photosynthesis

Part 2

Carbon reactions:
the Calvin-Benson cycle

- In plants, light energy drives the oxidation of water, produces molecular oxygen (O_2) in thylakoid membranes and yields ATP, and reduced pyridine nucleotide (NADPH). The ATP and NADPH released in the chloroplast stroma are used for the conversion of atmospheric CO_2 to carbohydrates

- The Calvin-Benson cycle in the chloroplast stroma utilizes the energy stored in the thylakoid membranes for the assimilation of atmospheric CO_2

- Conversion of inorganic carbon into carbon skeletons of organic material is required for building all the molecules necessary for life.

The pathway by which this conversion occurs is :
the Calvin-Benson cycle

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- The Calvin–Benson cycle proceeds through 13 biochemical reactions that can be analyzed separately in three highly coordinated phases:

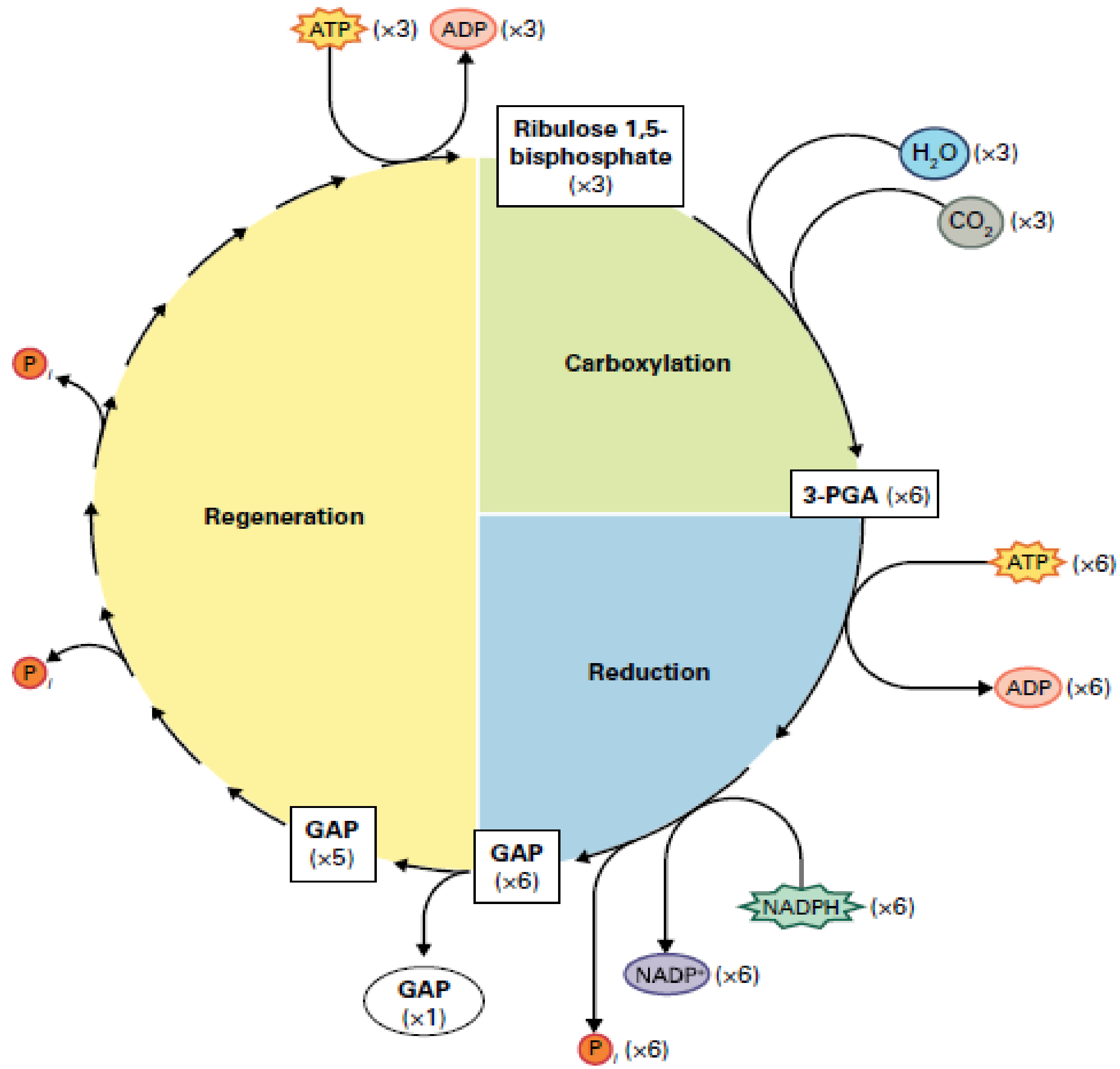
carboxylation, reduction, and regeneration

