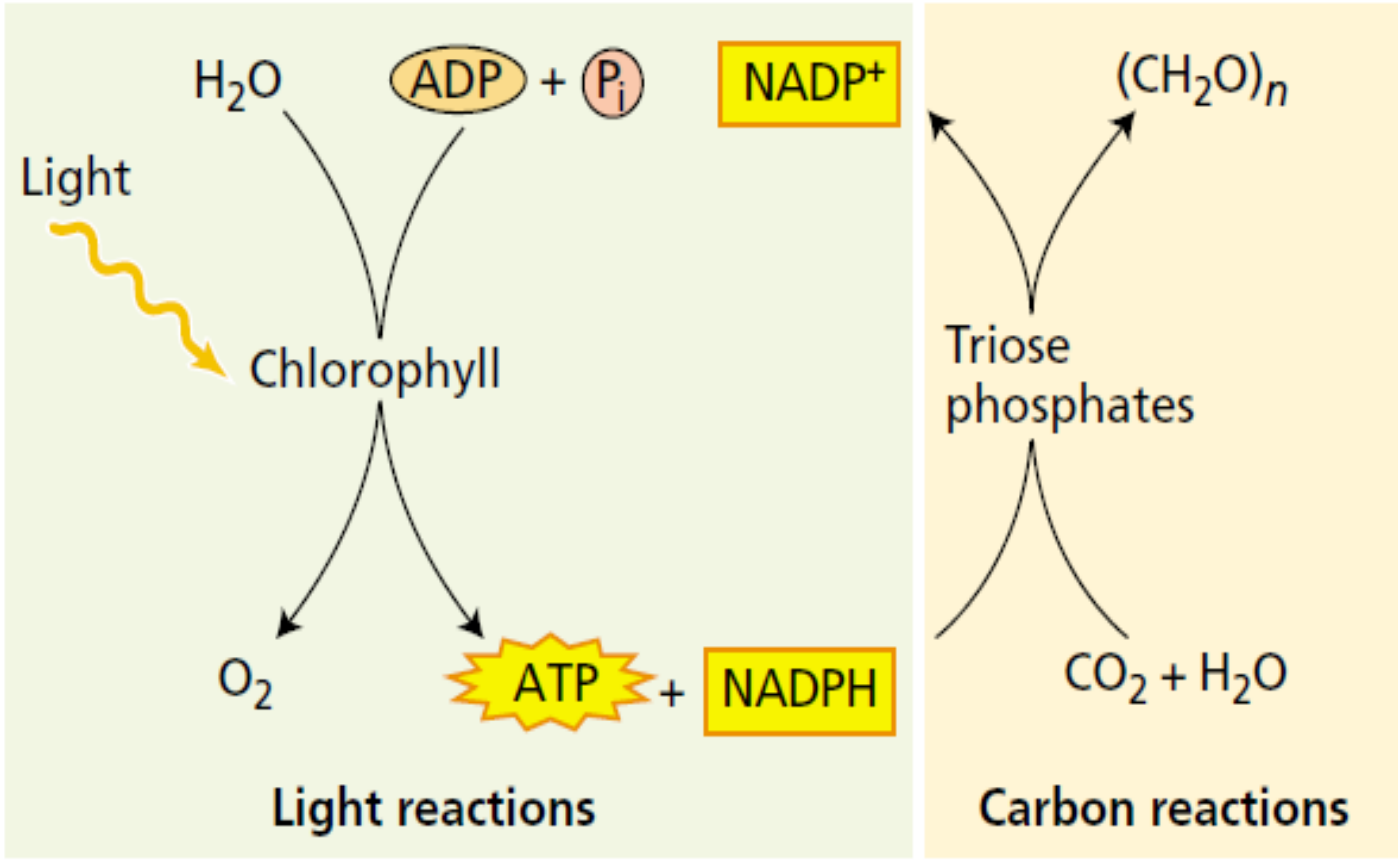
Photosynthesis-II

Carbon Reaction, Thermochemical Reaction, Biosynthetic Phase. Carbon Reaction of Photosvnthetic Phase



Products of light reactions are ATP, NADPH and O2.

Dark reaction

- second step in the mechanism of photosynthesis,
- takes place in the stroma of chloroplast
- purely enzymatic and it is slower than the light reaction. This phase does not directly depend on the light, but it's dependent on the products of the light reactions. It can be verified as follows: immediately after light becomes unavailable, the biosynthetic process continues for some time and then stops. In light is available, the synthesis starts again.
- In dark reaction, the sugars are synthesized from CO2 and H2O. The energy poor CO2 is fixed to energy rich carbohydrates using the energy rich compound, ATP and the assimilatory power, NADPH2 of light reaction. The process is called **carbon fixation** or **carbon assimilation**. Since Blackman demonstrated the existence of dark reaction, the reaction is also called as Blackman's reaction. In dark reaction two types of cyclic reactions (CO2 assimilation during photosynthesis) occur:

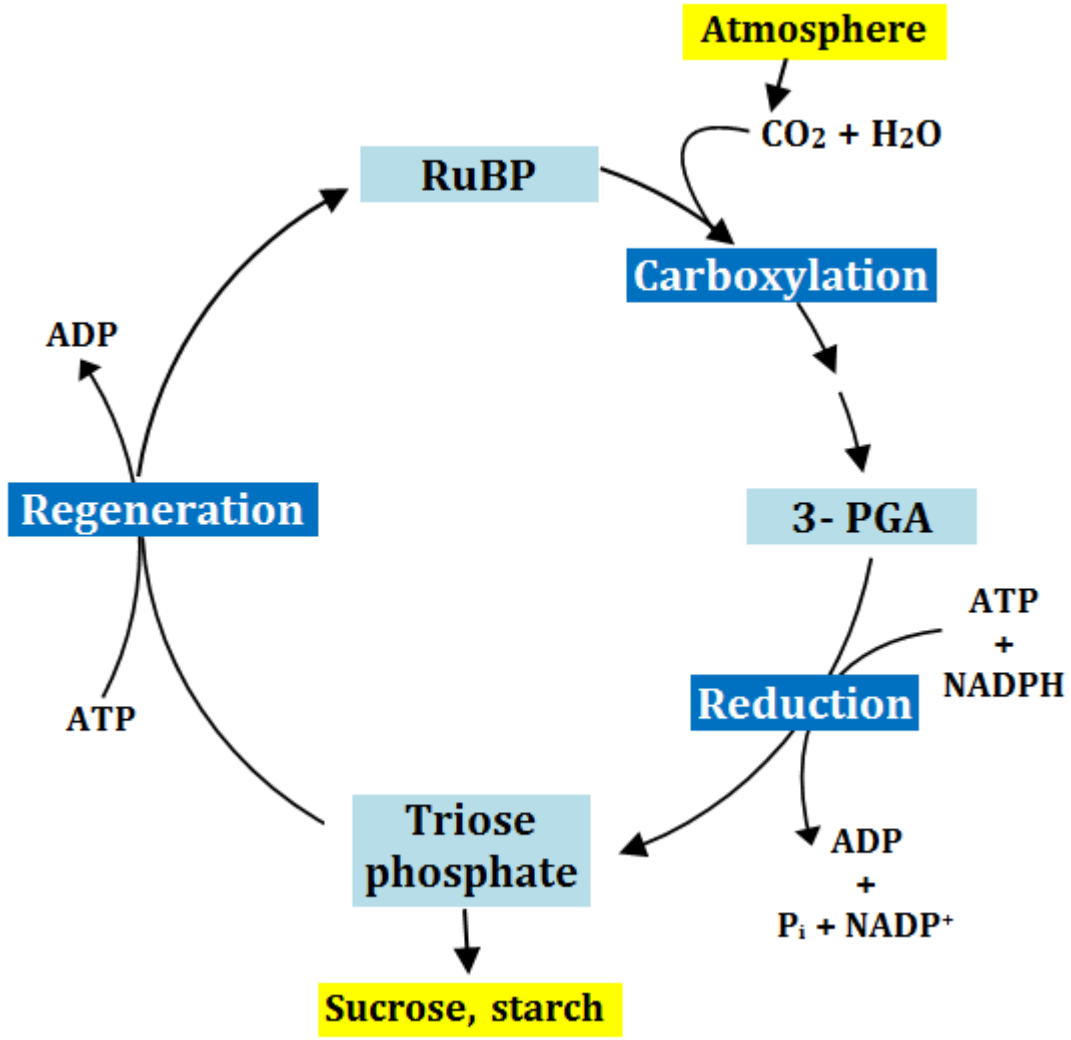
1. **C3 pathway or Calvin cycle** in this first stable product of CO2 fixation is a C3 acid (**3 phosphoglyceric acid PGA**). The Calvin cycle was first observed by Melvin Calvin using 14C in chlorella, unicellular green algae. Calvin was awarded Nobel Prize for this work in 1961. [Since the first stable compound in Calvin cycle is a 3 carbon compound (3 phosphoglyceric acid), the cycle is also called as C3 cycle.]
2. **C4 pathway or Hatch and Slack pathway**: in this first stable product is **oxaloacetic acid OAA,** a 4 carbon (C4) organic acid.

