Photosynthesis

(Light independent Phase)

CO_2 reduction, photorespiration, C_4pathways; Crassulacean acid metabolism; Factors affecting CO_2 reduction.

CC-12
UNIT-2

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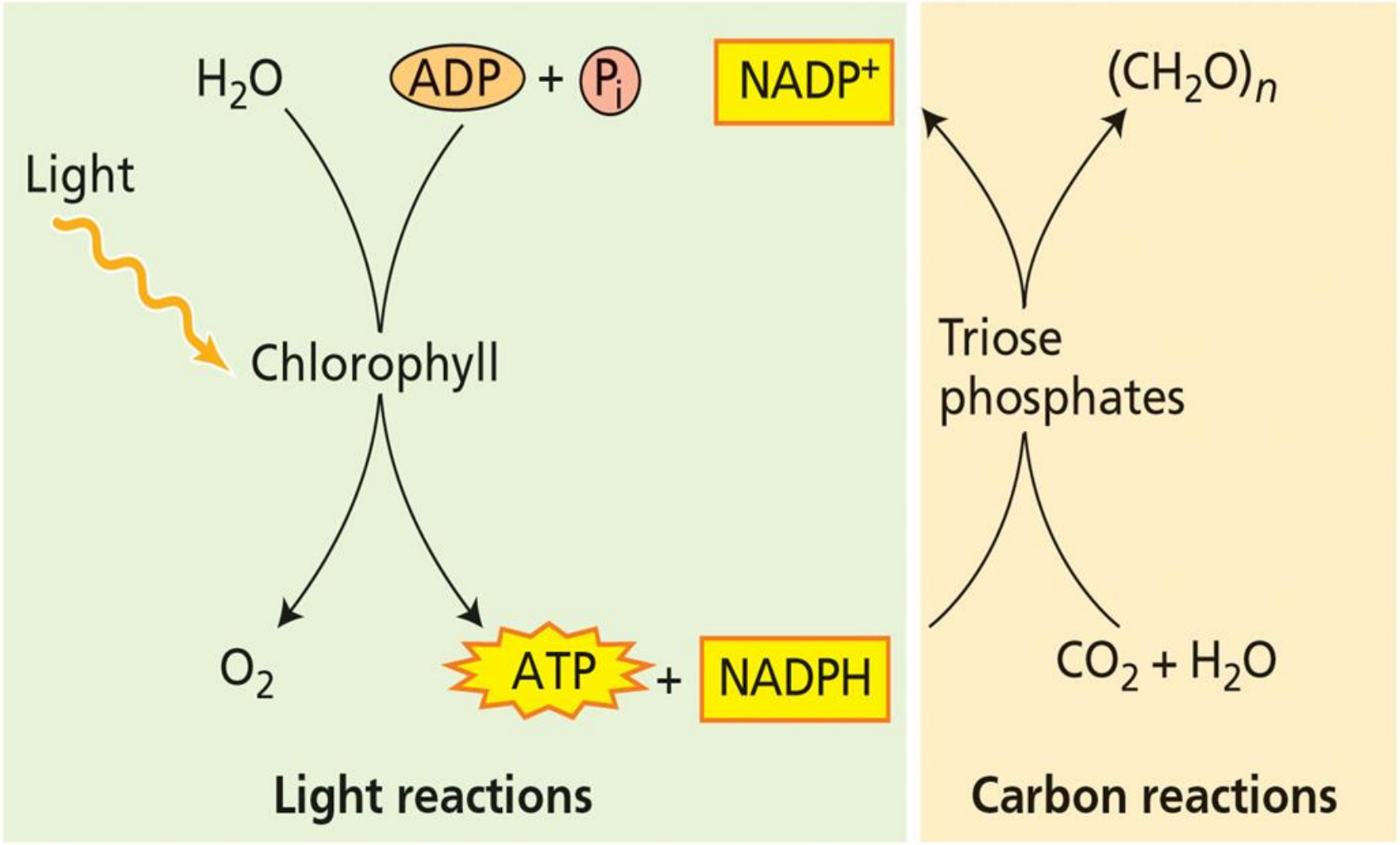
Photochemical Reactions and CO2 Reduction



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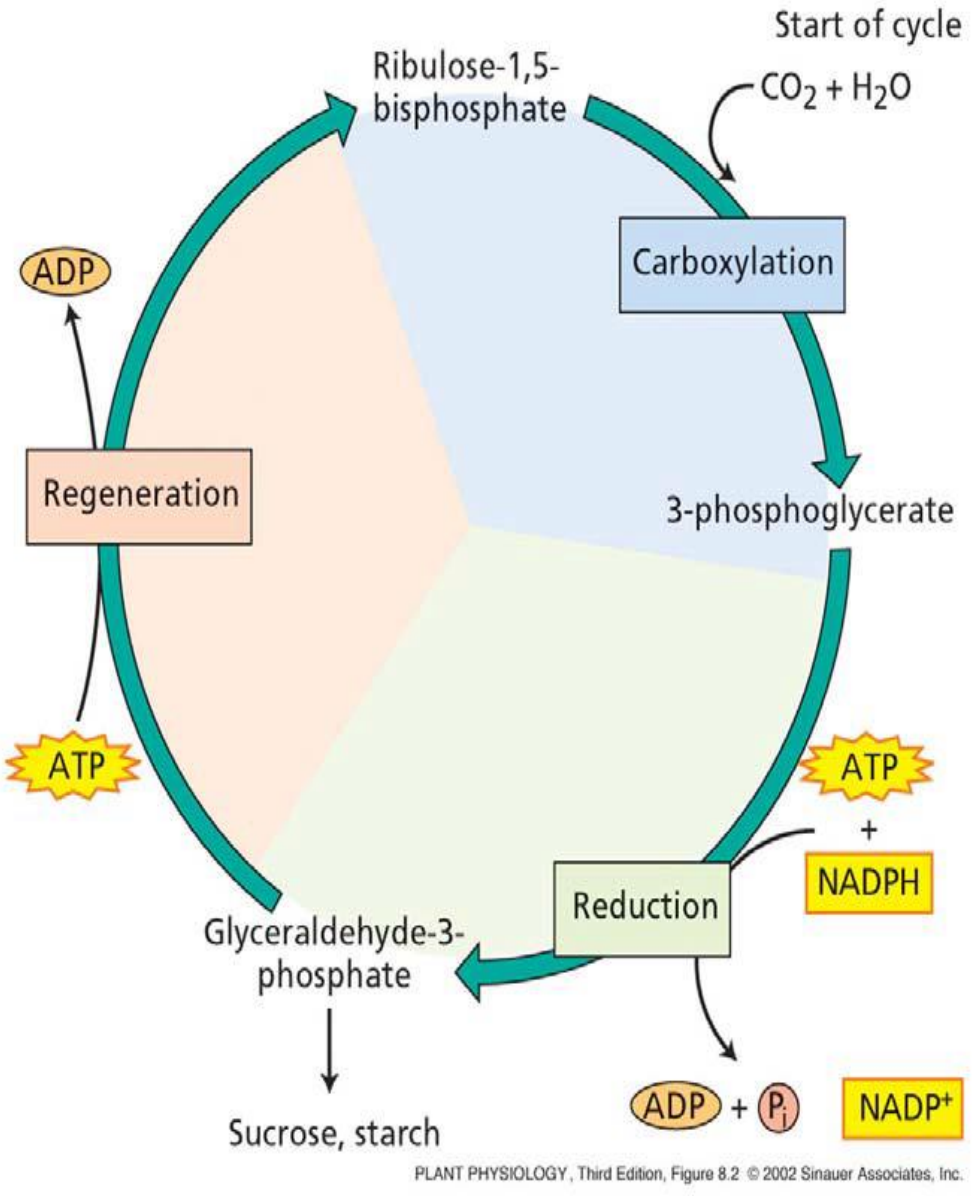
Light independent phase-C3 cycle

- Three stages.
- CO_2 reduced to triosephosphate .
- Uses ATP and NADPH from light reactions .
- Occurs in the stroma.
- Light energy converted to chemical energy of ATP and NADPH



Calvin-Benson cycle (C_3 pathway)

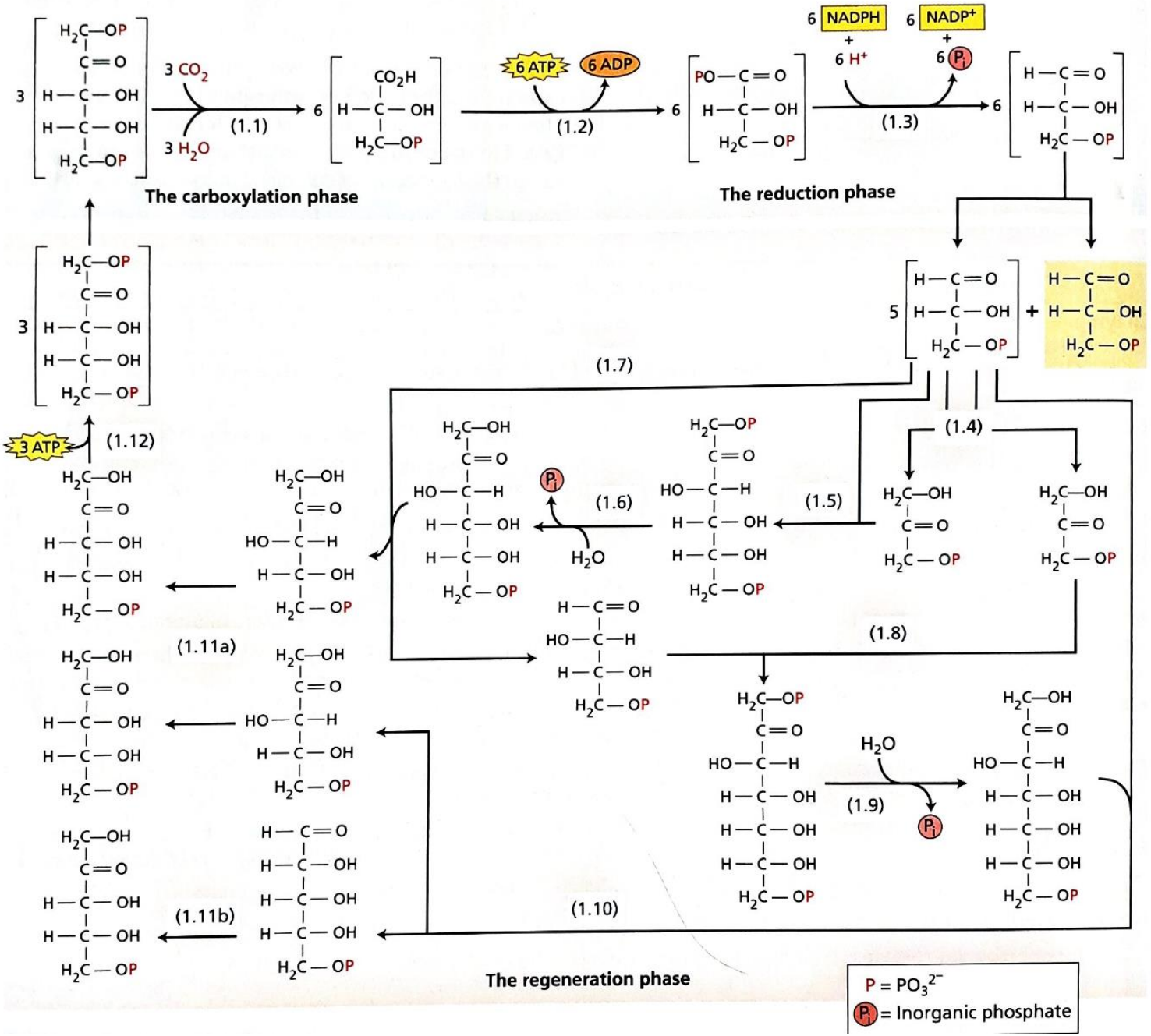
Discovery

- Use of 14 CO_2 and the green alga; Chlorella
- 1st product a C3acid
- Researchers found a 5 carbon acceptor molecule- Ribulose1,5-bisphosphate(RuBP)