●● Carboxylation of the CO2-acceptor molecule: ribulose 1,5-bisphosphate (RuBP; five-carbon acceptor molecule) reacts with one molecule each of CO2 and water to yield two molecules of 3-phosphoglycerate (3-PGA) in the first enzymatic step of the cycle.

●● Reduction of 3-PGA: Photochemically generated ATP and NADPH are used in two enzymatic reactions for the reduction of 3-PGA to glyceraldehyde 3-phosphate (GAP).

●● Regeneration of RuBP: The regeneration of the CO2 acceptor RuBP closes the cycle through ten enzyme-catalyzed reactions, one requiring ATP.

FIGURE 12.34 *Three phases of the Calvin–Benson cycle: carboxylation, reduction, and regeneration. Overall, the fixation of three molecules of CO_2 into one molecule of triose phosphate requires six molecules of NADPH and nine of ATP (3 CO_2 : 6 NADPH : 9 ATP ≡ CO_2 : 2 NADPH : 3 ATP). The net glyceraldehyde 3-phosphate (GAP) formed is utilized either for immediate metabolic needs or converted to a storage form of carbohydrate—starch in the chloroplast or sucrose in the cytosol. 3-PGA, 3-phosphoglycerate.*



Carboxylation of RuBP is the first committed reaction of the Calvin–Benson cycle

3 CO2 (3C)
3 H20 (0C)
3 RuBP(15C)
(18 C)

? PGA (3C)

- In the carboxylation phase, three molecules of CO2 and three molecules of H2O react with three molecules of RuBP to produce six molecules of 3-PGA.
- The enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes these reactions.
- Plants that form 3-PGA (a three-carbon molecule) as the first stable compound after feeding with 14CO2 in the light are called **C3 plants**.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)

$$3\ \text{Ribulose 1,5-bisphosphate (RuBP)} + 3CO_2 + 3H_2O \rightarrow 6\ \text{3-phosphoglycerate (3-PGA)} + 6H^+$$

Reduction of 3-PGA follows CO2 fixation and employs products of the light reactions

- The reductive phase of the Calvin-Benson cycle converts the six molecules of 3-PGA coming from the carboxylation stage into six molecules of GAP. This two-step process employs ATP and NADPH, the <u>products of the light reactions</u>.
- First, the enzyme 3-phosphoglycerate kinase catalyzes the reaction of ATP with the carboxyl group of 3-PGA, yielding the mixed anhydride 1,3-bisphosphoglycerate (1,3-bis-PGA).

$$6\ \text{3-Phosphoglycerate (3-PGA)} + 6\ \text{ATP} \rightarrow 6\ \text{1,3-bisphosphoglycerate (1,3-bis-PGA)} + 6\ \text{ADP}$$

- Next, NADPH converts 1,3-bis-PGA to GAP and inorganic phosphate (Pi) in a reaction catalyzed by the chloroplast enzyme NADP-glyceraldehyde-3-phosphate dehydrogenase (NADP-GAPD).

$$6\ \text{1,3-Bisphosphoglycerate (1,3 bis-PGA)} + 6\ \text{NADPH} + 6\text{H}^+ \rightarrow 6\ \text{glyceraldehyde 3-phosphate (GAP)} + 6\ \text{NADP}^+ + 6\ \text{P}_i$$