C3 pathway Calvin cycle. It occurs in all photosynthetic plants, C3 or C4 pathways. It has three stages carboxylation, reduction and regeneration.

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 C3 species (the Calvin cycle, or reductive pentose phosphate [RPP] cycle).
- 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.
- 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:



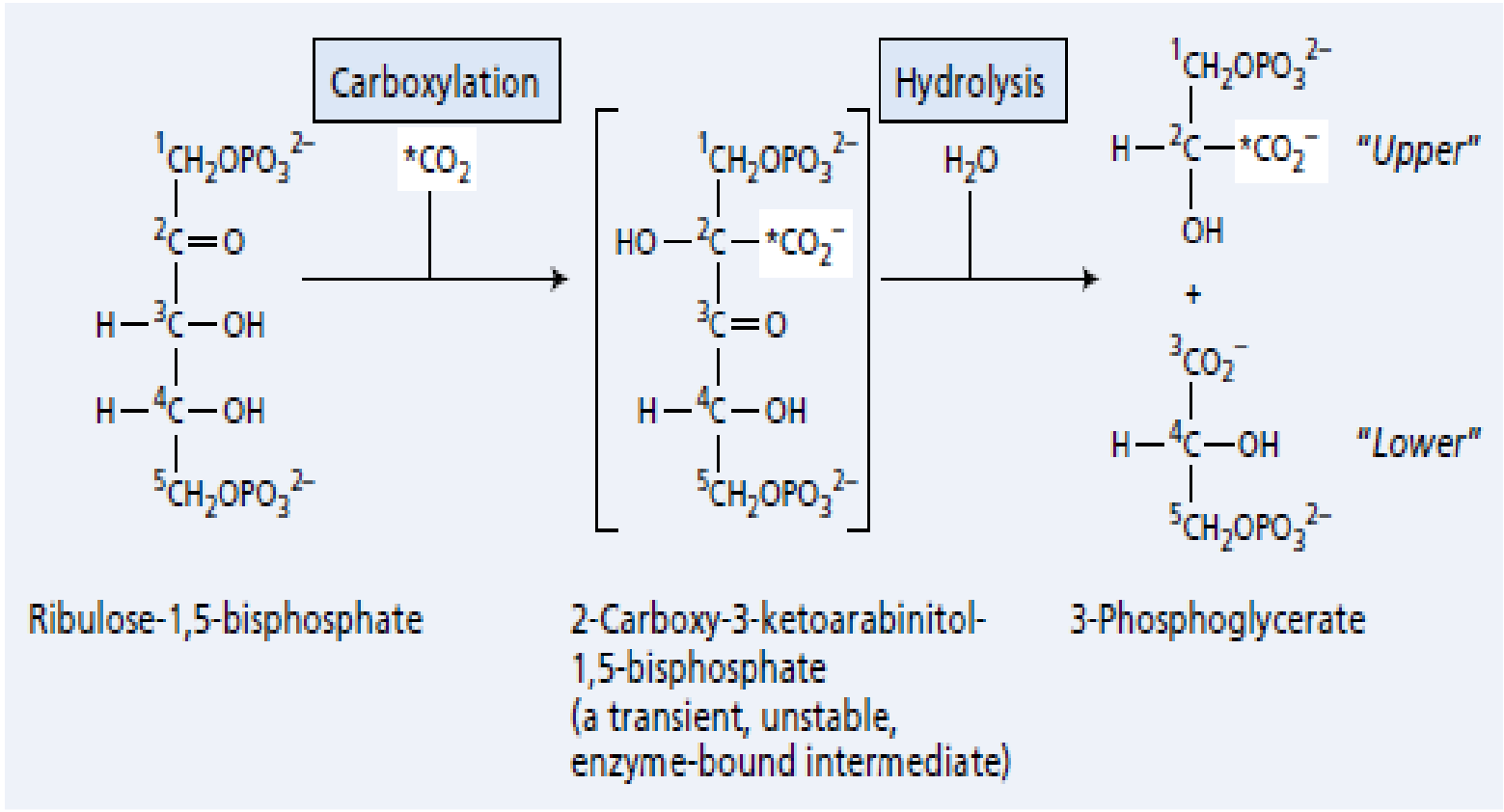
1. **Carboxylation of RuBP**, the CO_2 acceptor ribulose-1,5-bisphosphate, forming two molecules of 3-phosphoglycerate,the first stable intermediate of the Calvin cycle
2. **Reduction** of 3-phosphoglycerate, forming gyceraldehyde-3-phosphate, a carbohydrate
3. **Regeneration** of the CO_2 acceptor ribulose-1,5-bisphosphate 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 a reaction catalyzed by the chloroplast enzyme **ribulose bisphosphate carboxylase/oxygenase**, referred to as ***RuBisCO*** (most abundant enzyme in the world).
- As indicated 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.
- 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.
- 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 *CO2.

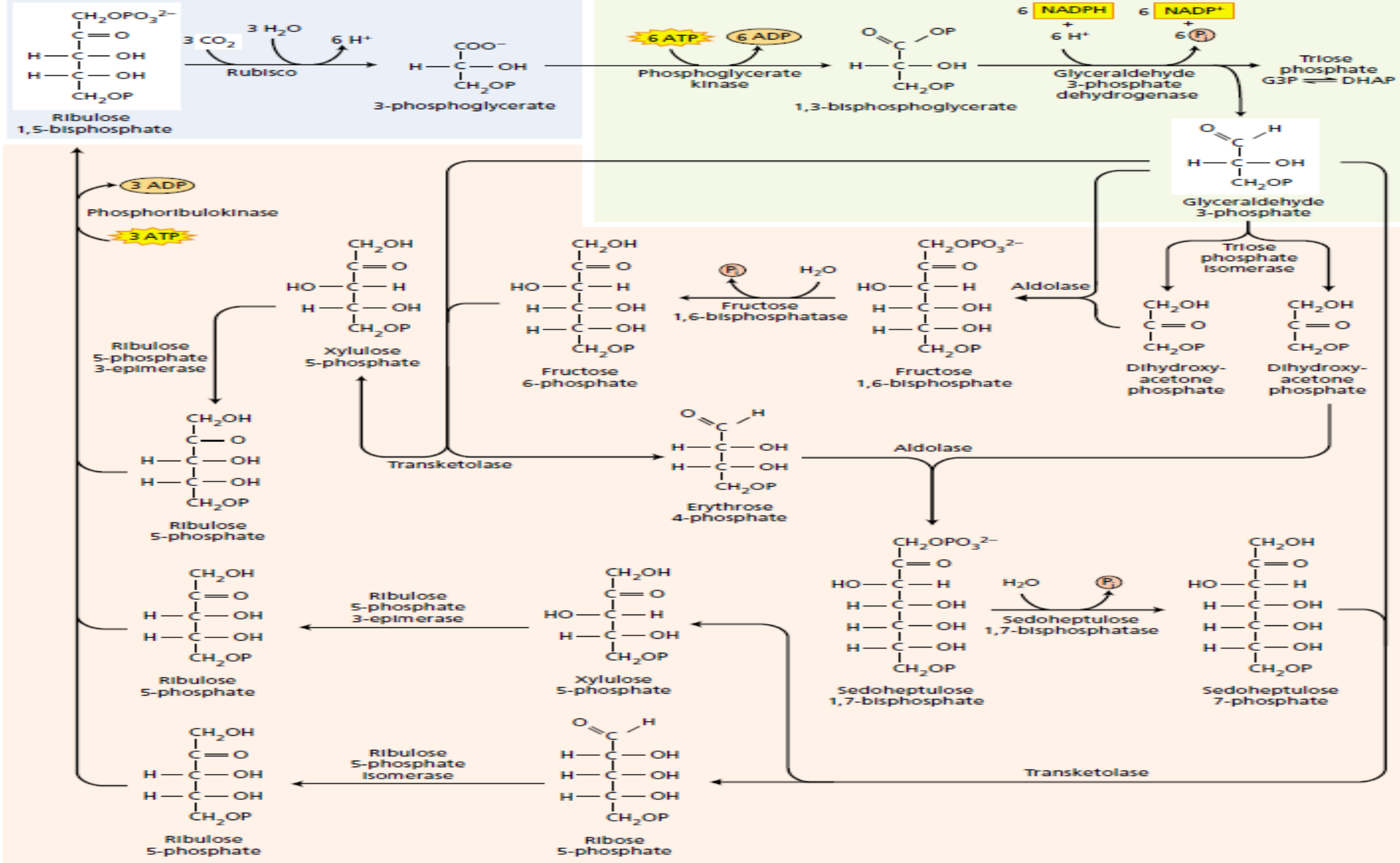

Carboxylation
*CO2
Hydrolysis
H2O
"Upper"
"Lower"
Ribulose-1,5-bisphosphate
2-Carboxy-3-ketoarabinitol-1,5-bisphosphate (a transient, unstable, enzyme-bound intermediate)
3-Phosphoglycerate

- RuBP (ribulose bis phosphate - a 5 C ketose sugar) is the primary CO2 acceptor.
- Two properties of the carboxylase reaction are especially important:

1. The negative change in 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 mM, or about 500 times greater than the concentration of its CO_2 substrate



Calvin cycle. Carboxylation of 3 molecules of ribulose-1,5- bisphosphate leads to net synthesis of 1 molecule glyceraldehyde-3-phosphate and regeneration of 3 molecules of starting material.

