase.

8.6 Operation of the C_2 Oxidative Photosynthetic Carbon Cycle

The enzymes of the C_2 oxidative photosynthetic carbon cycle are localized in three different organelles.

8.7 Three Variations of C_4 Metabolism

Certain reactions of the C_4 photosynthetic pathway differ among plant species.

Web Essay

8.1 Modulation of Phosphoenolpyruvate Carboxylase in C_4 and CAM Plants

The CO_2-fixing enzyme, phosphoenolpyruvate carboxylase is regulated differently in C_4 and CAM species.

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