Regeneration of RuBP is necessary for continuous operation of the Calvin-Benson cycle

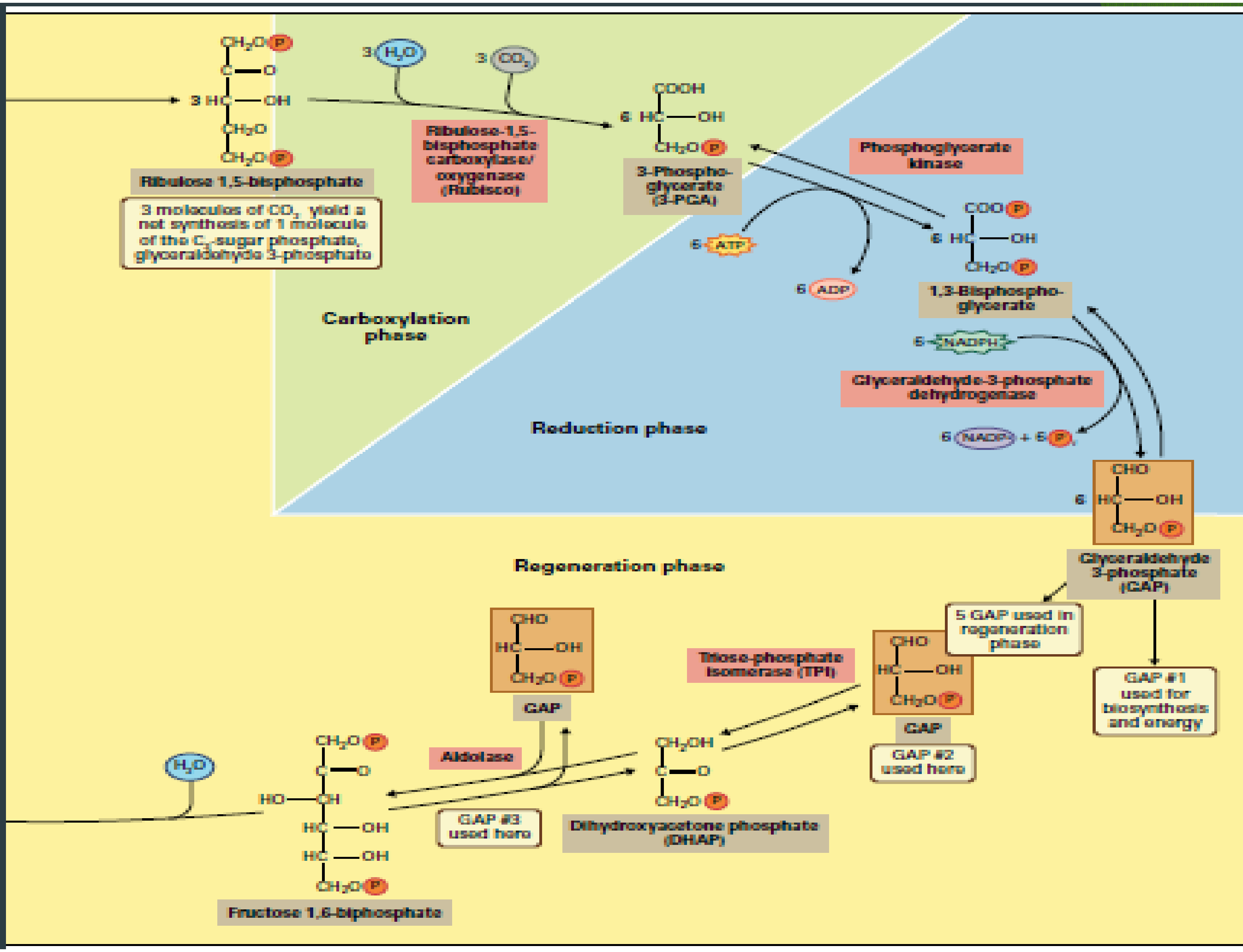
6 GAP (18C)