Stages of Calvin-Benson cycle

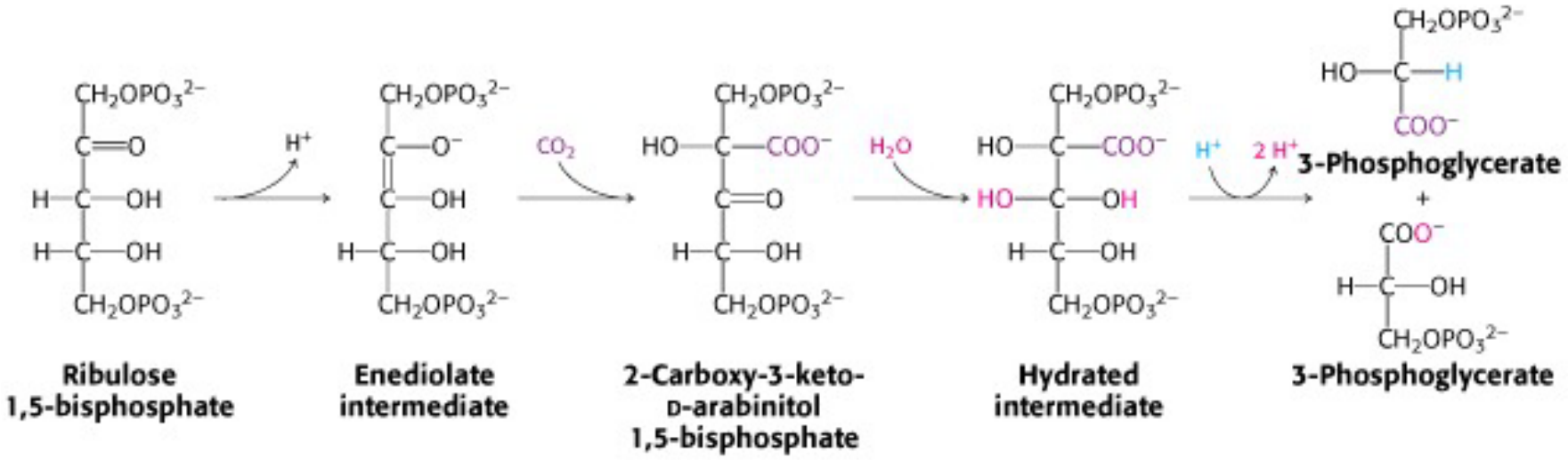
Three stages:-

- **Carboxylation**
- **Reduction**
- **Regeneration**


3 CO2
3 H2O
(1.1)
The carboxylation phase
6 ATP
6 ADP
(1.2)
6 NADPH
+
6 H+
6 NADP+
+
6 Pi
(1.3)
The reduction phase
5
+
(1.4)
(1.5)
(1.6)
Pi
H2O
(1.7)
(1.8)
(1.9)
H2O
Pi
(1.10)
3 ATP
(1.12)
(1.11a)
(1.11b)
The regeneration phase
P = PO3 2−
Pi = Inorganic phosphate

Carboxylation

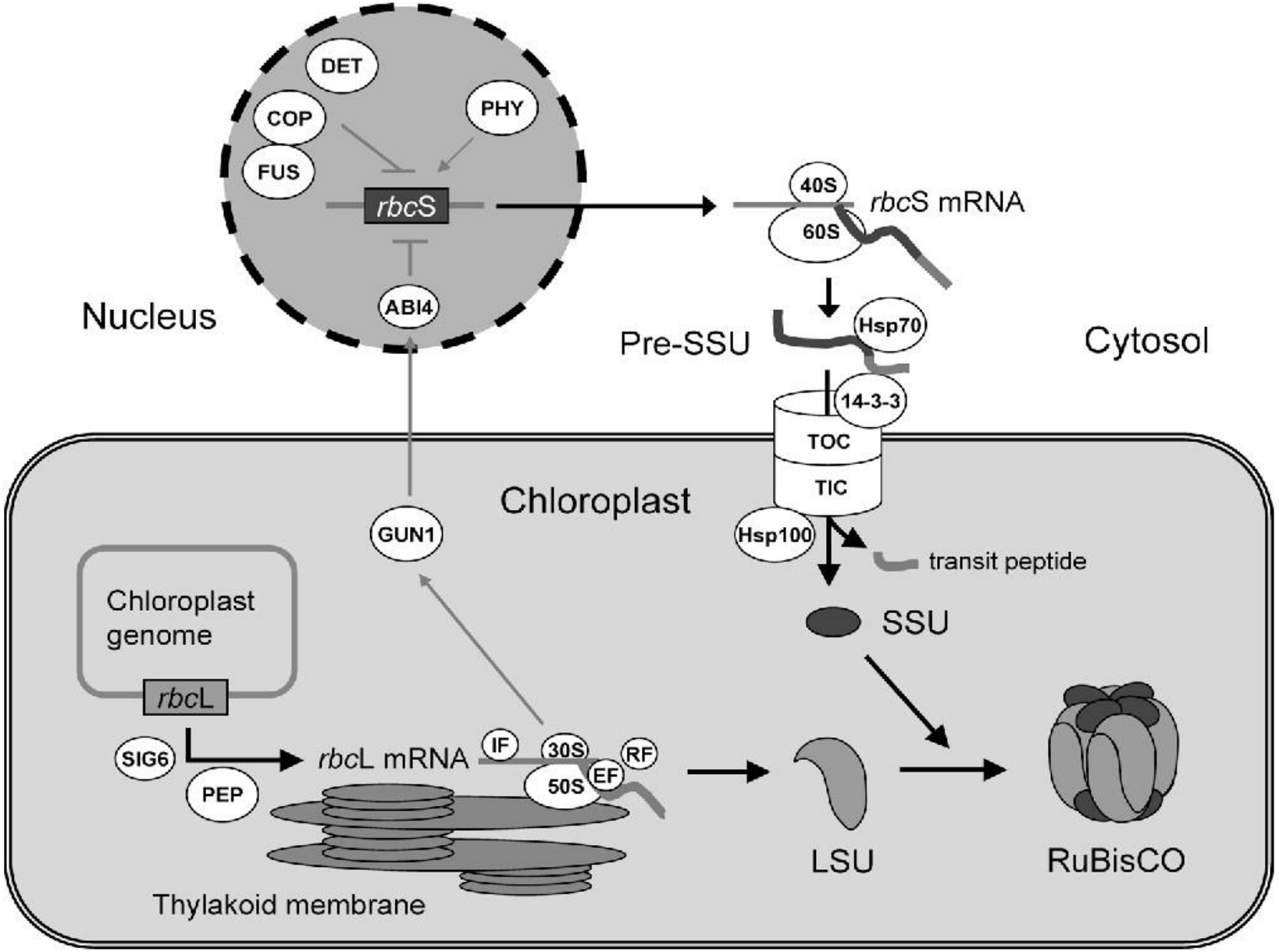
- Rubisco (large subunits= 55kd and small sub-unit 13kd), 30% of total leaf protein.
- coded by chloroplast (large) and nuclear (small) genes.
- spontaneous reaction, no energy required.
- forms 2x(3-PGA).



RubisCO

- The only enzyme that enables the fixation of atmospheric CO_2 for the formation of biomass
- A prerequisite for the existence of the present life on Earth
- In plants and cyanobacteria, it consists of eight identical large subunits (51–58 kDa) and eight identical small subunits (12–18 kDa). With its 16 subunits, RubisCO is one of the largest enzymes in nature
- In plants the genetic information for the large subunit is encoded in the plastid genome and for the small subunit in the nucleus
- Each large subunit contains one catalytic centre

RubisCO Biosynthesis



...BisCO biosynthesis in higher plants. Arrows indicate steps of RuBisCO biosynthesis: transcription, translation, imp...

Reduction

- Two steps
- Requires 2 ATP & NADPH
- Forms triosephosphate

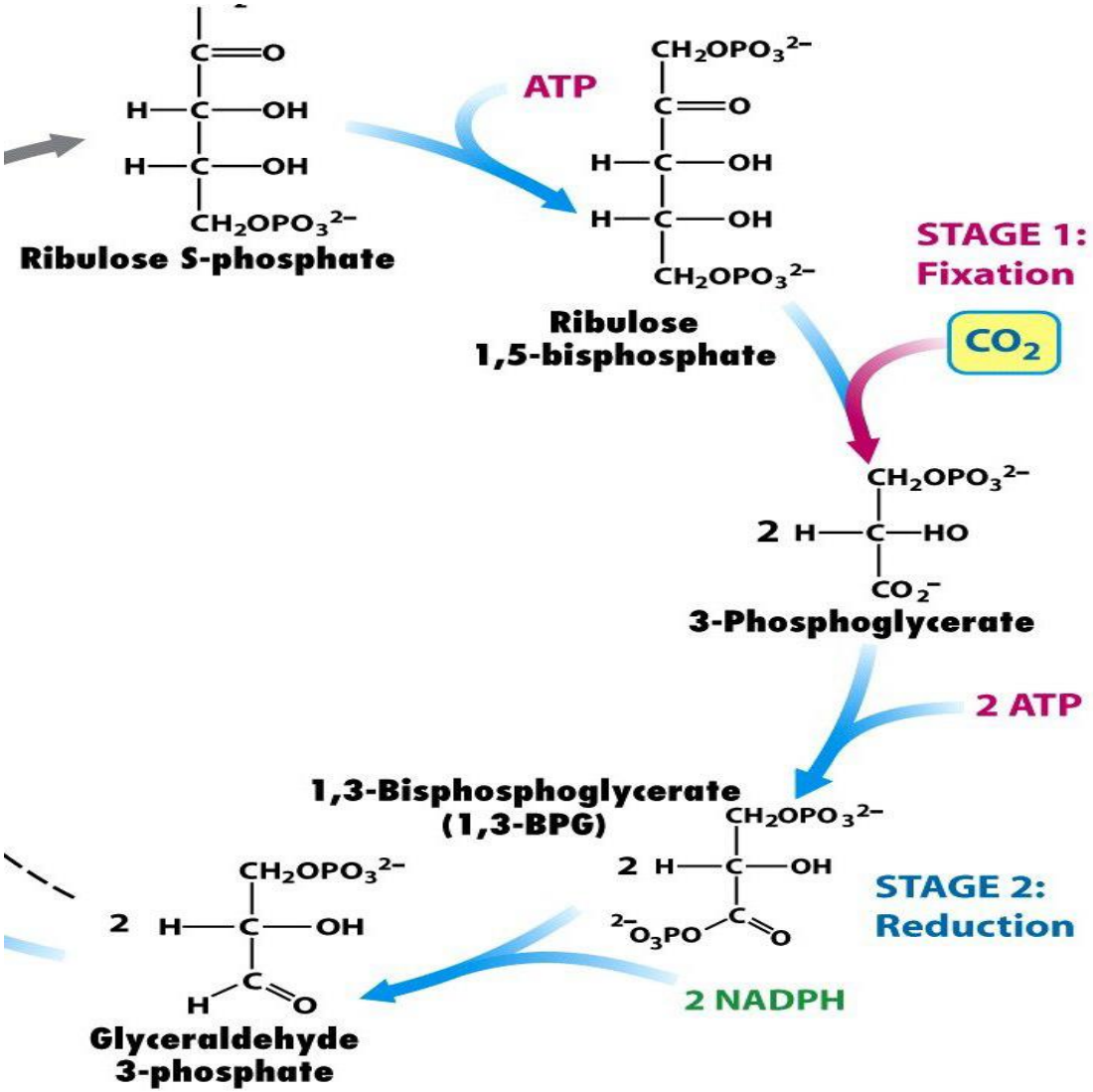


TABLE 8.1
Reactions of the Calvin cycle (Part 1)

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

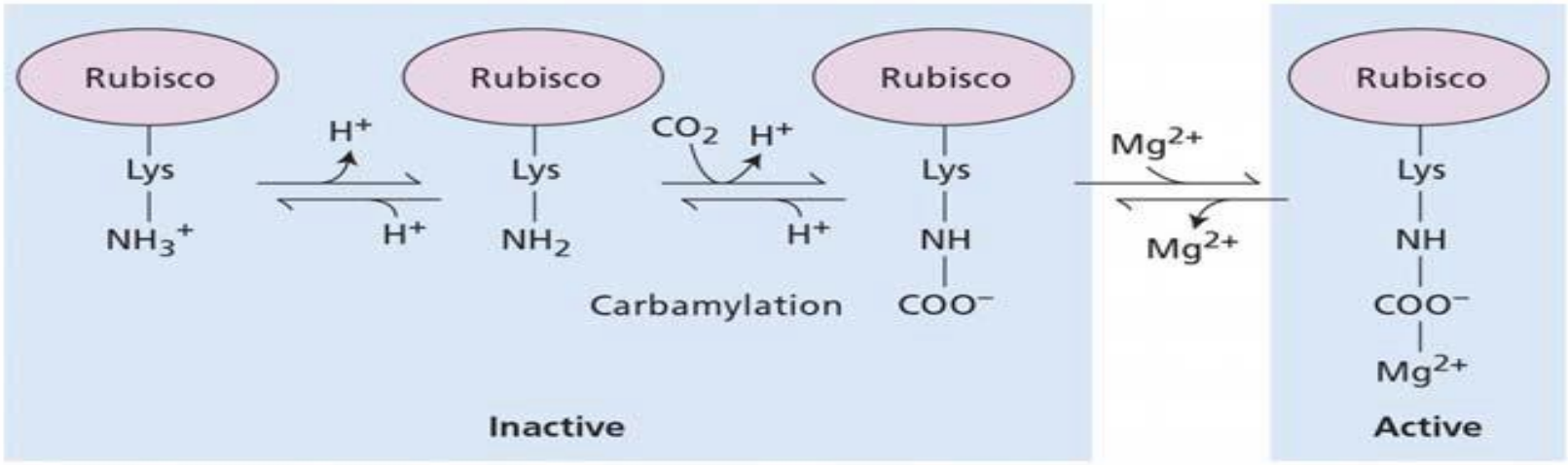
Note: P_i stands for inorganic phosphate.