Summary: **$3CO_2$ + 9ATP + 6 NADPH ---→ Glyceraldehyde 3 P + 9ADP + 8Pi + 6 $NADP^+$**

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

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

Next in the Calvin cycle, 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

2. Then it is reduced to glyceraldehyde-3-phosphate through use of the NADPH generated by the light reactions. The chloroplast enzyme NADP:glyceraldehyde-3-phosphate dehydrogenase catalyzes this step.

Note that the enzyme is similar to that of glycolysis 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.

Here, 2 ATP molecules for phosphorylation and 2 NADPH for reduction per CO2 molecule are used. Fixation of 6 CO2 molecules and 6 turns of the cycle are needed to remove 1 glucose molecule from the pathway.

Operation of Calvin cycle requires Regeneration of Ribulose-1,5-Bisphosphate

- The continued uptake of CO_2 requires that the CO_2 acceptor, ribulose-1,5-bisphosphate (RuBP), be constantly regenerated.
- To prevent depletion of Calvin cycle intermediates, three molecules of RuBP (15 carbons total) are formed by reactions that reshuffle the carbons from the five molecules of triose phosphate (5 × 3 = 15 carbons).
- This reshuffling takes place in following reactions:

1. One molecule of glyceraldehyde-3-phosphate is converted via triose phosphate isomerase to dihydroxyacetone- 3-phosphate (DHAP) in an isomerization reaction(reaction 4).

2. DHAP then undergoes aldol condensation with a 2nd molecule of glyceraldehyde-3-phosphate, reaction catalyzed by aldolase to give fructose-1,6-bisphosphate (Fr-1,6 P)(reaction 5).

3. Fr-1,6 P occupies a key position in the cycle and is hydrolyzed to fructose-6-phosphate (Fr-6-P) (reaction 6).

4. A two-carbon unit (C-1 and C-2 of Fr-6-P) 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 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:

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

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.
- 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.
- Glyceraldehyde 3 Phosphate may be converted to other CHO:
  - Metabolites (e.g. Fructose-6-Phosphate, glucose-1-phosphate)
  - Energy stores (e.g. sucrose, starch)
  - Cell wall constituents (e.g. cellulose)
- Glyceraldehyde 3 Phosphate-also utilized as Carbon source for synthesis of other compounds as fatty acids or amino acids etc.
- An input of energy, provided by ATP and NADPH, is required in order to keep the cycle functioning in the fixation of CO_2.
- Calculation- 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.

$$6\,CO_2 + 18\,ATP + 12\,NADPH + 12\,H_2O \longrightarrow C_6H_{12}O_6 + 18\,ADP + 18\,P_i + 12\,NADP^+ + 6\,H^+$$

- Thus, **three molecules of ATP and two molecules of NADPH are consumed in incorporating a single CO_2 molecule into a hexose** such as glucose or fructose
- 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.
- There is a fundamental distinction between NADPH and NADH in biochemistry: *NADH is oxidized by the respiratory chain to generate ATP, whereas NADPH serves as a reductant in biosynthetic processes*
- Evidence of multienzyme complexes of Calvin cycle within chloroplast stroma.

- 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.
- Therefore, the minimum light energy needed to reduce 6 moles of CO_2 to a mole of hexose is approximately 6 × 8 × 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 rather than during operation of the Calvin cycle.
- Efficiency of the Calvin cycle - 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.
- Synthesis of 1 molecule of fructose-6-phosphate from 6 molecules of CO_2 uses 12 NADPH and 18 ATP.
- Therefore the Calvin cycle consumes (12X 217) + (18 X 29) = 3126 kJ (750 kcal) in the form of NADPH and ATP.

- 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 × 52 kcal mol^{-1} = 104 kcal, but 3 mol ATP × 7 kcal mol^{-1} = 21 kcal.
- Thus, 83% (104 of 125 kcal) of the energy stored comes from the reductant NADPH.

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***.
- 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

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 composition of the cellular milieu (e.g., pH).

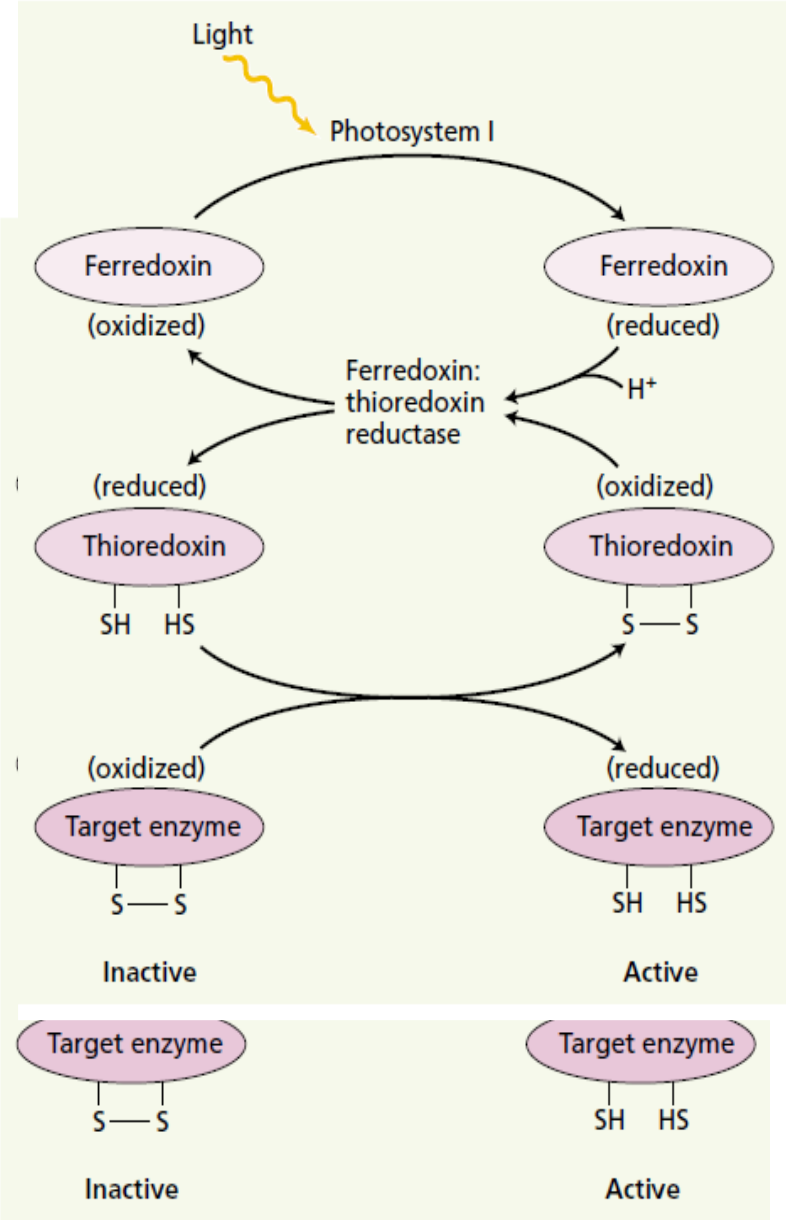
In addition, the binding of the enzymes to the thylakoid membranes enhances the efficiency of the Calvin cycle, thereby achieving a higher level of organization that favors the channeling and protection of substrates.-

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 .
- 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.