3 RuBP(15C)
? GAP (3C)

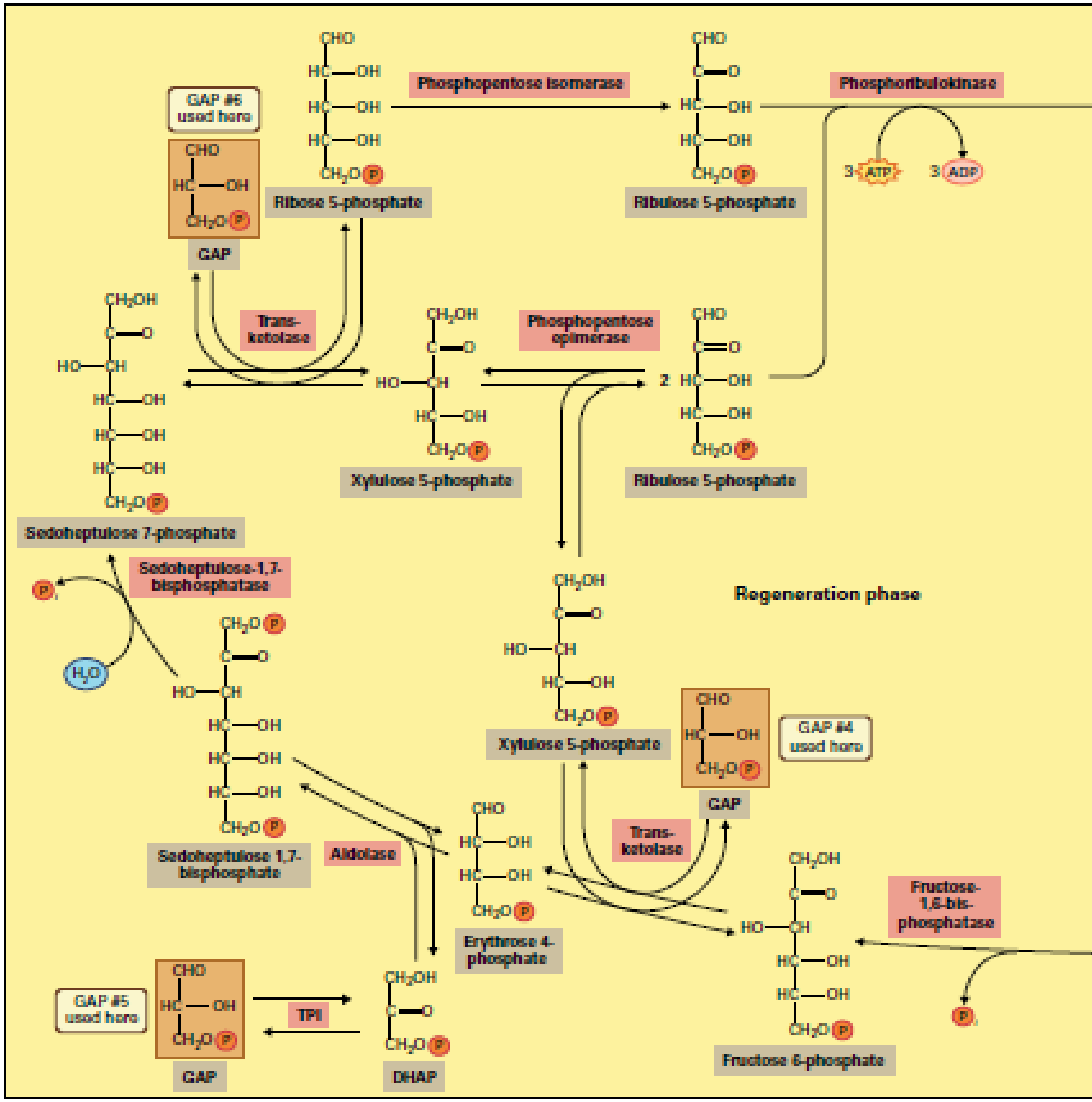
- One of the six molecules of GAP (1 molecule × 3 carbons/molecule = 3 carbons) accounts for the net fixation of three molecules of CO_2 (3 molecules × 1 carbon/molecule = 3 carbons) and represents the newly formed photosynthetic product.