Regulation of Calvin Cycle

Rubisco

- light activates electron transport
- pH stroma goes up from 7 to 8
- Mg2+increases in stroma
- NADPH allosteric activator
- Rubisco Activase catalyzes carbamate formation

–CO2required



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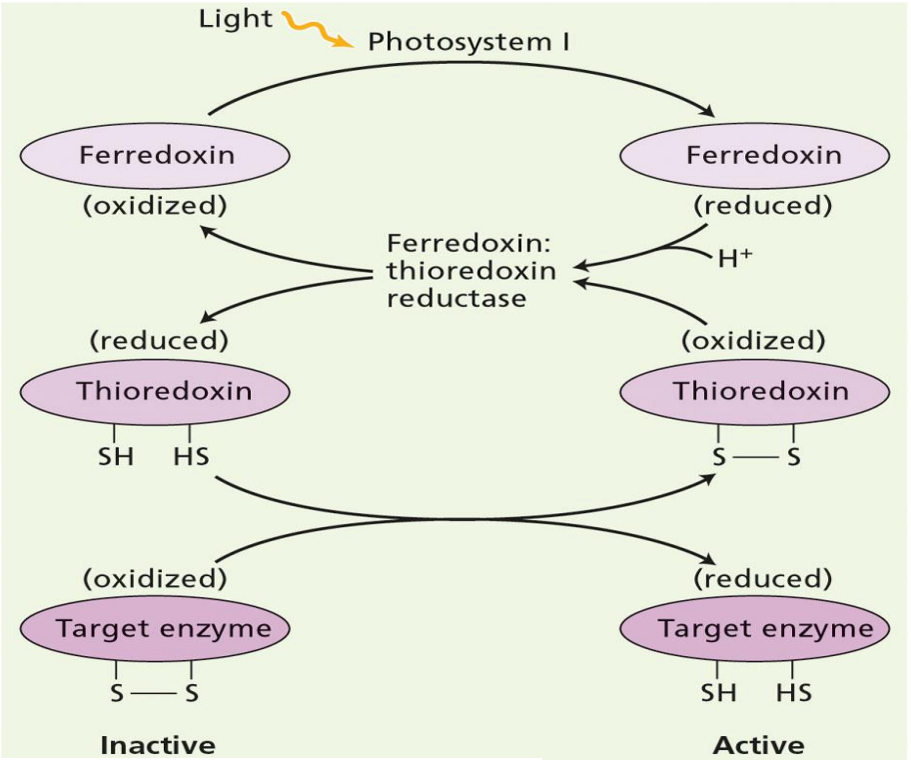
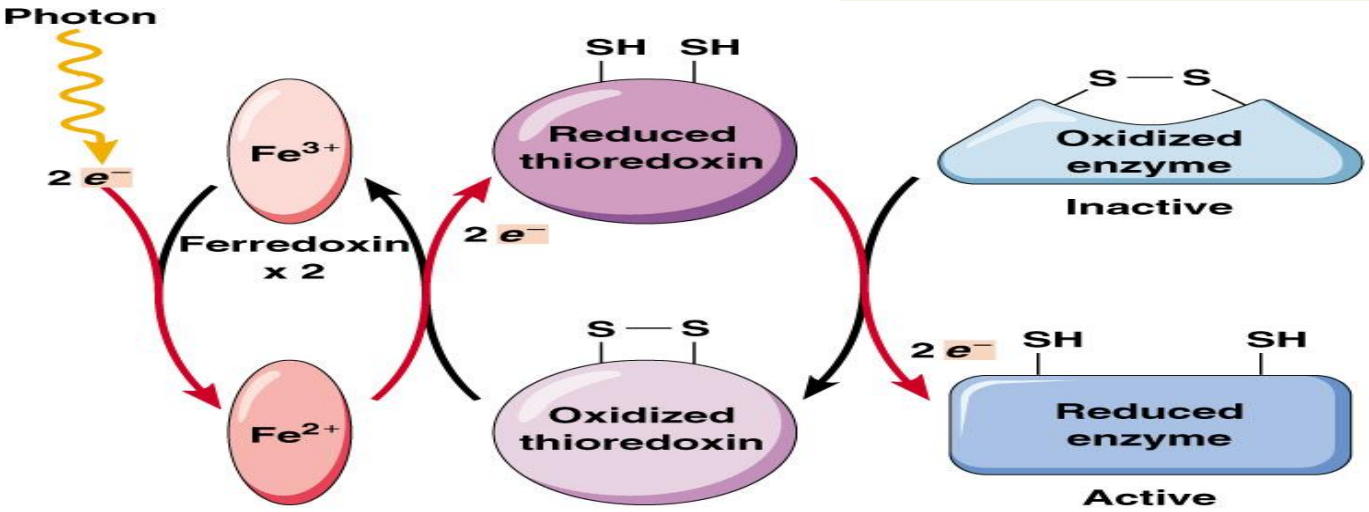
Regulation of Regeneration Enzymes

- Light activated through PS I
- Ferrodoxin-Thioredoxin

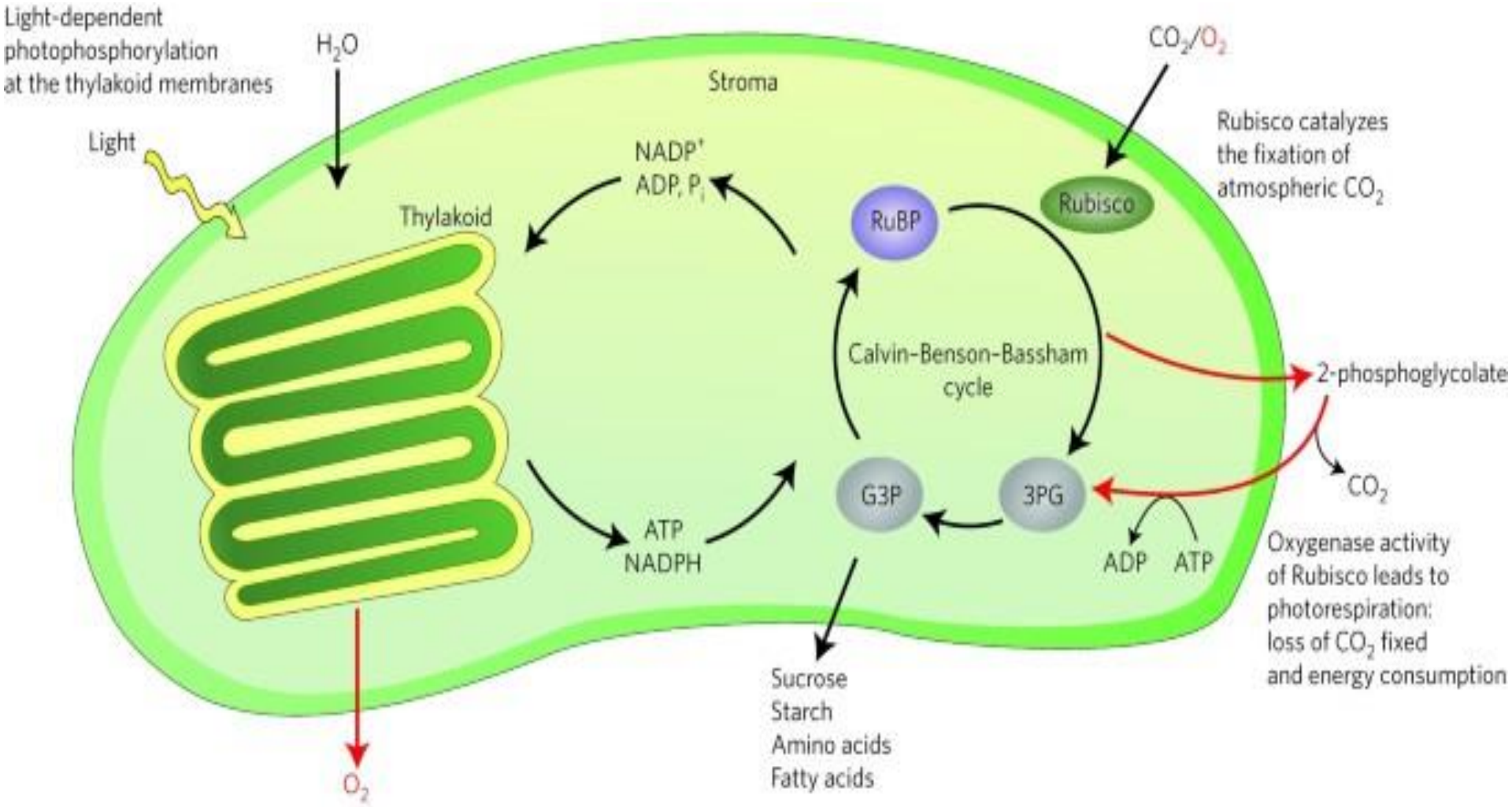
1. Gly3-P dehydrogenase
2. FBPase
3. Sedoheptulose1,7 Bisphosphotase
4. Ribulose5-P kinase





ABLE 20.1 Enzymes regulated by thioredoxin

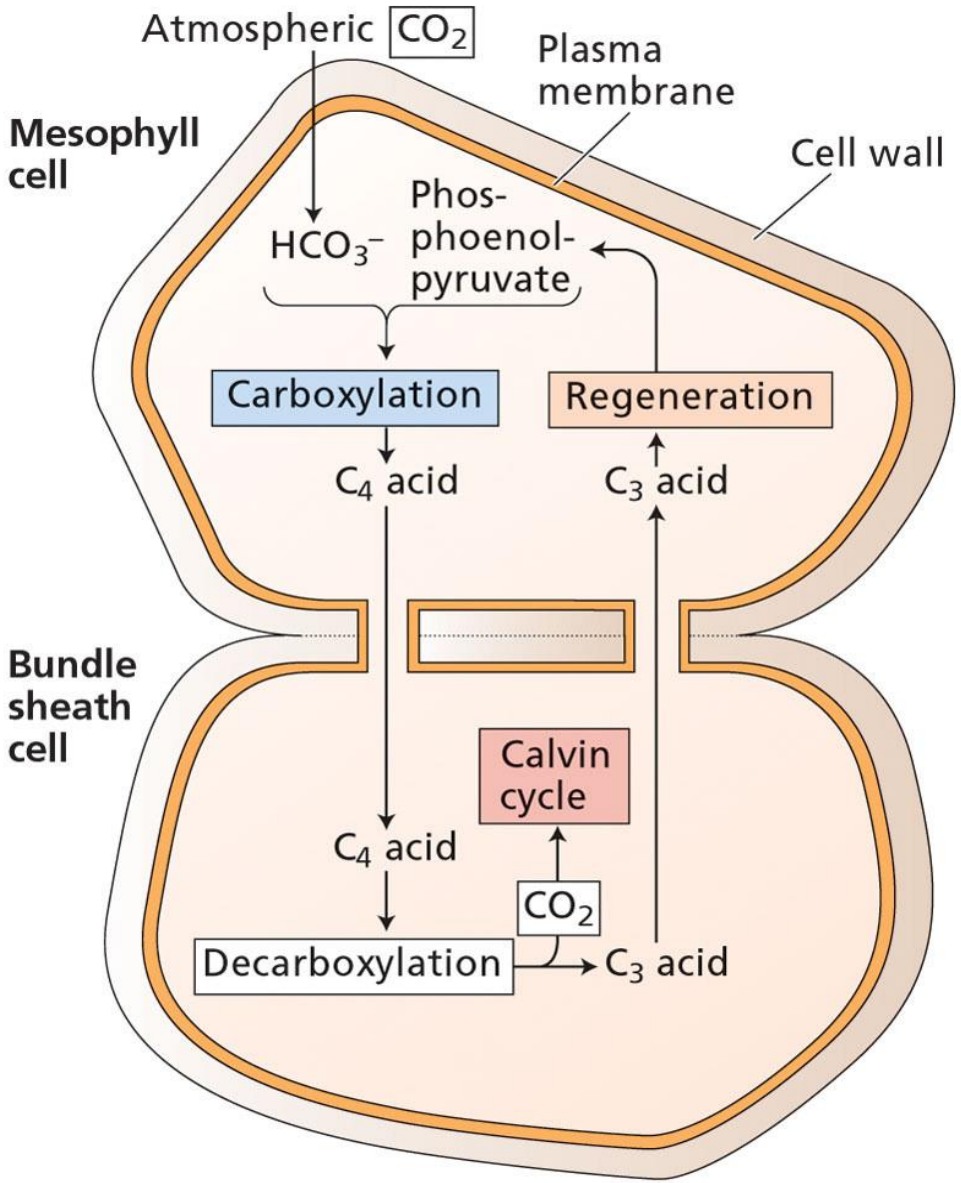
Enzyme	Pathway
Rubisco	Carbon fixation in the Calvin cycle
Fructose 1,6-bisphosphatase	Gluconeogenesis
Glyceraldehyde 3-phosphate dehydrogenase	Calvin cycle, gluconeogenesis, glycolysis
Sedoheptulose bisphosphatase	Calvin cycle