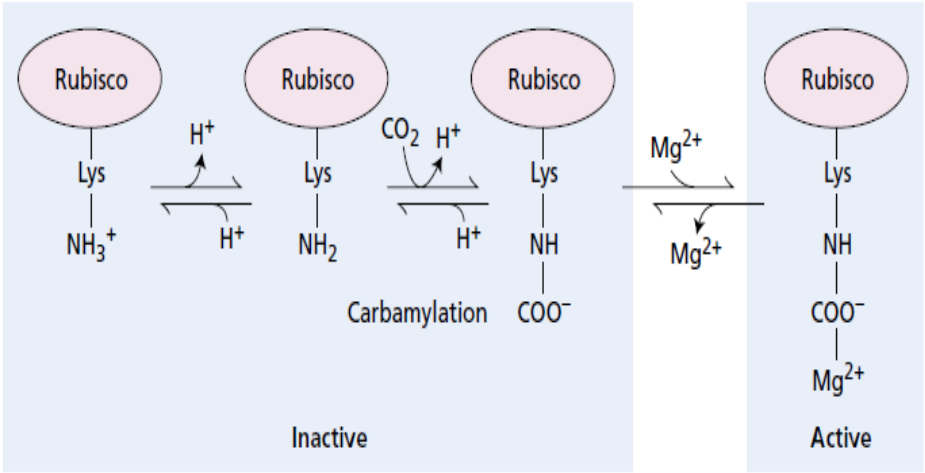


- The ferredoxin–thioredoxin system reduces specific enzymes in the light.
- Upon reduction, biosynthetic enzymes are converted from an inactive to an active state.
- The activation process starts in the light by a reduction of ferredoxin by photosystem I.
- The reduced ferredoxin plus two protons are 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.
- 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.
- Rubisco is activated when **activator CO_2** (a different molecule from the substrate CO_2 that becomes fixed) **reacts slowly with an uncharged ε-NH2 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.
- Two protons are released during the formation of the **ternary complex rubisco–CO2–Mg2+,** so activation is promoted by an increase in both pH and Mg^{2+} concentration.
- Thus, light-dependent stromal changes in pH and Mg2+ 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—CH2—COH=COH—CHOH—CH2O—P) yielding 2-carboxy-3-ketoribitol 1,5-bisphosphate. 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
- 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.
- In some plants rubisco activase is regulated by the ferredoxin–thioredoxin system
- 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.



One way in which **rubisco is activated** involves the formation of a **carbamate–Mg2+ 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 Mg2+ concentration and higher pH** (low H+ concentration) that result from illumination.
The CO2 involved in the carbamate–Mg2+ reaction is not the same as the CO2 involved in the carboxylation of ribulose-1,5-bisphosphate.

Light-Dependent Ion Movements Regulate Calvin Cycle Enzymes

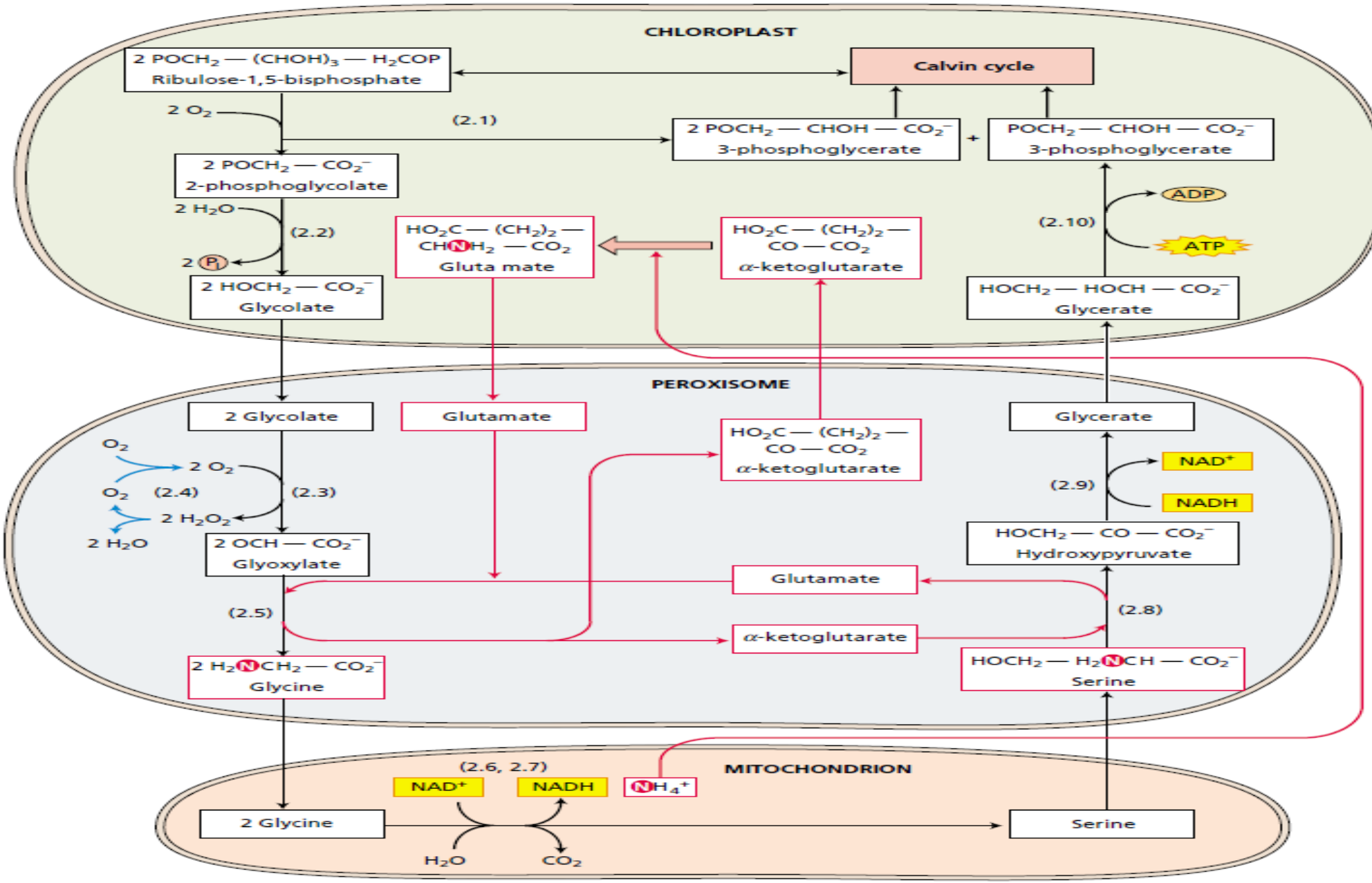
- Light causes reversible ion changes in the stroma that influence the activity of rubisco and other chloroplast enzymes.
- Upon illumination, protons are pumped from the stroma into the lumen of the thylakoids.
- The proton efflux is coupled to Mg^{2+} uptake into the stroma. These ion fluxes decrease the stromal concentration of H+ (pH 7 $\rightarrow$ 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 Mg2+ as a cofactor for catalysis.
- Hence these light-dependent ion fluxes enhance the activity of key enzymes of the Calvin cycle

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.
- To ensure continued operation of the Calvin cycle, at least five-sixths of the triose phosphate must be recycled.
- Thus, at most one-sixth can be exported for sucrose synthesis in the cytosol or diverted to starch synthesis within the chloroplast.

THE C2 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.
- **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**.
- The ability to catalyze the oxygenation of ribulose-1,5-bisphosphate is a property of all rubiscos, regard-less of taxonomic origin. Even the rubisco from anaerobic, autotrophic bacteria catalyzes the oxygenase reaction when exposed to oxygen.


CHLOROPLAST
2 POCH2 — (CHOH)3 — H2COP
Ribulose-1,5-bisphosphate
Calvin cycle
2 O2
(2.1)
2 POCH2 — CHOH — CO2−
3-phosphoglycerate
POCH2 — CHOH — CO2−
3-phosphoglycerate
2 POCH2 — CO2−
2-phosphoglycolate
2 H2O
(2.2)
2 Pi
HO2C — (CH2)2 — CHNH2 — CO2
Gluta mate
HO2C — (CH2)2 — CO — CO2
α-ketoglutarate
(2.10)
ADP
ATP
2 HOCH2 — CO2−
Glycolate
HOCH2 — HOCH — CO2−
Glycerate
PEROXISOME
2 Glycolate
Glutamate
HO2C — (CH2)2 — CO — CO2
α-ketoglutarate
Glycerate
O2
2 O2
O2
(2.4)
(2.3)
2 H2O2
2 H2O
NAD+
NADH
(2.9)
2 OCH — CO2−
Glyoxylate
HOCH2 — CO — CO2−
Hydroxypyruvate
Glutamate
(2.5)
(2.8)
α-ketoglutarate
2 H2NCH2 — CO2−
Glycine
HOCH2 — H2NCH — CO2−
Serine
MITOCHONDRION
(2.6, 2.7)
NAD+
NADH
NH4+
2 Glycine
Serine
H2O
CO2

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^+

- As alternative substrates for rubisco, CO_2 and O2 compete for reaction with ribulose-1,5-bisphosphate because carboxylation and oxygenation occur within the same active site of the enzyme.
- The C2 oxidative photosynthetic carbon cycle acts as a scavenger operation to recover fixed carbon lost during photorespiration by the oxygenase reaction of rubisco.
- The 2-phosphoglycolate formed in the chloroplast by oxygenation of ribulose-1,5-bisphosphate is rapidly hydrolyzed to glycolate by a specific chloroplast phosphatase.
- Subsequent metabolism of the glycolate involves the cooperation of two other organelles: peroxisomes and mitochondria.
- 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.
- The vast amounts of hydrogen peroxide released in the peroxisome are destroyed by the action of catalase 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.
- 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.
- 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).