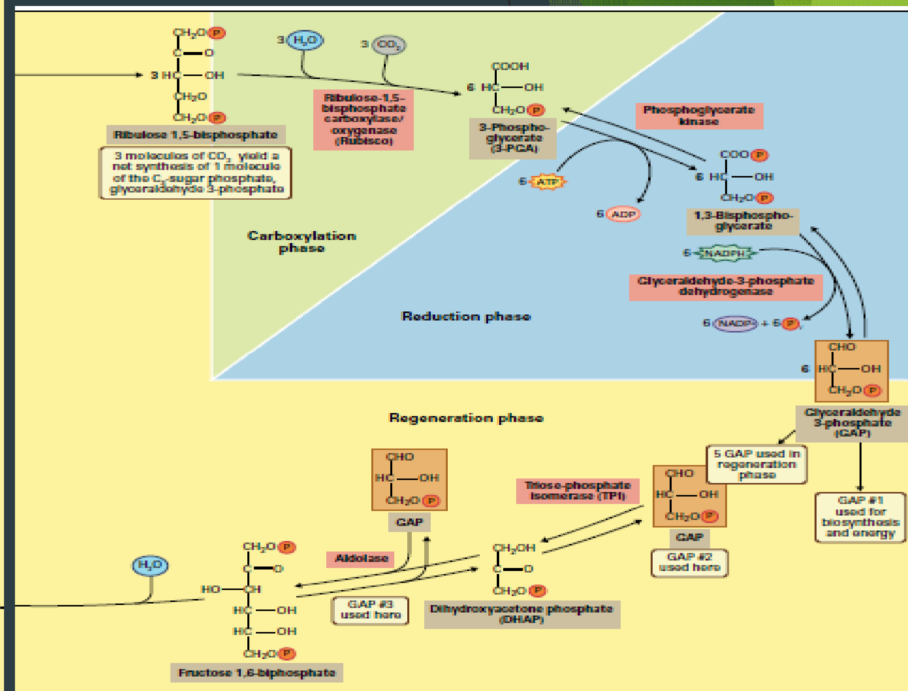
- The other five molecules of GAP (5 molecules × 3 carbons/molecule = 15 carbons) enter the last and largest set of reactions to regenerate three molecules of RuBP (3 molecules × 5 carbons/molecule = 15 carbons) and allow continuous uptake of atmospheric CO_2.

- Ten of the 13 enzymes of the Calvin-Benson cycle catalyze the reshuffling of the carbons from five molecules of GAP to form three molecules of RuBP.



3 H_2O
3 CO_2
Ribulose 1,5-bisphosphate
3 molecules of CO_2 yield a net synthesis of 1 molecule of the C_3-sugar phosphate, glyceraldehyde 3-phosphate
Ribulose-1,5-bisphosphate carboxylase/ oxygenase (Rubisco)
3-Phospho-glycerate (3-PGA)
Phosphoglycerate kinase
6 ATP
6 ADP
1,3-Bisphospho-glycerate
6 NADPH
Glyceraldehyde-3-phosphate dehydrogenase
6 NADP + 6 P_i
Carboxylation phase
Reduction phase
Regeneration phase
Glyceraldehyde 3-phosphate (GAP)
5 GAP used in regeneration phase
GAP #1 used for biosynthesis and energy
GAP
GAP #2 used here
Triose-phosphate isomerase (TPI)
GAP
Aldolase
H_2O
GAP #3 used here
Dihydroxyacetone phosphate (DHAP)
Fructose 1,6-biphosphate