Light-dependent
photophosphorylation
at the thylakoid membranes
H_2O
Light
Thylakoid
Stroma
NADP+
ADP, P_i
ATP
NADPH
O_2
CO_2/O_2
Rubisco catalyzes
the fixation of
atmospheric CO_2
Rubisco
RuBP
Calvin-Benson-Bassham
cycle
2-phosphoglycolate
CO_2
G3P
3PG
ADP
ATP
Oxygenase activity
of Rubisco leads to
photorespiration:
loss of CO_2 fixed
and energy consumption
Sucrose
Starch
Amino acids
Fatty acids

C4 Pathway

C4 Pathway

- C4acid 1st product
- Discovered by Hatch and Slack in sugar cane
- Shuttle system
- PEP carboyxlase
- Increase CO2 at site of Calvin cycle
- Under high light/high temperature conditions



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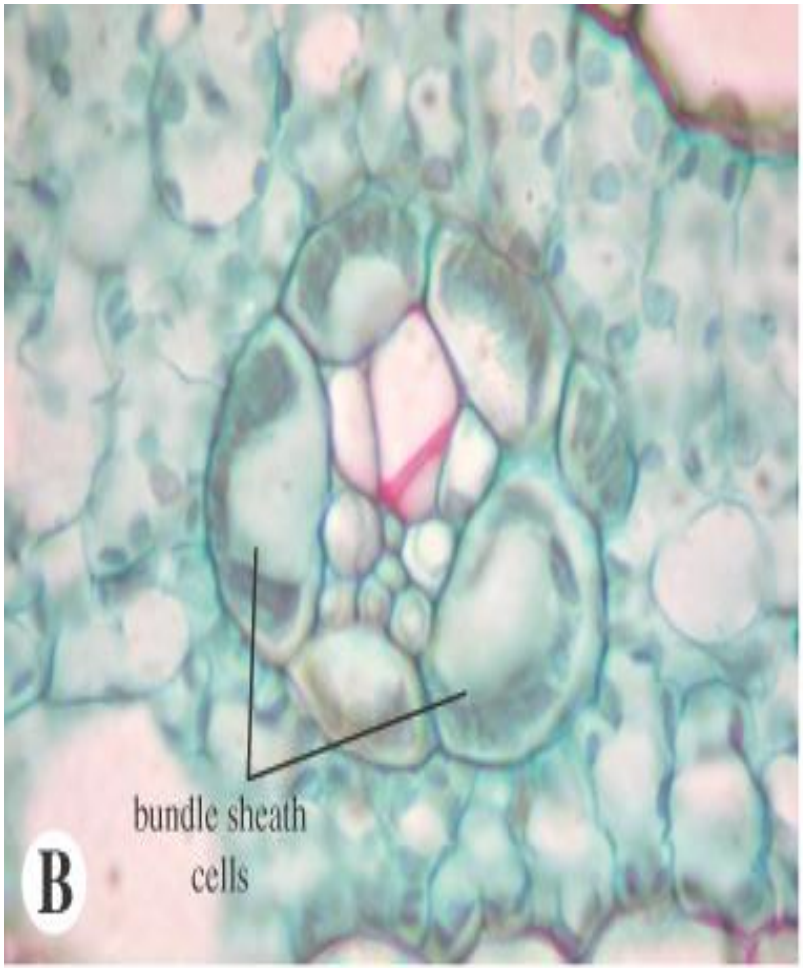
Kranz Anatomy

a typical C4 leaf has two distinct chloroplast-containing cell types: mesophyll and bundle sheath cells.

There is considerable anatomic variation in the arrangement of the bundle sheath cells with respect to the mesophyll and vascular tissue.

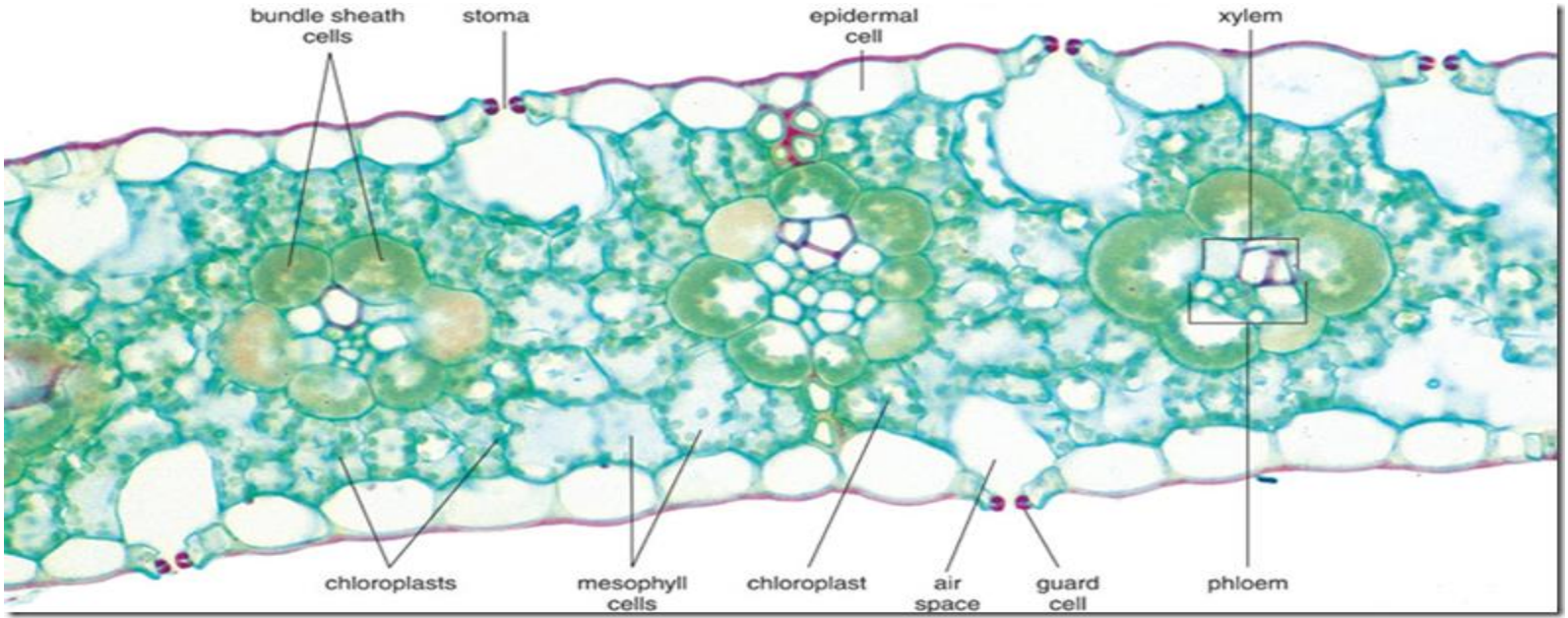
No mesophyll cell of a C4plant is more than two or three cells away from the nearest bundle sheath cell.



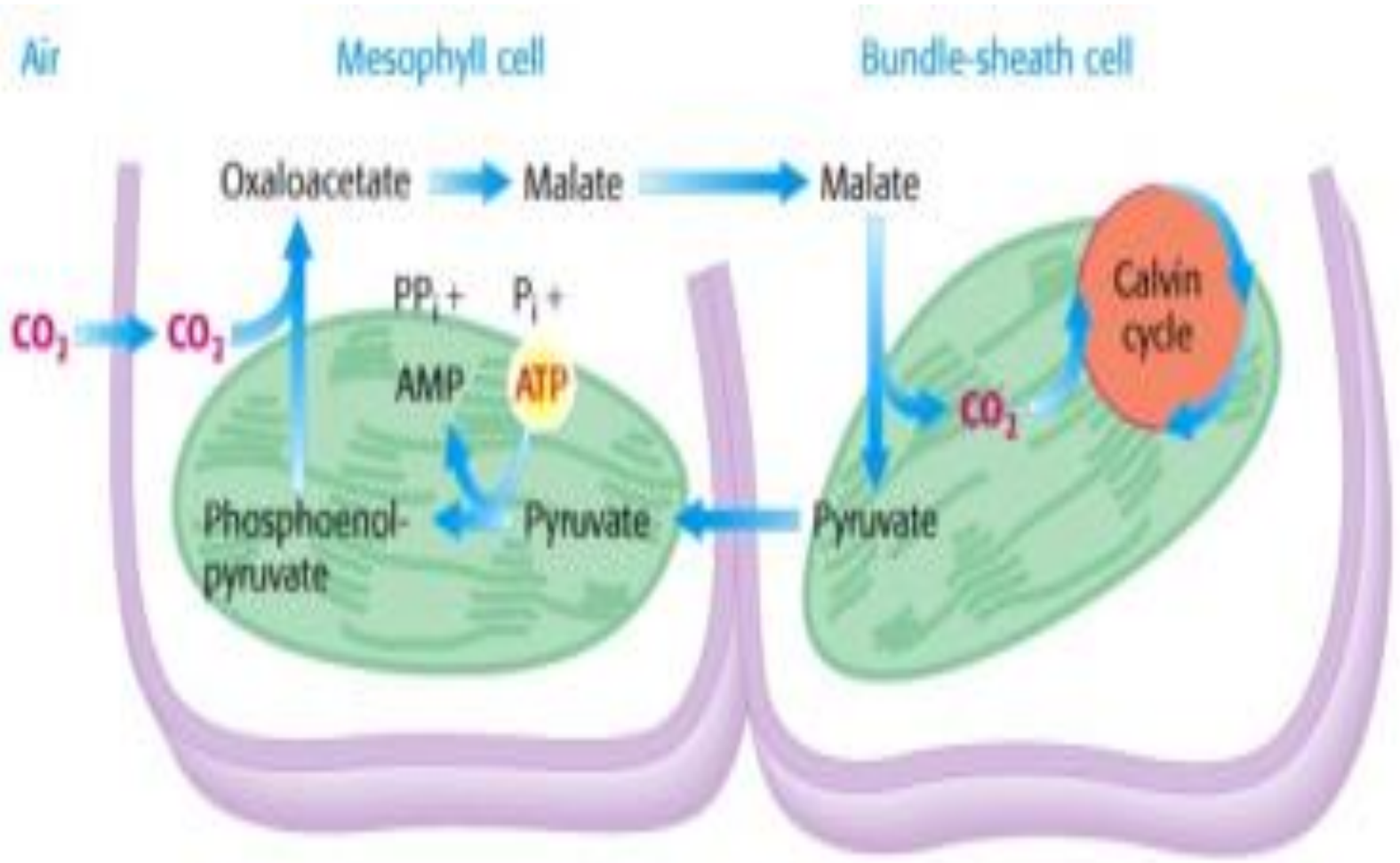
Kranz Anatomy

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.

Theparticipating 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.