- 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).
- 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).
- 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 CO2.
- In other words, 75% of the carbon lost by the oxygenation of ribulose-1,5-bisphosphate is recovered by the C2 oxidative photosynthetic carbon cycle and returned to the Calvin cycle.
- On the other hand, the total organic nitrogen remains unchanged because the formation of inorganic nitrogen (NH4+) 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).

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.

Three mechanisms for concentrating CO_2 at the site of carboxylation:

1. C4 photosynthetic carbon fixation (C4)
2. Crassulacean acid metabolism (CAM)
3. CO_2 pumps at the plasma membrane

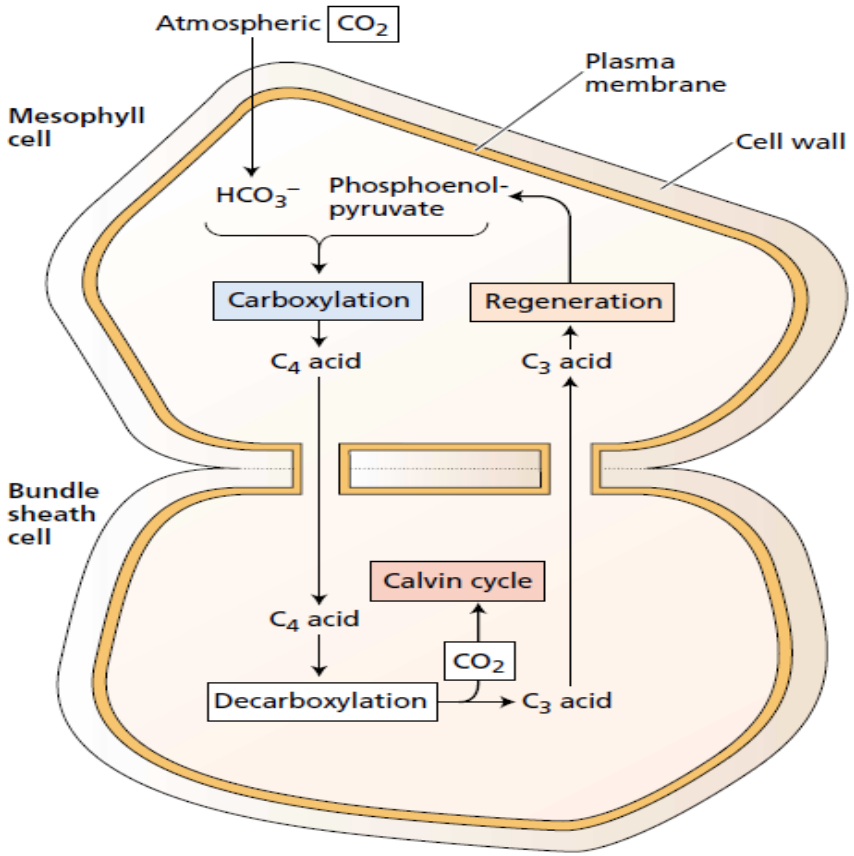
- When algal and cyanobacterial cells are grown in air enriched with 5% CO_2 and then transferred to a low-CO_2 medium, they display symptoms typical of photorespiration (O2 inhibition of photosynthesis at low concentration of CO_2).
- But if the cells are grown in air containing 0.03% CO_2, they rapidly 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 HCO3–. Total inorganic carbon inside some cyanobacterial cells can reach concentrations of 50 mM.
- 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 CO2 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 CO2**.

CO2-CONCENTRATING MECHANISMS II:THE C4 CARBON CYCLE

- There are differences in leaf anatomy between plants that have a C4 carbon cycle (called C4 plants) and those that photosynthesize solely via the Calvin photosynthetic cycle (C3 plants).
- Across section of a typical C3 leaf reveals one major cell type that has chloroplasts, the mesophyll.
- In contrast, a **typical C4 leaf has two distinct chloroplast-containing cell types: mesophyll and bundle sheath (or Kranz**, German for “wreath”) cells.
- 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 C4 cycle requires the cooperative effort of both cell types.
- No mesophyll cell of a C4 plant is more than two or three cells away from the nearest bundle sheath cell.
- In addition, an **extensive network of plasmodesmata** 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 C4 Cycle

- **M. D. Hatch and C. R. Slack** elucidated that the C4 photosynthetic cycle (C4 cycle).
- They established that the C4 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.
- The **primary carboxylation** in these leaves is catalyzed not by rubisco, but **by PEP (phosphoenyl pyruvate) carboxylase**.
- 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.



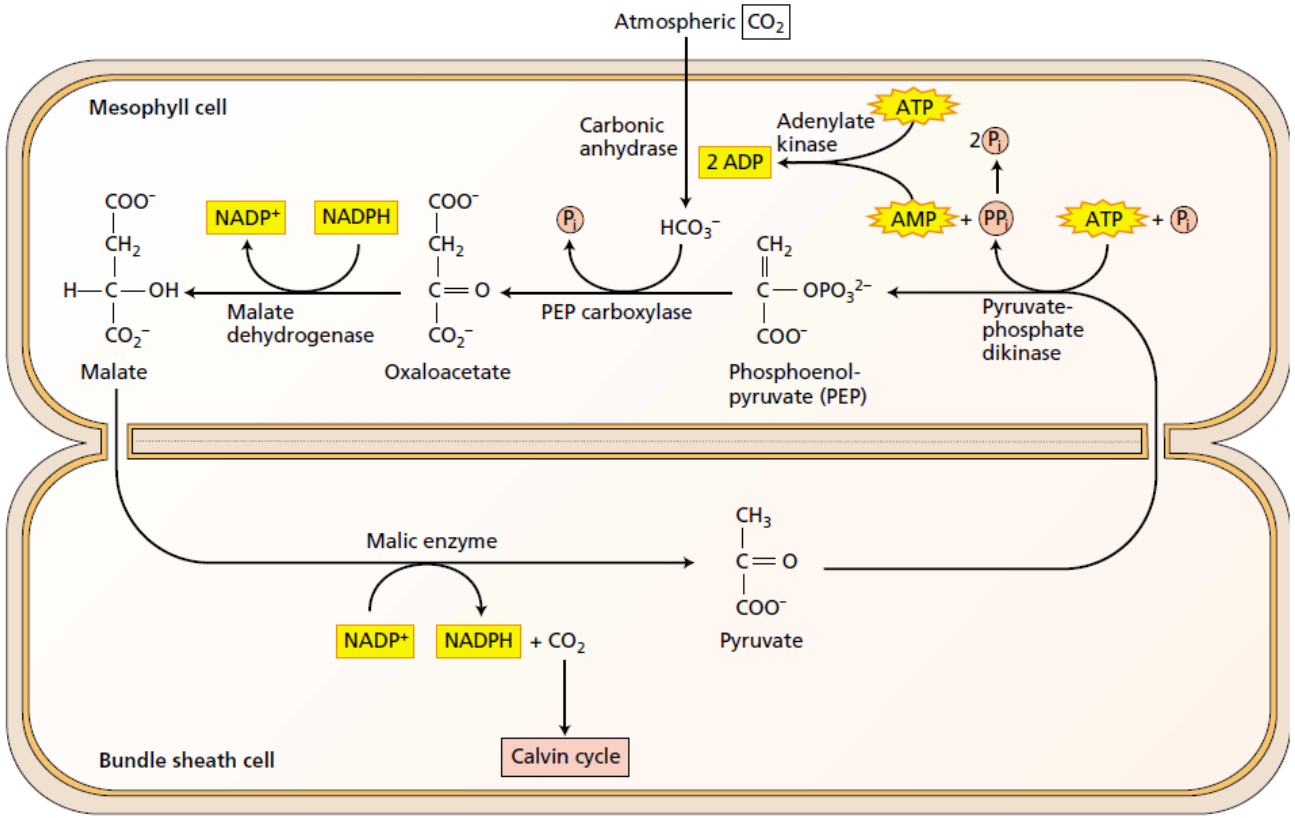
The basic C4 photosynthetic carbon cycle involves four stages in two different cell types:

(1) Fixation of CO2 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 CO2 concentration in the bundle sheath cell. The CO2 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 CO2 acceptor, phosphoenolpyruvate, is regenerated.



The C4 photosynthetic pathway.

The hydrolysis of two ATP drives the cycle in the direction of the arrows, thus pumping CO2 from the atmosphere to the Calvin cycle of the chloroplasts from bundle sheath cells.