Phosphopentose isomerase
Phosphoribulokinase
GAP #6 used here
Ribose 5-phosphate
Ribulose 5-phosphate
3 ATP
3 ADP
GAP
Trans-ketolase
Phosphopentose epimerase
Xylulose 5-phosphate
Ribulose 5-phosphate
Sedoheptulose 7-phosphate
Sedoheptulose-1,7-bisphosphatase
H_2O
Regeneration phase
Xylulose 5-phosphate
GAP #4 used here
GAP
Sedoheptulose 1,7-bisphosphate
Aldolase
Trans-ketolase
Erythrose 4-phosphate
Fructose-1,6-bis-phosphatase
GAP #5 used here
GAP
TPI
DHAP
Fructose 6-phosphate


Ribulose 1,5-bisphosphate
3 molecules of CO_2 yield a net synthesis of 1 molecule of the C_3 sugar phosphate, glyceraldehyde 3-phosphate
Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco)
3-Phosphoglycerate (3-PGA)
Phosphoglycerate kinase
1,3-Bisphosphoglycerate
Carboxylation phase
Reduction phase
Glyceraldehyde-3-phosphate dehydrogenase
Glyceraldehyde 3-phosphate (GAP)
Regeneration phase
5 GAP used in regeneration phase
GAP #1 used for biosynthesis and energy
Triose-phosphate isomerase (TPI)
GAP
GAP #2 used here
Aldolase
GAP #3 used here
Dihydroxyacetone phosphate (DHAP)
Fructose 1,6-biphosphate

In summary, the addition of 3 molecules of CO_2 to 3 molecules of the five-carbon sugar RuBP yields 6 molecules of 3-PGA, which in turn generate 6 molecules of GAP after successive phosphorylation and reduction with 6 molecules of ATP and 6 molecules of NADPH, respectively.
5 molecules of GAP and 3 additional molecules of ATP regenerate the 3 molecules of RuBP that set the Calvin-Benson cycle for another turn of CO_2 fixation. The remaining (1) molecule of GAP formed in the carboxylation and reduction phases of the Calvin-Benson cycle constitutes the net product of carbon fixation that plants use to build storage carbohydrates and other cellular constituents.

3 molecules of CO_2

3 molecules of RuBP

6 molecules of 3-PGA

6 molecules of NADPH

6 molecules of ATP

3 add. molecules of ATP

---

3 C

Glucose: 6C

↓

6 CO_2

12 NADPH

18 ATP

Rubisco

- Rubisco, the predominant protein in plant leaves, catalyzes the initial reaction of the Calvin–Benson cycle, the carboxylation of RuBP. In addition, Rubisco catalyzes a competing oxygenation reaction (**photorespiration**), which reduces the efficiency of photosynthetic carbon fixation.
- In addition to its role as a carboxylase, Rubisco has an oxygenase activity that uses O_2 as a substrate instead of CO_2 to produce 3-PGA and the two-carbon molecule, 2-phosphoglycolate.
- The two substrates, CO_2 and O_2, compete for the same active site on the enzyme, and the activity of the enzyme toward these substrates is dictated by the relative amounts of O_2 and CO_2 in the environment. In air, the carboxylation reaction proceeds approximately three times faster than the oxygenation reaction; the oxygenase activity, however, proceeds at a rate that can have profound effects on the overall efficiency of CO_2 fixation in C3 plants: in some cases, an estimated 50% of CO_2 fixed via photosynthesis is lost through this process of photorespiration.

photorespiration

The oxygenation of RuBP (ribulose bisphosphate) in the presence of O_2 is first reaction of photorespiration that leads to the formation of one molecule of phosphoglycolate, a two-carbon compound and one molecule of PGA. P.361