Chloroplast Dimorphism



General Mechanism

***Four steps*:**

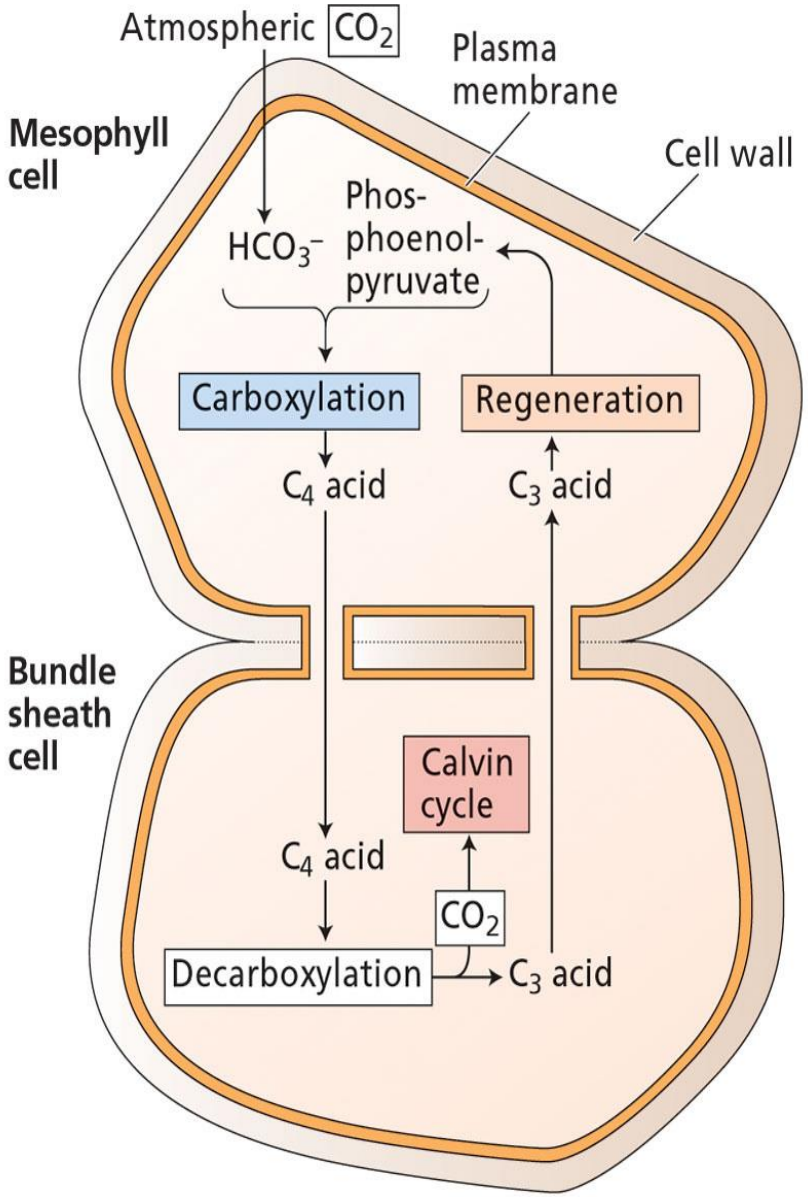
Step 1:

In Mesophyll cell

- **Fixation of CO_2 by the carboxylation of *phosphenol-pyruvate* (primary acceptor molecule)**
- **forms a C_4 acid molecule**
- **Malic acid and/or aspartate**

Step 2:

- **Transport of the C_4 acid molecule to the bundle sheath cell**



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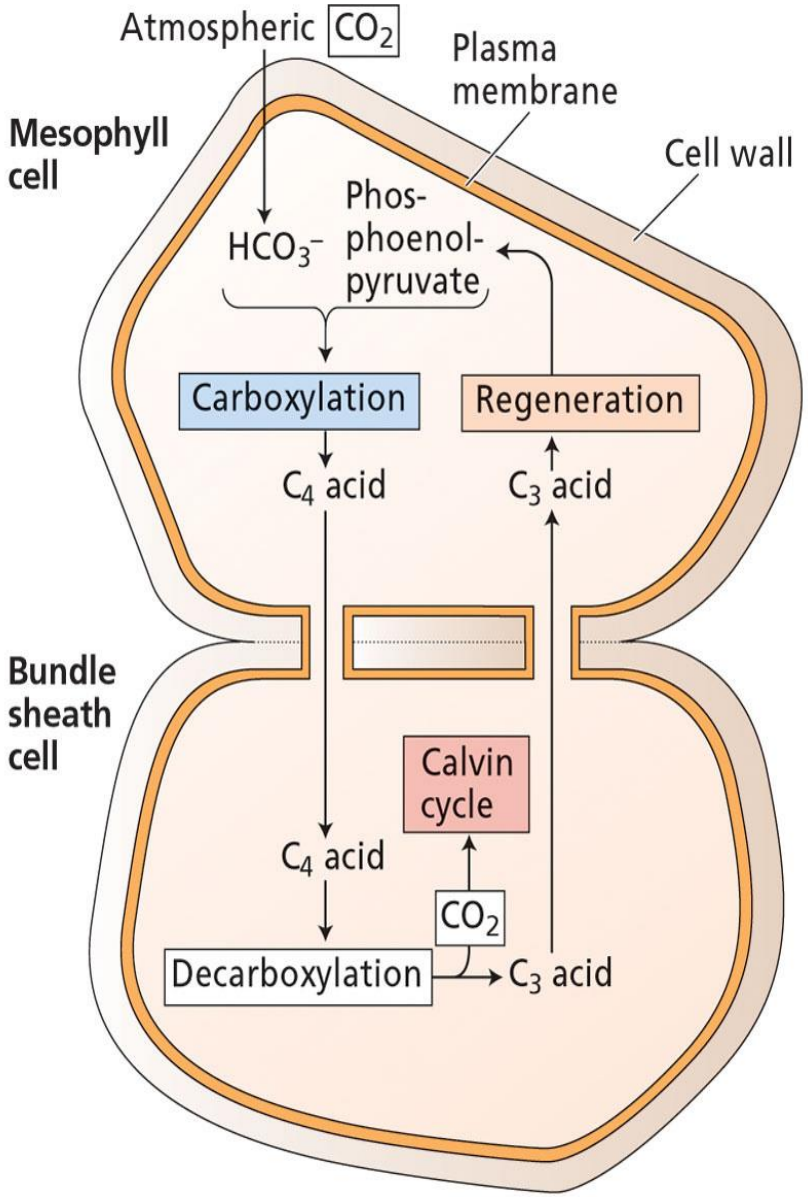
General Mechanism

Sep 3:

- Decarboxylation of the C_4 acid molecule (in bundle sheath)
- Makes a C_3 acid molecule
- This generates CO_2
- This CO_2 is reduced to carbohydrate by the Calvin cycle

Step 4:

- The C_3 acid molecule *(pryuvate)* is transported back to mesophyll cells
- Regeneration of *phosphenol-pyruvate*