TABLE 8.3
Reactions of the C_4 photosynthetic carbon cycle

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

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

The C4 Cycle Concentrates CO_2 in Bundle Sheath Cells

- The basic C4 cycle consists of four stages:
- 1. Fixation of CO_2 by the carboxylation of phosphoenolpyruvate in the mesophyllcells to form a C4 acid (malate and/or aspartate)
- 2. Transport of the C4 acids to the bundle sheath cells
- 3. Decarboxylation of the C4 acids within the bundle sheath cells and generation of CO2, which is then reduced to carbohydrate via the Calvin cycle
- 4. Transport of the C3 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 and another in the conversion of Ppi to 2Pi catalyzed by pyrophosphatase .
- 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 C4 cycle is now known to occur in 16 families of both monocotyledons and dicotyledons, and it is particularly prominent in Gramineae, Chenopodiaceae, and Cyperaceae.
- About 1% of all known species have C4 metabolism,There are three variations of the basic C4 pathway that occur in different species.
- The variations differ principally in the C4 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 C4 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. Thermodynamics tells us that work must be done to establish and maintain CO2 concentration gradient in bundle sheath.
- The calculation shows that the CO_2- concentrating process consumes two ATP equivalents (2 "high-energy" bonds) per CO2 molecule transported. Thus the total energy requirement for fixing CO_2 by the combined C4 and Calvin cycles is five ATP plus two NADPH per CO2 fixed.
- Because of this higher energy demand, C4 plants photosynthesizing under non photorespiratory conditions (high CO_2 and low O_2) require more quanta of light per CO_2 than C3 leaves do.
- In normal air, the quantum requirement of C3 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 C4 plants remains relatively constant under different environmental conditions

Light Regulates the Activity of Key C4 Enzymes

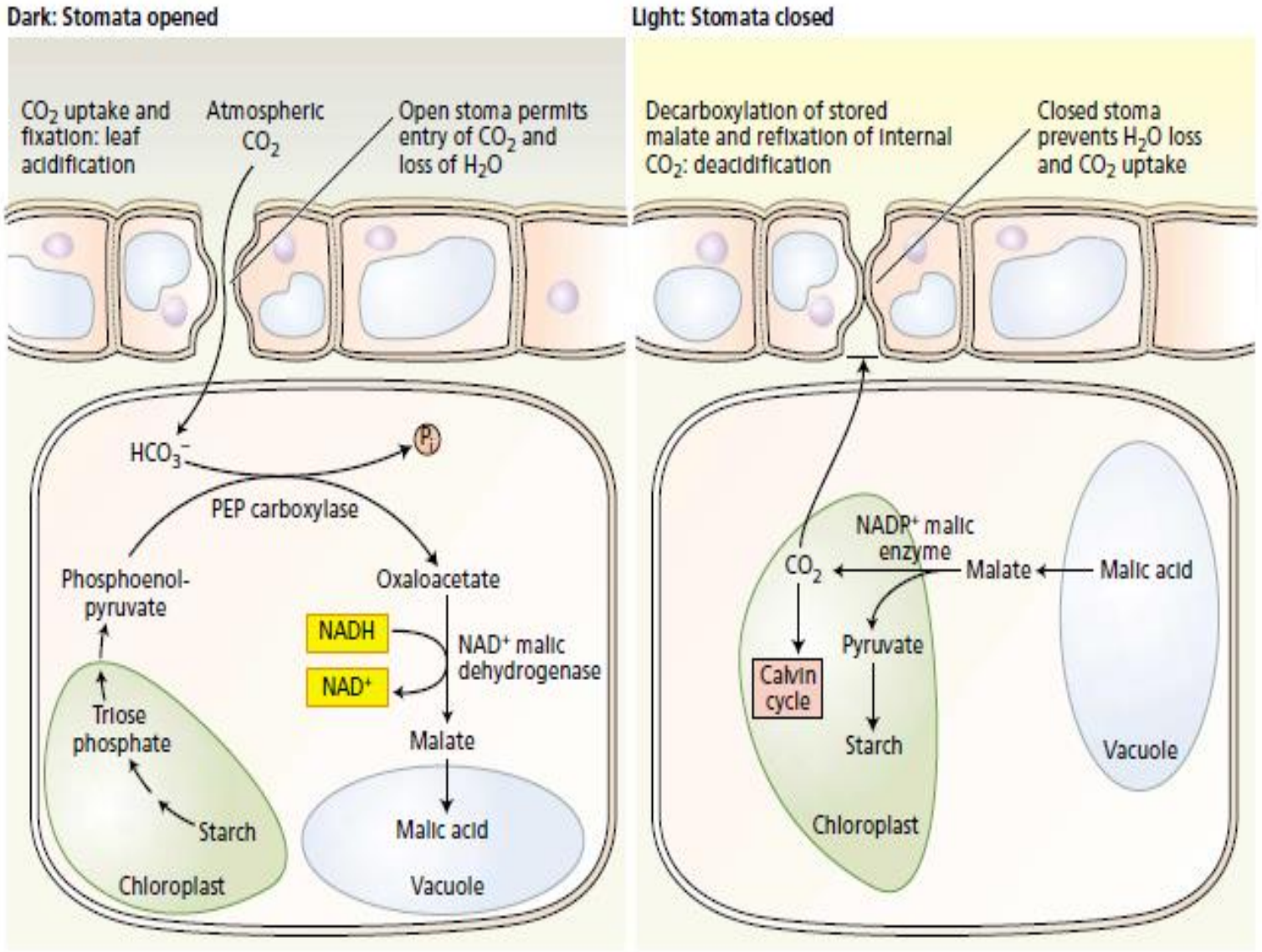
- Light is essential for the operation of the C4 cycle because it regulates several specific enzymes.
- For example, the activities of PEP carboxylase, NADP:malate dehydrogenase, and pyruvate–orthophosphate dikinase are regulated in response to variations in photon flux density by two different processes: reduction–oxidation of thiol groups and phosphorylation–dephosphorylation.
- NADP:malate dehydrogenase is regulated via the thioredoxin system of the chloroplast.
- 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 C4 pathway, pyruvate– orthophosphate dikinase, is rapidly inactivated by an unusual ADP-dependent phosphorylation of the enzyme when the photon flux density drops.
- 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 C4 Cycle Reduces Photorespiration and Water Loss

- Two features of the C4 cycle in C4 plants overcome the deleterious effects of higher temperature on photosynthesis.
- 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 C4 plants to reduce the stomatal aperture and thereby conserve water while fixing CO_2 at rates equal to or greater than those of C3 plants.
- The second beneficial feature is the suppression of photorespiration resulting from the concentration of CO_2 in bundle sheath cells.
- These features enable C4 plants to photosynthesize more efficiently at high temperatures than C3 plants, and they are probably the reason for the relative abundance of C4 plants in drier, hotter climates. Depending on their natural environment, some plants show properties intermediate between strictly C3 and C4 species.

CO2-CONCENTRATING MECHANISMS III: CRASSULACEAN ACID METABOLISM

- A third mechanism for concentrating CO2 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 C4 and C3 plants, respectively. Thus, CAM plants have a competitive advantage in dry environments.
- The CAM mechanism is similar in many respects to the C4 cycle. In C4 plants, formation of the C4 acids in the mesophyll is spatially separated from decarboxylation of the C4 acids and from refixation of the resulting CO_2 by the Calvin cycle in the bundle sheath. In CAM plants, formation of the C4 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.
- 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.



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.

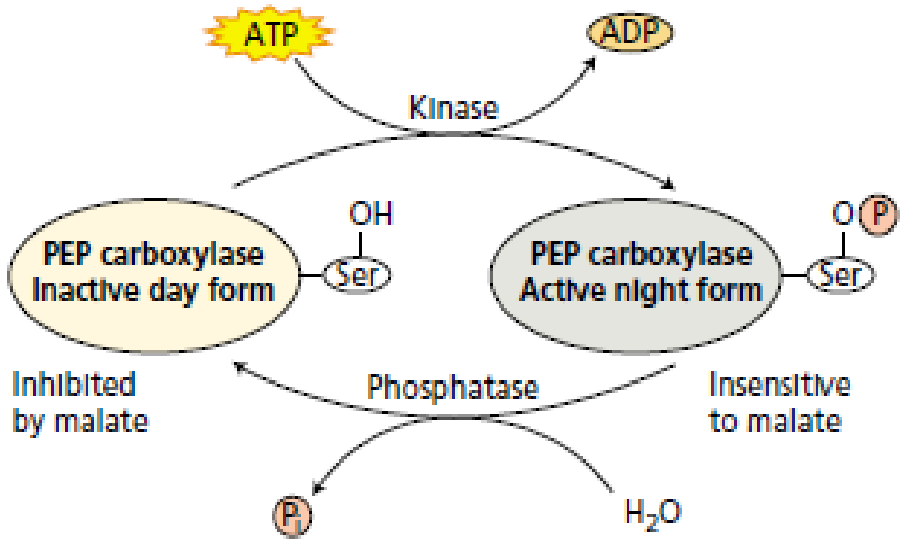
The Stomata of CAM Plants Open at Night and Close during the Day

- 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.
- The accumulation of substantial amounts of malic acid, equivalent to the amount of CO2 assimilated at night, has long been recognized as a nocturnal acidification of the leaf.
- 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.
- 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.
- The elevated internal concentration of CO_2 effectively suppresses the photorespiratory oxygenation of ribulose bisphosphate and favors carboxylation.
- The C3 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 C4 and CAM Plants

- In addition to the spatial and temporal separation exhibited by C4 and CAM plants, respectively, a futile cycle is avoided by the regulation of PEP carboxylase.
- In C4 plants the carboxylase is “switched on,” or active, during the day and in CAM plants during the night.
- In both C4 and CAM plants, PEP carboxylase is inhibited by malate and activated by glucose-6-phosphate.
- 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. 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.