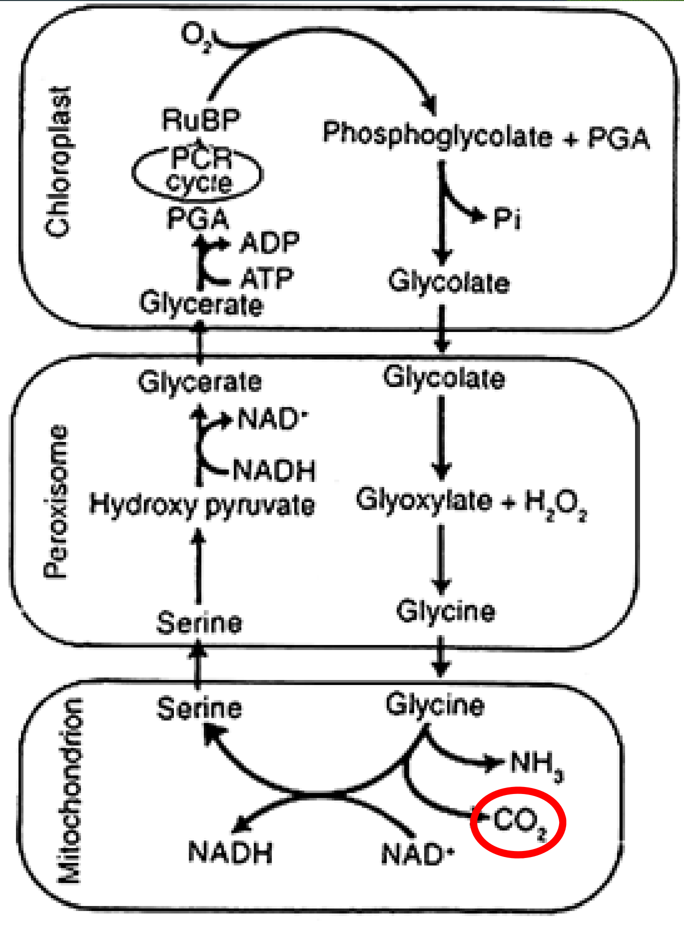


Fig. 5.10. Photorespiration.

1 RuBP(5C)

1 PGA (3C)

1 phosphoglyco. (2C)

Regulation of the Calvin–Benson cycle by light

- Chloroplasts ensure appropriate rates of biochemical transformations through modification of both the level and catalytic activity of enzymes. Specific regulatory mechanisms activate enzymes of the Calvin-Benson cycle when needed in the light and turn them off at night; degradative enzymes, such as glucose-6-phosphate dehydrogenase of the pentose phosphate pathway, behave in the opposite manner and are inactive in the light and active in the dark, adjusting production of triose phosphates and preventing futile cycles caused by competing processes
- Light regulates flux through the Calvin–Benson cycle. This regulation is critical because the chloroplast stroma contains enzymes of the Calvin–Benson cycle that catalyze carbohydrate synthesis as well as oxidative enzymes of pathways that catalyze carbohydrate degradation. To prevent futile cycling and ensure optimal activity of these competing pathways, it is essential for the synthetic apparatus to be "on" and the degradative apparatus "off " in the light.

Light modulates the levels of stromal enzymes encoded in nuclear and chloroplast genomes. P 355

Variations in mechanisms of CO_2 fixation

- Some autotrophic bacteria and archaea do not fix carbon via the Calvin-Benson cycle
- In plants, C4 and CAM metabolism increase the efficiency of Rubisco carboxylation

Rubisco catalyzes both carboxylation and oxygenation of RuBP, and photosynthetic organisms have developed strategies to overcome the problems caused by photorespiration. Land plants have adapted two different mechanisms to suppress photorespiration and increase the use of atmospheric CO_2 (Fig): **C4 photosynthetic carbon fixation** and **Crassulacean acid metabolism** (CAM). These two mechanisms are additions to the Calvin-Benson cycle that separate the uptake of atmospheric CO_2 from the supply of substrate to Rubisco inside the leaf. They act as CO_2 pumps to increase the concentration of CO_2 around the carboxylation site of Rubisco, thereby reducing the competing effect of O_2.