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Variation of C4 Pathway

1. **NADP+ Malic enzyme type**

   E.g., *Zea mays, Saccharum officinarum, Sorghum sp*

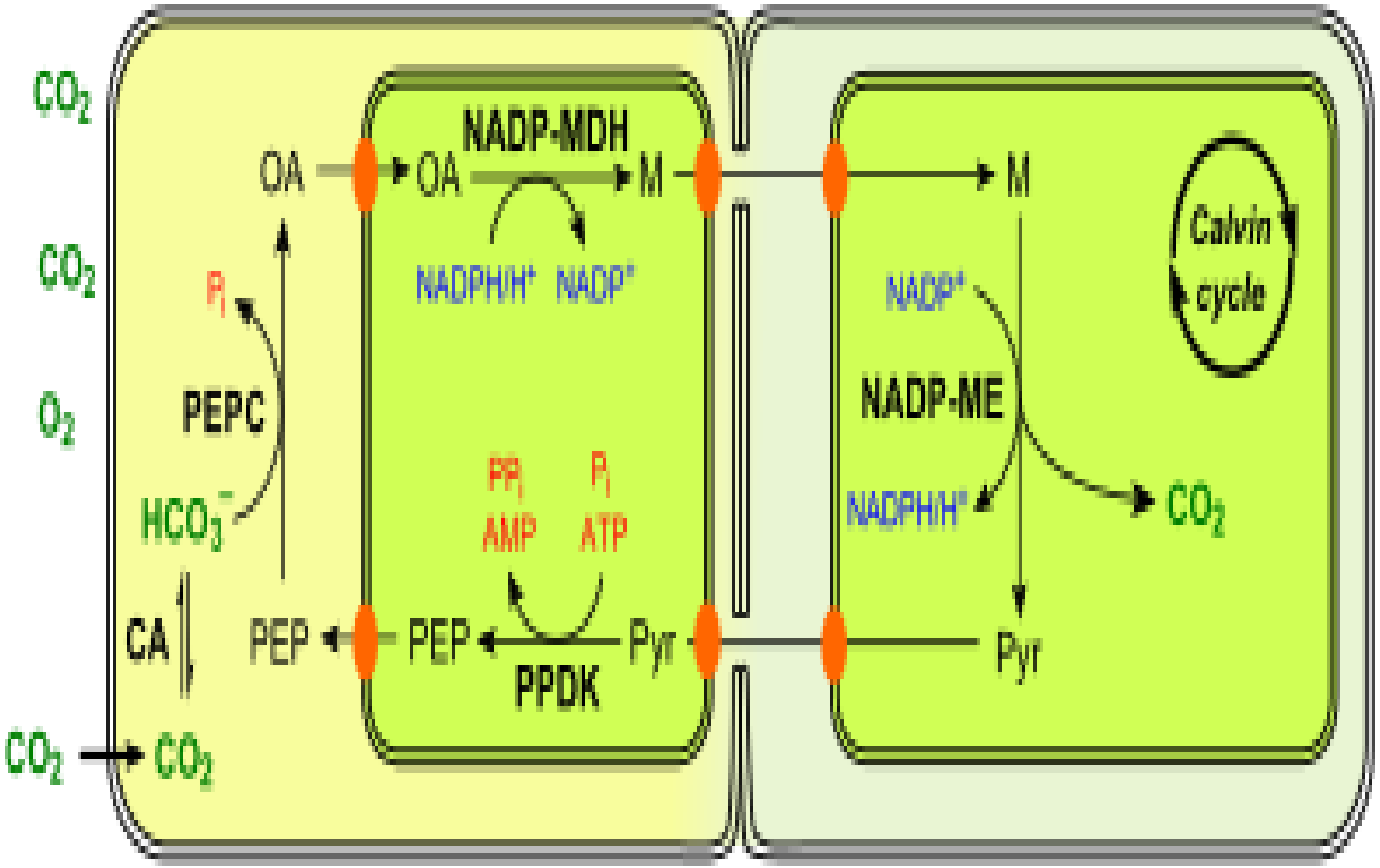
2. **NAD+ Malic enzyme type**

   E.g., *Atriplex spongiosa, Portulaca oleracea, Amaranthus edulis*

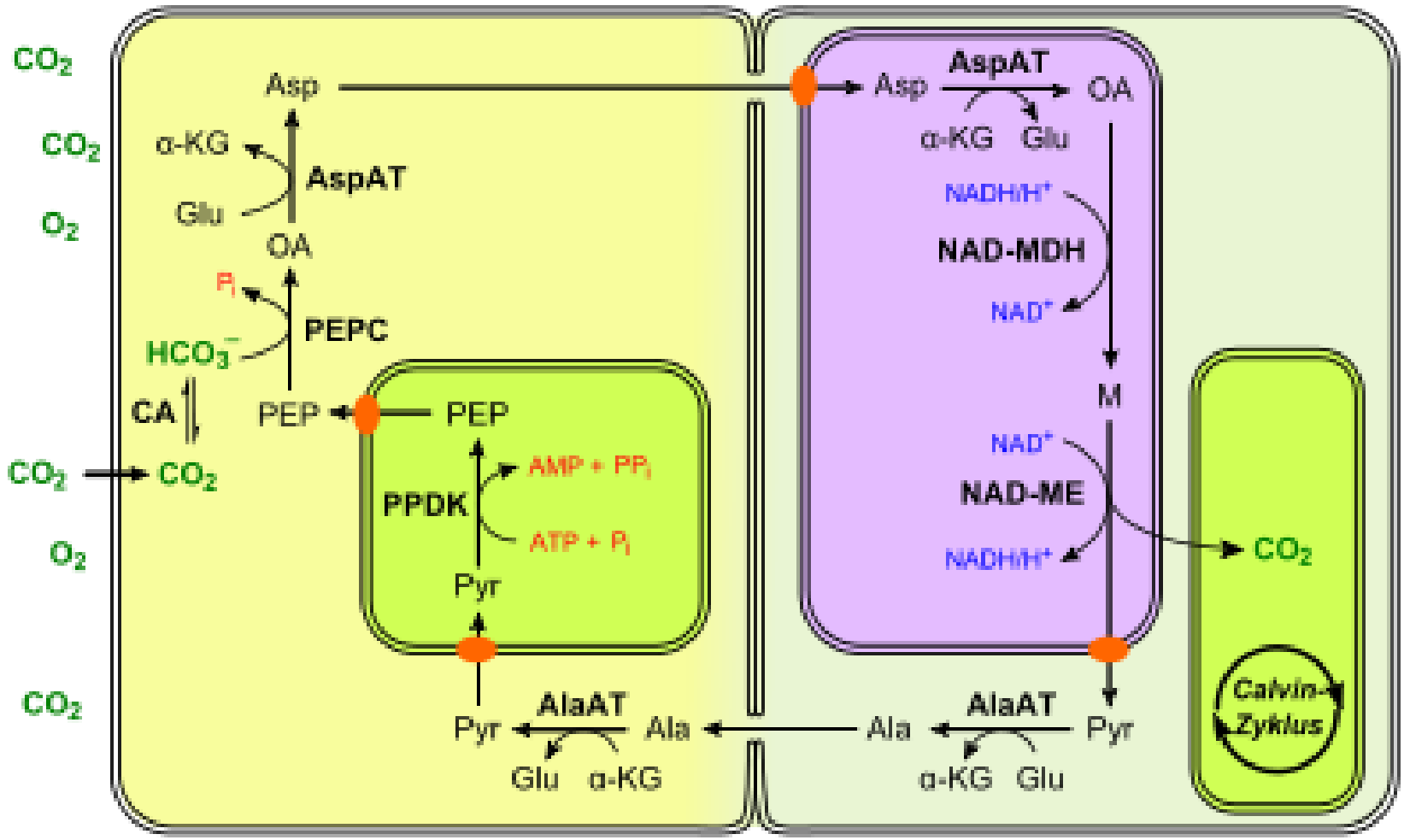
3. **PEP Carboxykinase type**

   E.g., *Panicum maximum, Chloris gayana*

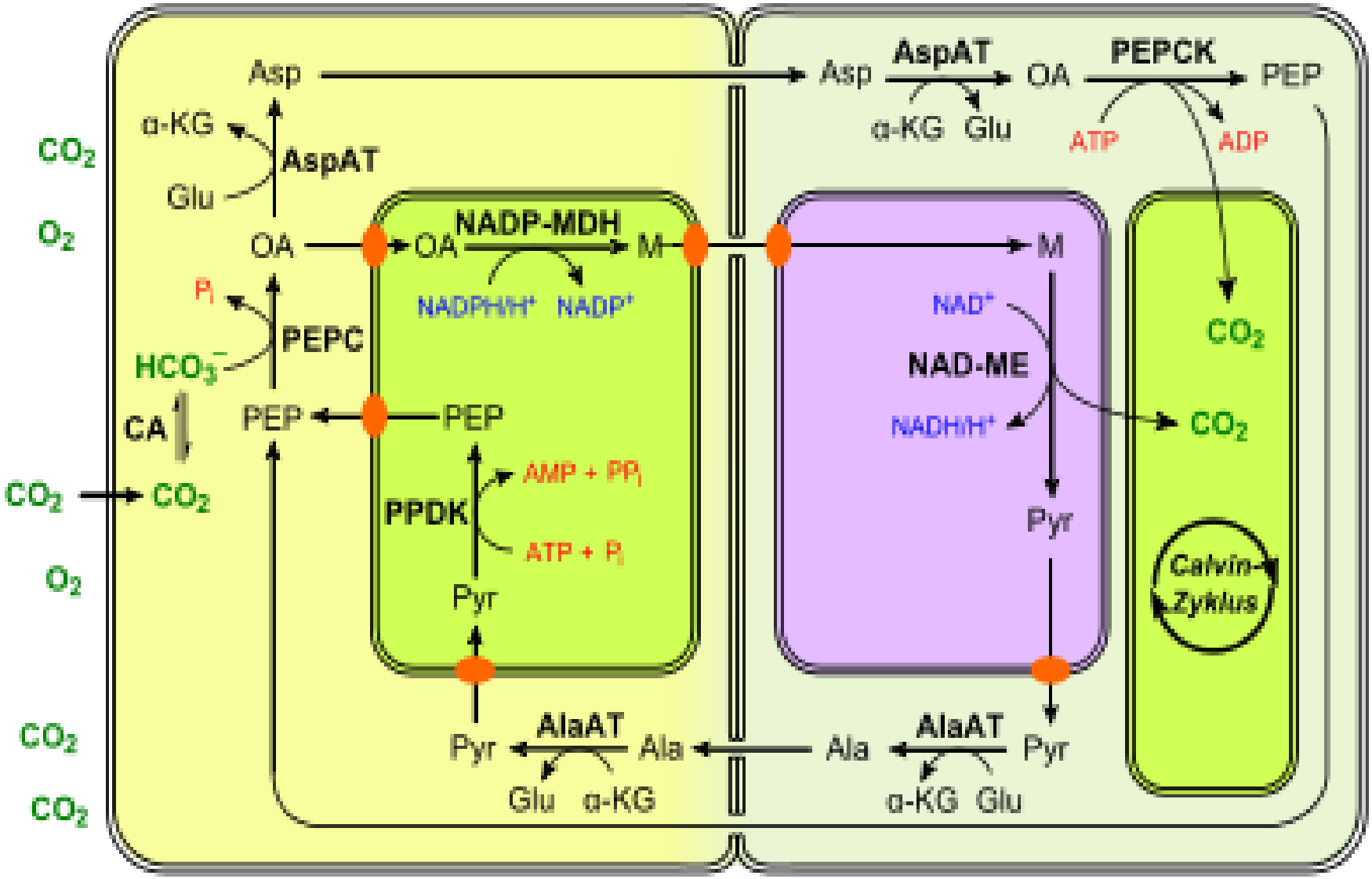
NADP-Malic enzyme type



NAD-Malic enzyme type



PEP-Carboxykinase type



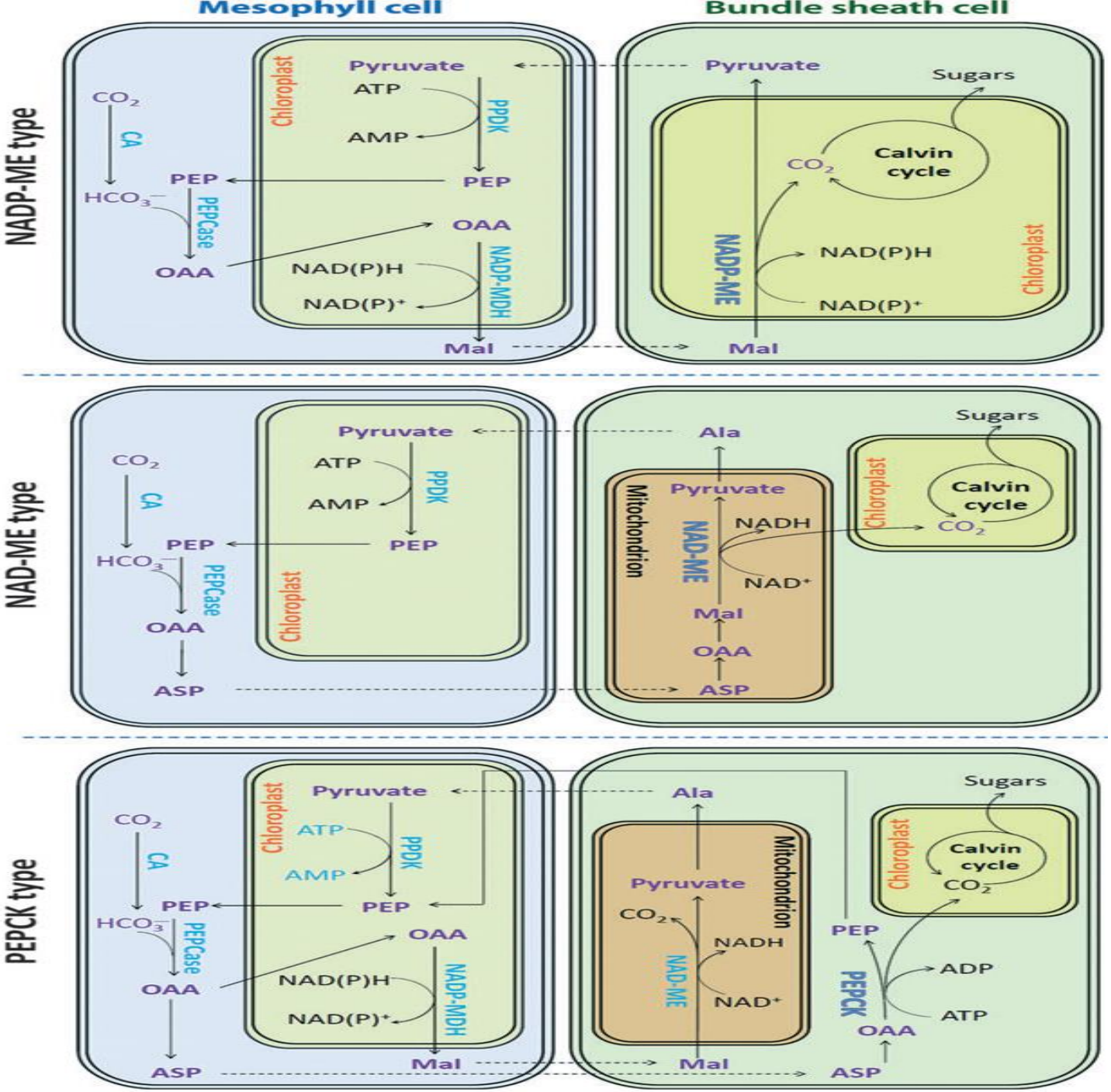
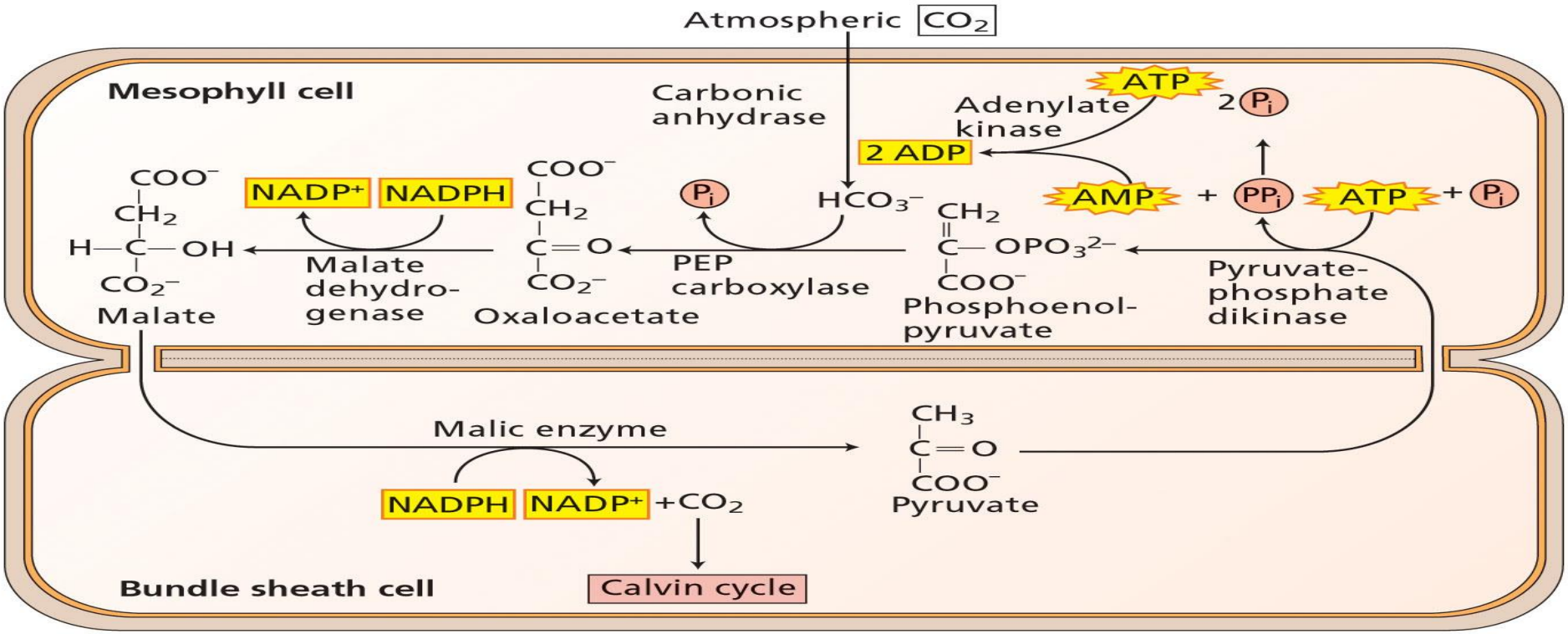


Fig. 1 Major types of C_4 photosynthesis. C_4 photosynthesis can be

C4 Pathway

- Regeneration of *phosphenol-pyruvate* consumes two high energy bonds from ATP.
- Movement between cells is by diffusion via plasmodesmata.
- Movement within cells is regulated by concentration gradients.
- This system generates a higher CO_2 conc in bundle sheath cells than would occur by equilibrium with the atmosphere.
- *Prevents photorespiration*



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The net effect of the C_4 carbon Cycle is to convert a dilute CO_2 solution in the mesophyll into a concentrated solution in the bundle sheath cells.