Diurnal regulation of CAM phosphoenolpyruvate (PEP) carboxylase

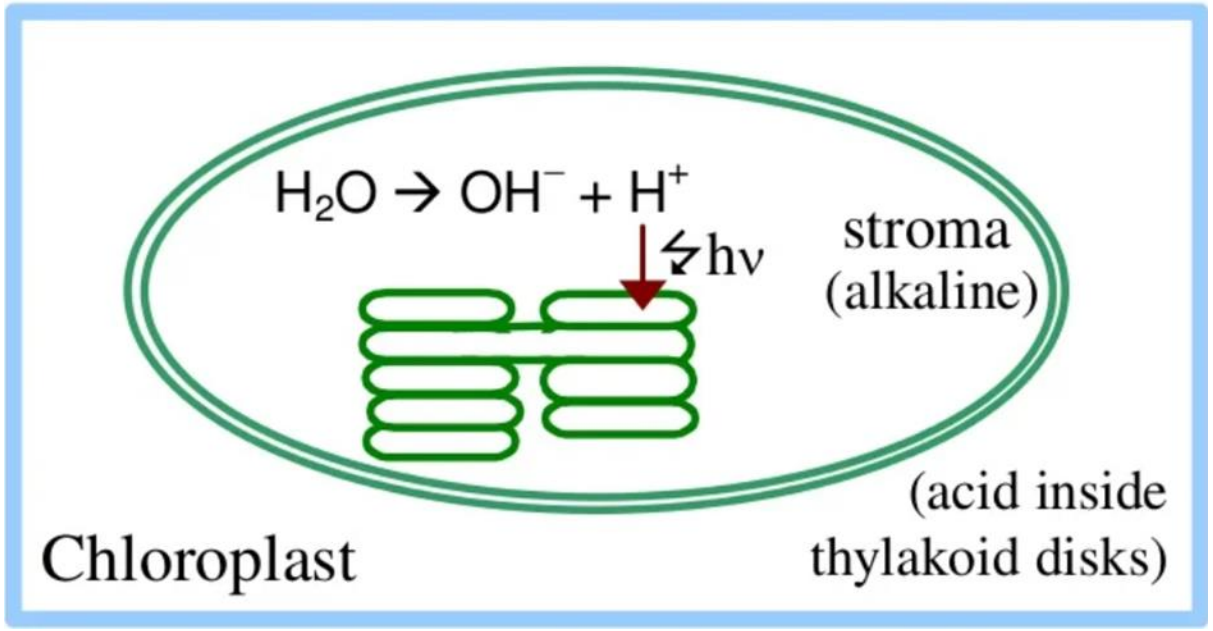


- Phosphorylation of the serine residue (Ser-OP) yields a form of the enzyme which is active during the night and relatively insensitive to malate.
- During the day, dephosphorylation of the serine (Ser-OH) gives a form of the enzyme which is inhibited by malate.

SUMMARY

Regulation of Calvin cycle

- Regulation prevents Calvin cycle from being active in the dark, when it might function in a futile function with glycolysis & pentose phosphate pathway, wasting ATP and NADPH.
- Light activates, or dark inhibits the Calvin cycle (previously called dark reaction) in several ways:
- **Light activated** e- transfer is linked to pumping of H+ into thylakoid disks. pH in the stroma increases to about 8.
- **Alkaline pH activates stromal calvin cycle enzymes** RuBP carboxylase, Fructose 1,6-bis phosphatase and Sedoheptulose bis phosphatase.
- The light activated H+ shift is countered by **Mg++ release from thylakoids to stroma**. RuBP carboxylase in stroma requires Mg binding to carbamate at the active site.
- Some plants **synthesize a transition state inhibitor** carboxyarabinitol-1-phosphate (CA-1-P) **in the dark.**
- **RuBP carboxylase activase** facilitates release of CA1P from RuBP carboxylase, when it is activated under conditions of light by thioredoxin.



THE C4 PATHWAY

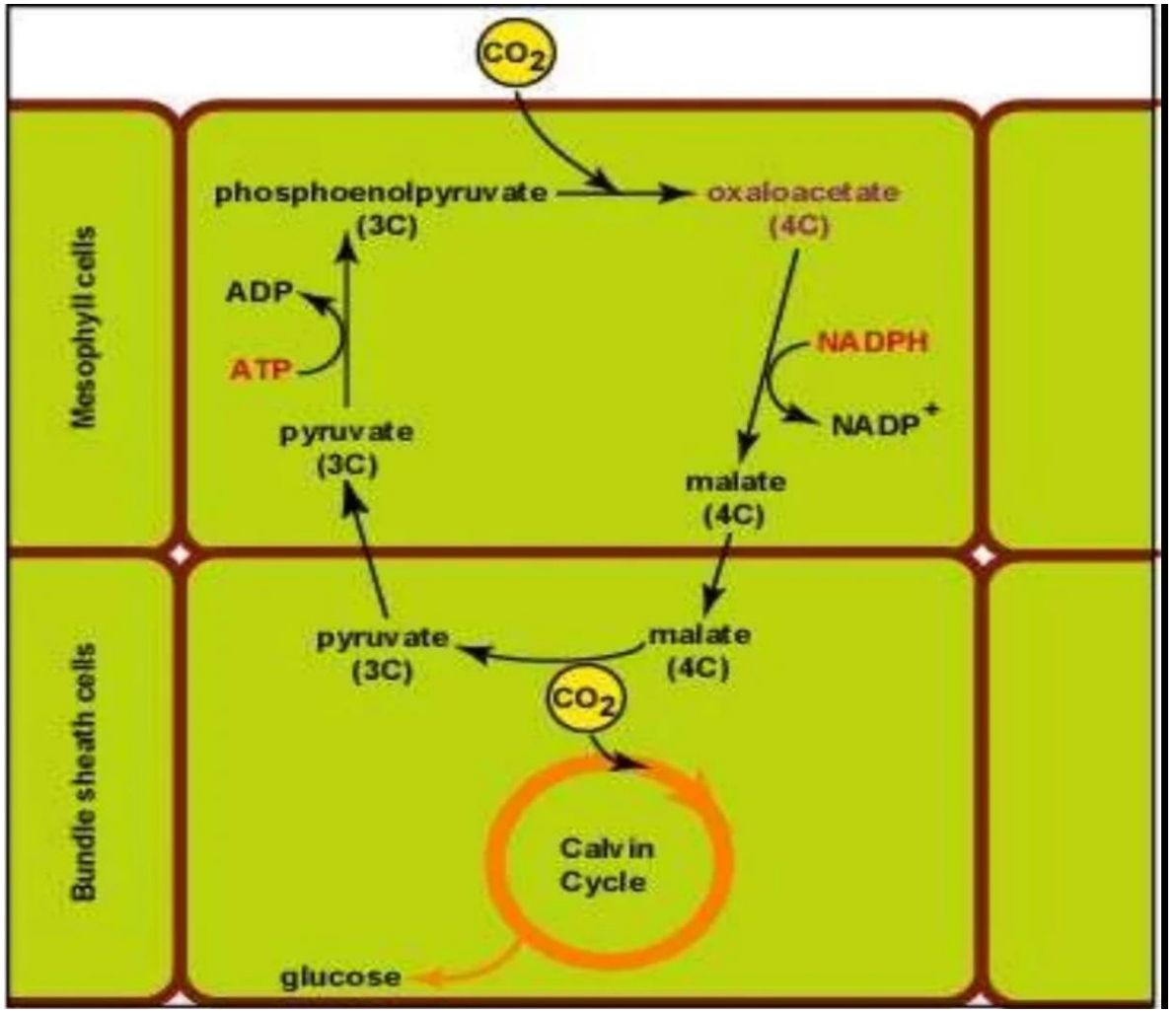
- The C4 pathway is designed to efficiently fix CO_2 at low concentrations. And plants that efficiently use this pathway are known as C4 plants.
- These plants fix CO_2 into a 4 C compound – oxaloacetate.
- This occurs in mesophyll cells.