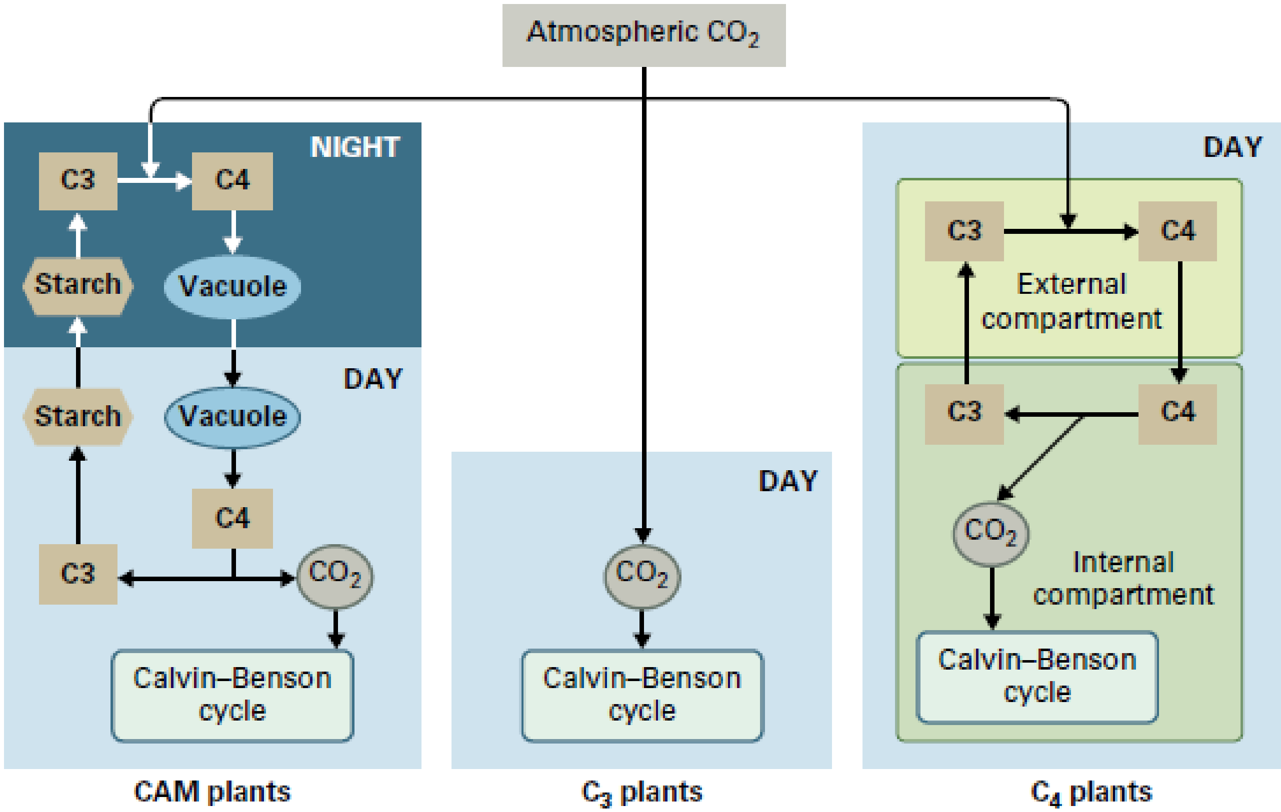


FIGURE 12.42 *Photosynthetic CO_2 assimilation in C_3, C_4, and CAM plants. The C_4 cycle and CAM are additions to the Calvin–Benson cycle for the efficient capture of CO_2. The C_4 cycle and CAM separate spatially and temporally, respectively, the capture of atmospheric CO_2 from the CO_2 fixation by the Calvin–Benson cycle.*

C4 plants utilize two distinct metabolic compartments for fixing CO_2

- Not all organisms that contain the Calvin–Benson cycle produce 3-PGA as the first stable photosynthetic intermediate.

- several plant species form large amounts of four-carbon organic acids as the first products of CO_2 fixation. On this basis, plants are classified as C3 or **C4 plants** based on the primary product of carbon fixation in photosynthesis: three-carbon (3-PGA) and four-carbon (oxaloacetic acid, OAA) compounds are the primary products of carbon fixation in C3 and C4 plants, respectively.