This requires more energy than C_3 carbon plants.

But,this energy requirement is constant no matter what the environmental conditions.

Allows more efficient photosynthesis in hotter conditions.

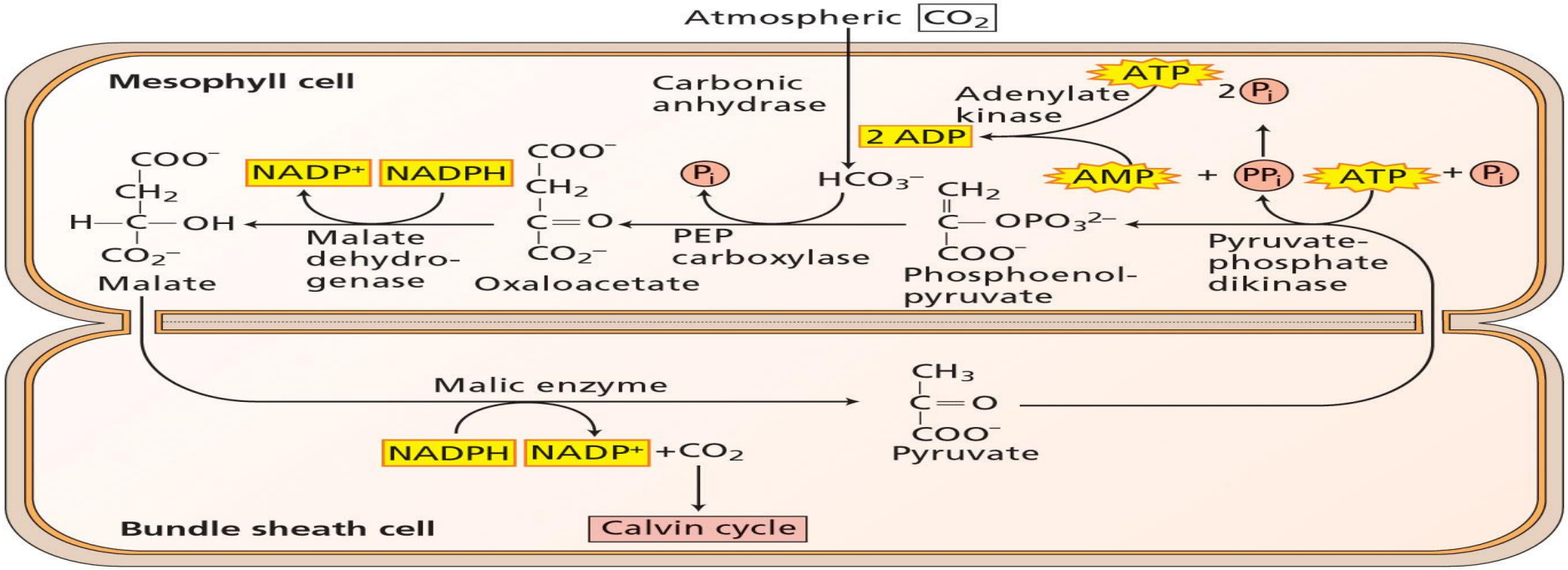

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TABLE 8.3
Reactions of the C_4 photosynthetic carbon cycle

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.

Energetics of C4 Pathway

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^-.

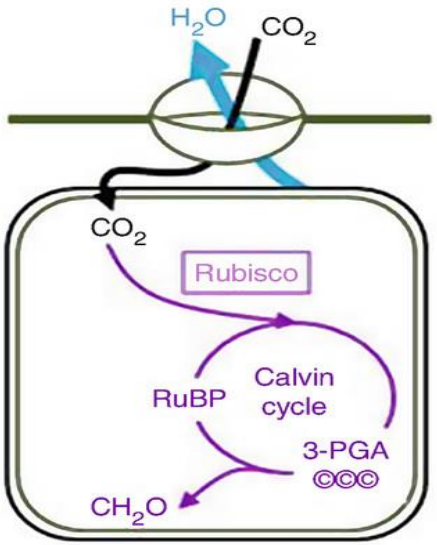
P_i and PP_i stand for inorganic phosphate and pyrophosphate, respectively.

Regulation of C4 Pathway

- **Thioredoxin: NADP: malate dehydrogenase**
- **PEP carboxylase: covalent modification by phosphorylation/dephosphorylation; regulated by phosphorylationby PEP carboxylase-kinase to make active**
- **PyruvatePi dikinase: ADP-dependent phosphorylation when light intensity drops**

C3 Cycle Vs C4 Cycle

a



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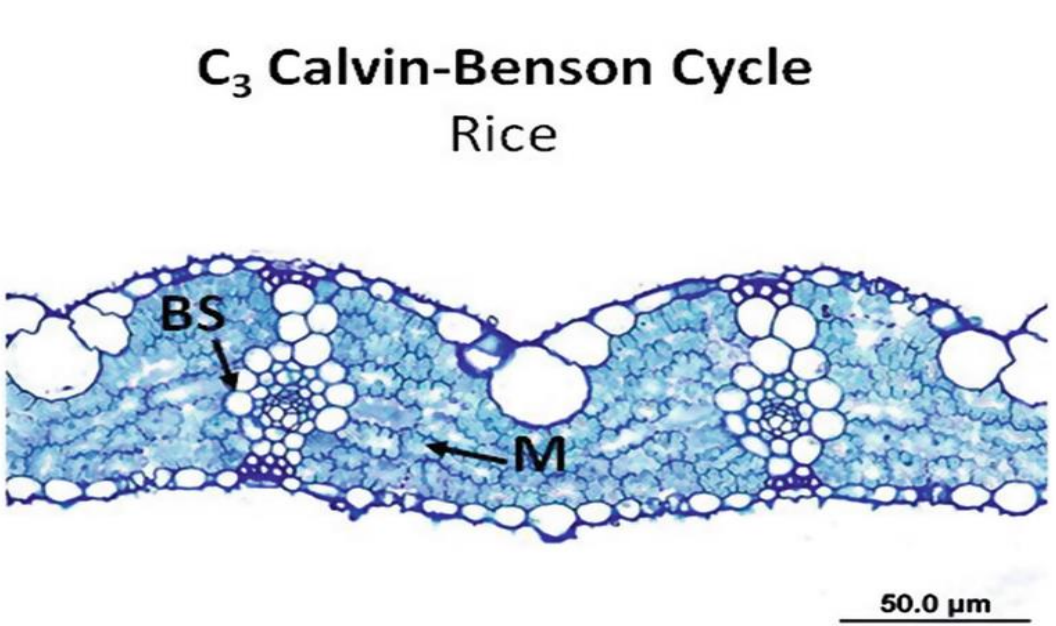

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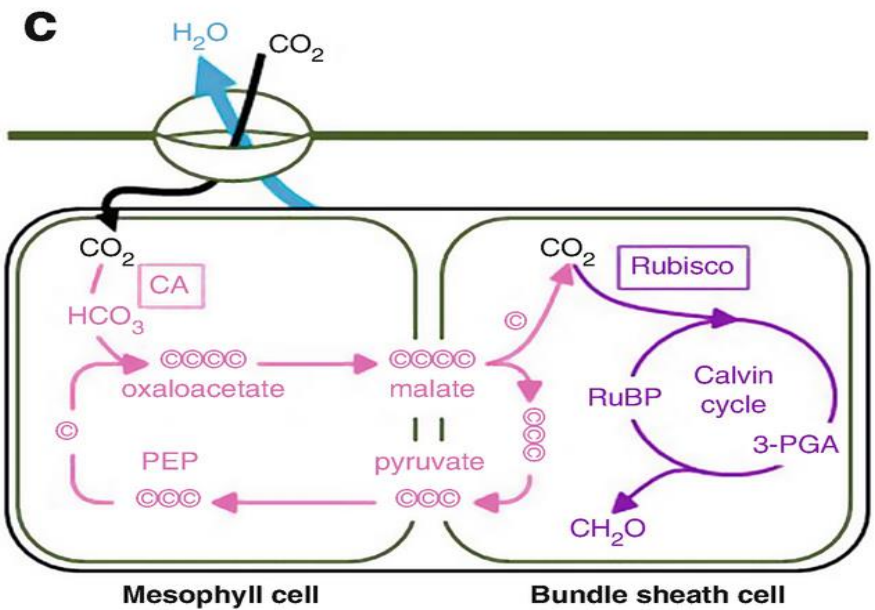

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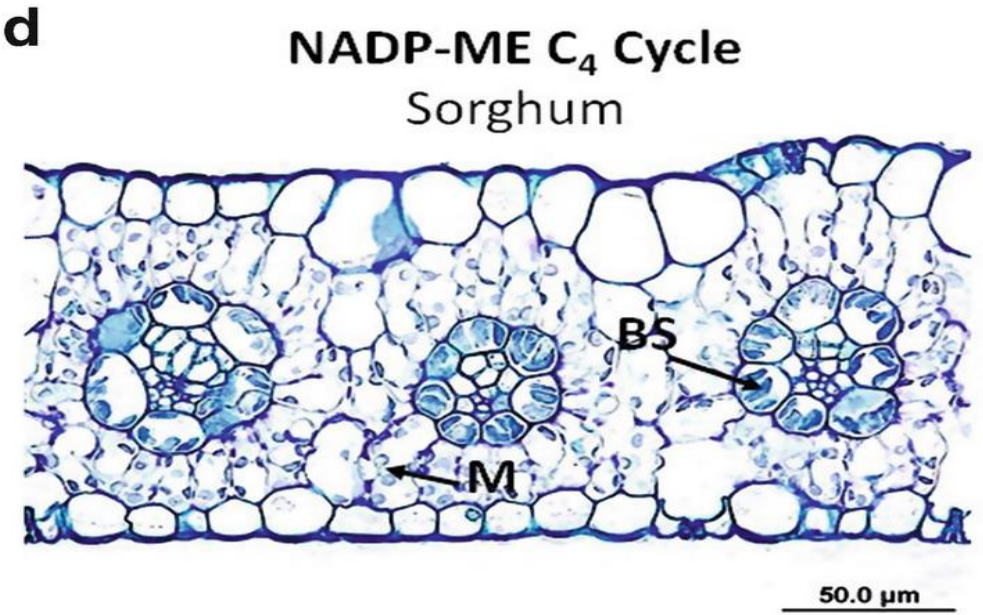


Significance of C4 Mechanism

1. The low transpiration ratio for C4 plants reflects their capacity to maintain high rates of photosynthesis while effectively conserving water.

2. Unlike C3 plants, photosynthesis of C4 plants is not inhibited by O2, and they exhibit no post-illumination CO2 burst and have a very low CO2 compensation point.

3. The high level of CO2 developed in the bundle sheath cells would tend to suppress photorespiration by outcompeting O2 for binding to Rubisco.

4. C4 leaves are not only efficient CO2 absorbers, but also effectively trap and recirculate any CO2 that might be produced in the leaf.

CAM Pathway

Occurance

So named because it was originally studied most extensively in the family Crassulaceae.

This specialized pattern of photosynthesis has now been found in some 23 different families of flowering plants (including the Cactaceae and Euphorbiaceae), one family of ferns (the Polypodiaceae), and in one Gymnoispermous plant *Welwitschia*.

Characteristics of CAM plants

1. The unique features of CAM permit a remarkable degree of water conservation, especially adapted to survival in extremely dry, or xerophytic, habitats.

2. They are also succulent plants—characterized by thick, fleshy leaves whose cells contain large, water-filled vacuoles.