1. CO2 is fixed into a 3 C compound called **phosphoenolpyruvate** to produce the 4 C compound **oxaloacetate**. The enzyme catalyzing this reaction, **PEP carboxylase**, fixes CO_2 very efficiently so that C4 plants don't need to have their stomata open as much. The **oxaloacetate** is then converted into a 4 C compound called ***malate*** in a step requiring the reducing power of NADPH.

2. The **malate** then exits mesophyll cells and enters the chloroplasts of specialized cells called **bundle sheath cells**.

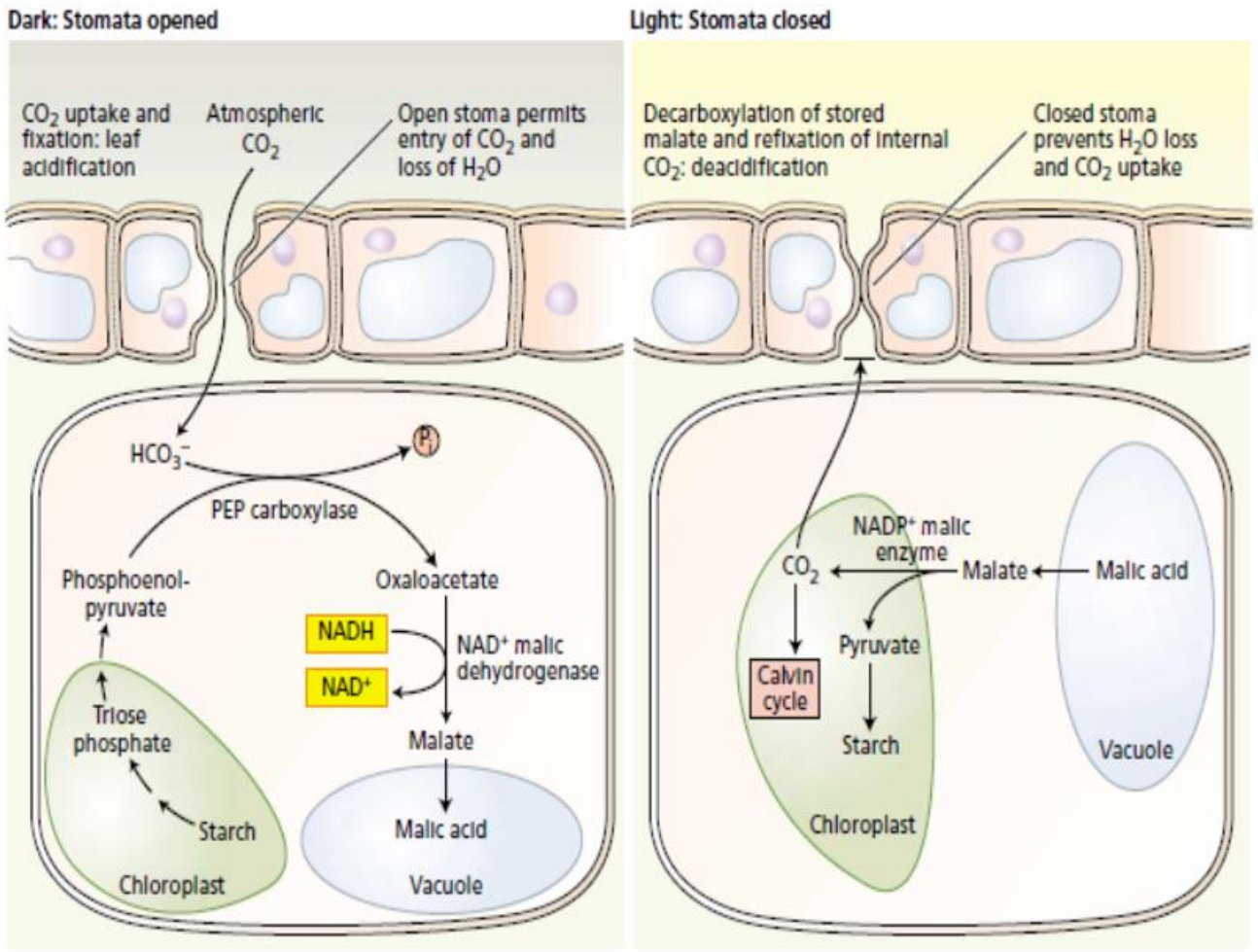
Here the 4 C malate is decarboxylated to produce CO_2, a 3 C compound called **pyruvate** and **NADPH**. The **CO_2** combines with RuBP and goes through Calvin cycle.

3. The pyruvate re-enters mesophyll cells, reacts with ATP and is converted back into **phosphoenolpyruvate**, the starting compound of C4 cycle.



THE CAM PATHWAY

- **CAM plants live in very dry condition, and unlike other plants open their stomata to fix CO_2 <u>only at night</u>.**
- Like C4 plants, they use **PEP carboxylase** to fix CO2, forming **oxaloacetate**.
- The oxaloacetate is converted into malate, which is stored in **<u>cell vacuoles</u>**. During the day, when stomata are closed, CO2 is removed from the stored malate and enters the Calvin cycle.



Differences between C3 and C4

C3

- Temperature 15-25^0 C
- Absence of malate
- One carboxylation reaction
- CO2 is the substrate
- Usual leaf structures

C4

- Temperature 30-35^0 C
- Presence of malate
- 2 carboxylation reaction
- HCO3 is the substrate
- Closed stomata to reduce water loss and concentrating CO2 in bundle sheath cells
- Additional ATP is required

Comparison between C3, C4, and CAM

	C3	C4	CAM
product	G3P Day &night	Malate Day &night	Malate Night only
Anatomy	No bundle sheet cell	Bundle sheet cell	No bundle sheet cell
No of stomata	2000-31000	10000-16000	100-800
Photorespiration	Up to 40%	Not detectable	Not detectable
Species	Wheat, rice, potato	Sugar cane	Pineapple, vanilla, cacti

Table 5.2 Comparison of some features of C_3, C_4, and CAM plants

Characteristic	C_3	C_4	CAM
Typical species/ plant groups	Wheat, barley, rice	Maize, sugarcane, millet (*Panicum* spp.), crab grass (*Digitaria sanguinalis*), Bermuda grass (*Cynodon dactylon*)	Pineapple, Cacti, succulents and many epiphytes
Leaf anatomy	Bundle sheath cells, if present, are not green (nonphotosynthetic), photosynthesis occurs in mesophyll cells	Kranz anatomy with photosynthesis occurring in mesophyll and bundle sheath cells	Photosynthesis occurs in mesophyll cells, these contain large vacuoles for malic acid storage
Carboxylating enzyme	Rubisco	Rubisco and PEP carboxylase active in the light only	Rubisco in the light, PEP carboxylase in the dark
First product of CO_2 fixation	3-PGA	OAA	OAA in the dark, 3-PGA in the light
Theoretical energy requirement (CO_2:ATP:NADPH) (with no photorespiration)	1:3:2	1:5:2 (NADP- and NAD- malic enzyme type), 1:6:2 (PEP carboxykinase type)	1:6.5:2
Water use efficiency (mg dry weight produced per g H_2O lost)	1.05–2.22	2.85–4.00	8.00–55.0
Maximum rates of net photosynthesis[a] (µmol CO_2 fixed per unit leaf area (m^2) per second)	20–40	30–60	5–12 (in the light); 6–10 (in the dark)
Photorespiration detectable?	Yes	Sometimes in bundle sheath—very low rates	Only in Phase IV
Temperature optimum for photosynthesis (°C)	15–25	30–47	35
Dry matter production (tonnes per hectare per year)	22–39	39–54	Low and very variable, (generally less than 10)
Maximum rates of productivity[b] (net assimilation rate g dry matter produced per unit leaf area (m^2) per day)	10–25	40–80	6–10
Typical range of $\delta^{13}C$ values[c]	–32 to –20‰	–17 to –9‰	–17 to –9‰ (drought) –32 to –20‰ (well-watered)

Factors affecting photorespiration

- O_2 : CO_2 ratio
- If cells have low O_2 but higher CO_2, normal photosynthesis, i.e. Calvin cycle dominates
- C4 plants have little photorespiration because they carry CO_2 to the bundle sheath cells and can build up high [CO_2].
- Calvin cycle reactions always favored over photorespiration.
- If cells have higher O_2 and lower CO_2, photorespiration dominates.
- Temperature: photorespiration increases with temperature.

***Disclaimer note**: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline.*

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