3. One of the most striking features of CAM plants is an inverted stomatal cycle—the stomata open mainly during the night time hours and are usually closed during the day. This means that CO2 uptake also occurs mainly at night.

4. In addition, CAM plants are characterized by an accumulation of malate at night and its subsequent depletion during daylight hours and storage carbohydrate levels that fluctuate inversely with malate levels.

Crassulacean Acid Metabolism

- Initial CO2fixation step which occurs at night.
- After the initial carboxylation, malic acid (the first stable product after fixation) is then sequestered into the central vacuole during the night period.
- In the following light period, the stomata close and the malic acid returns to the cytoplasm for decarboxylation.
- The released CO2is then assimilated through the C3pathway

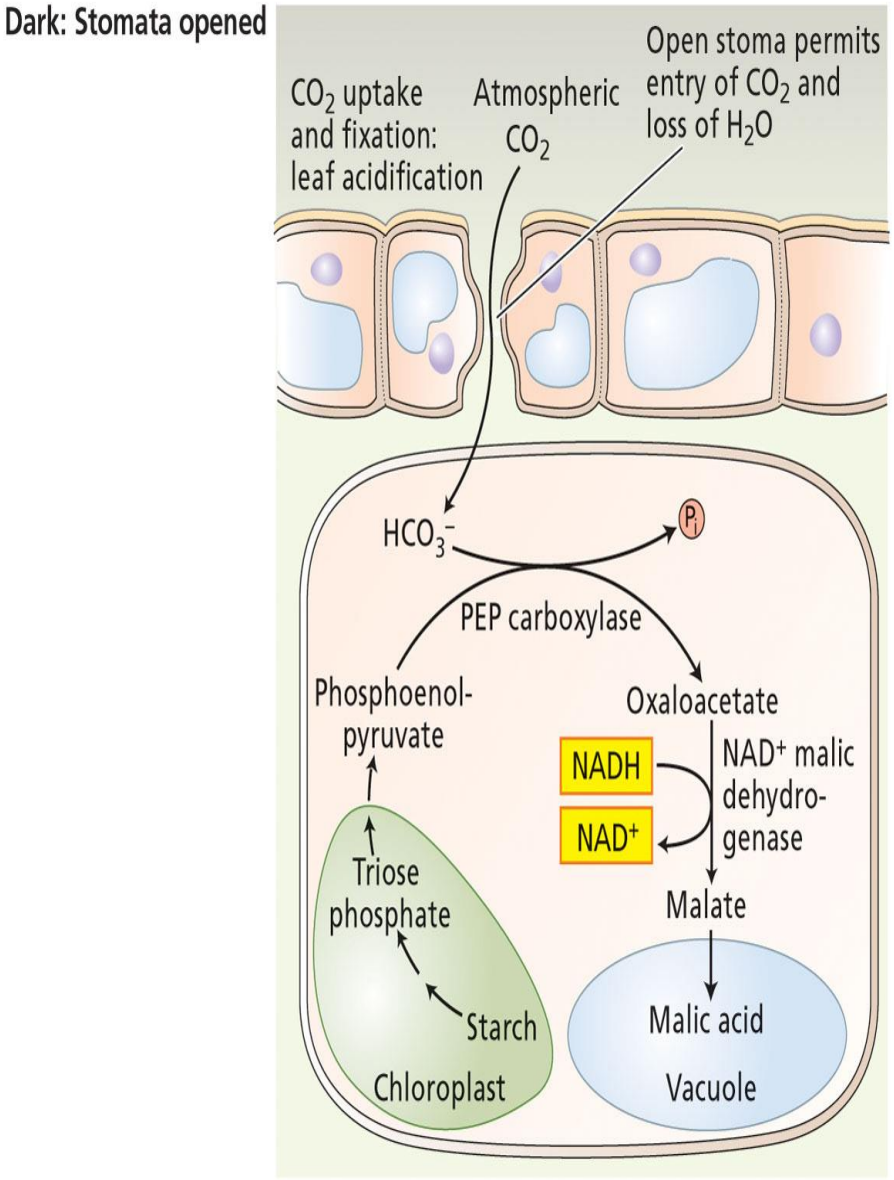
At night

Stomata only open at night when it is cool

CO_2 is captured by *phosphenol-pyruvate carboxylase* in the cytosol – *leaves become acidic*

The malic acid formed is stored in the vacuole.

Amount of malic acid formed is equal to the amount of CO_2 taken in



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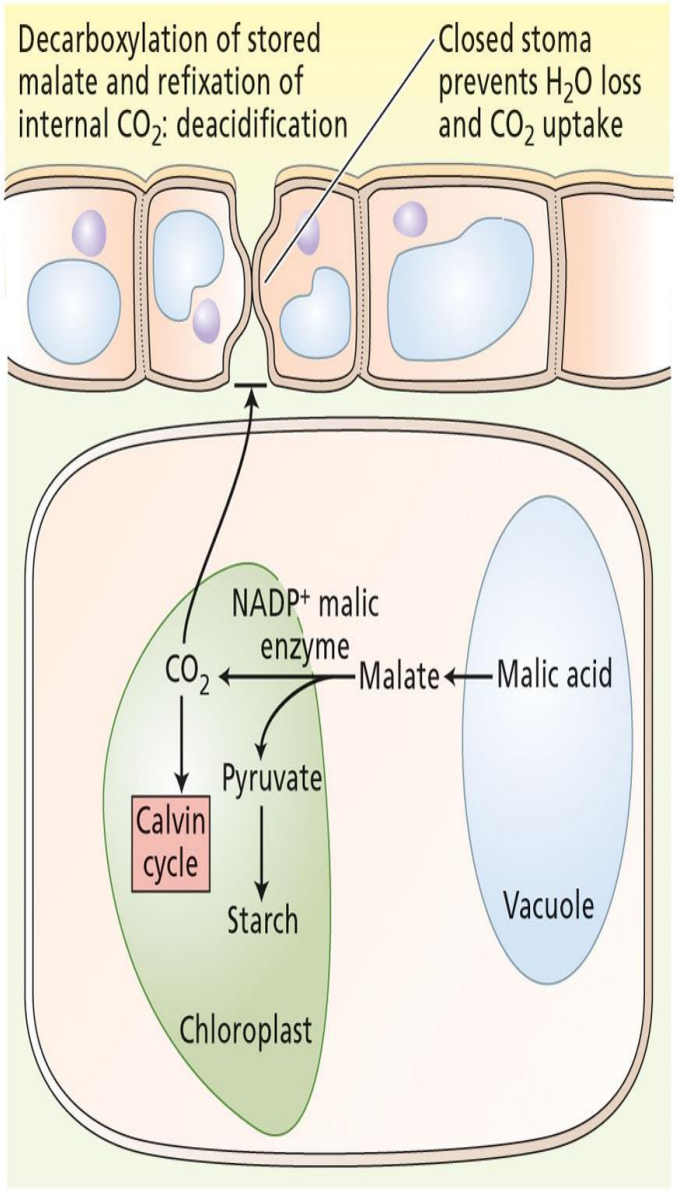
During the day

Stomata close, preventing water loss, and further uptake of CO2

Malic acid is transported to the chloroplast and decarboxylated to release CO2

This enters the Calvin cycle as it can not escape the leaf

Pyruvate is converted to starch in the chloroplast – regenerates carbon acceptor



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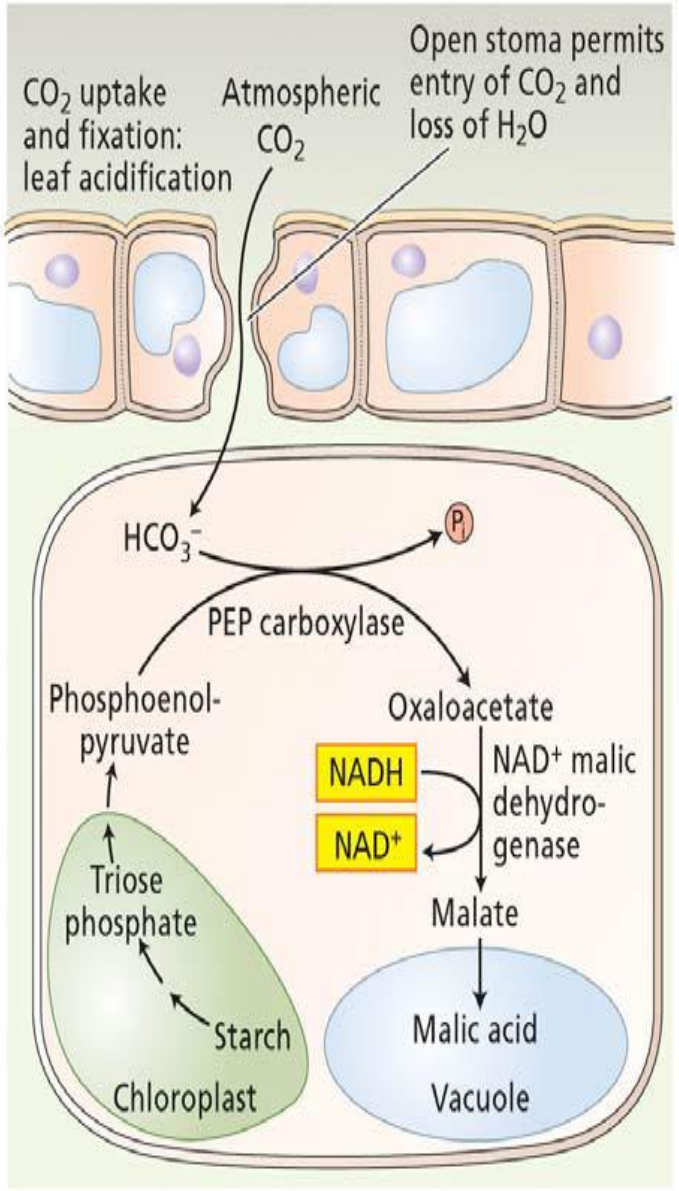
The Total Pathway

Dark: Stomata opened



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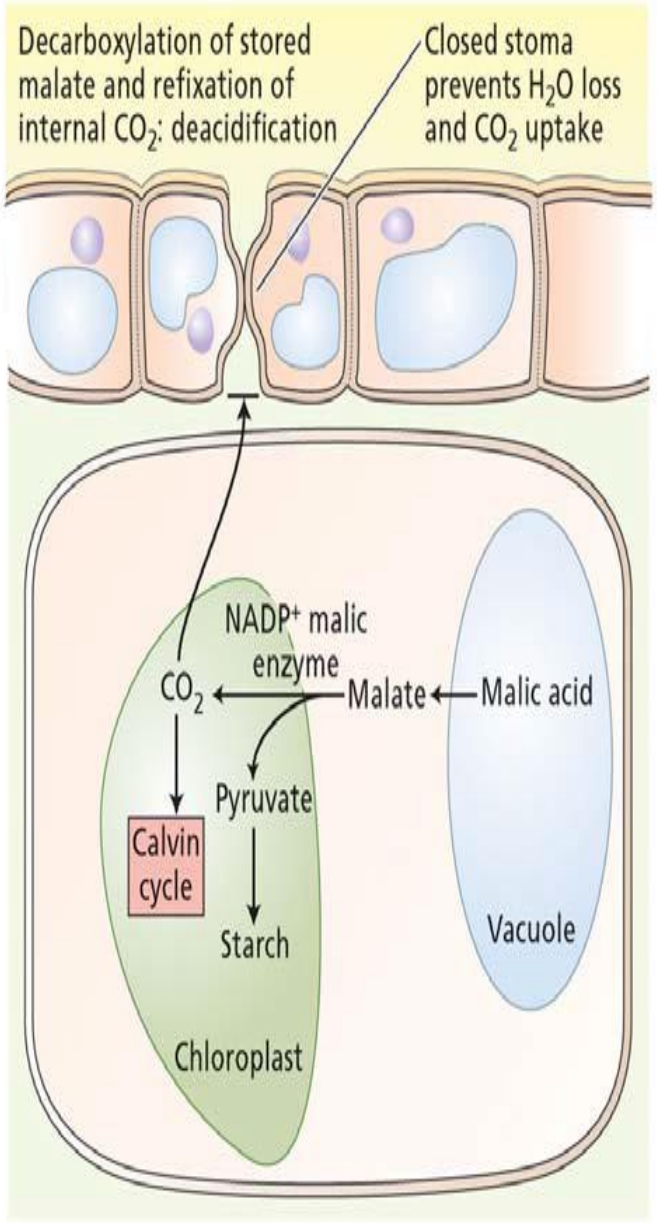
Light: Stomata closed



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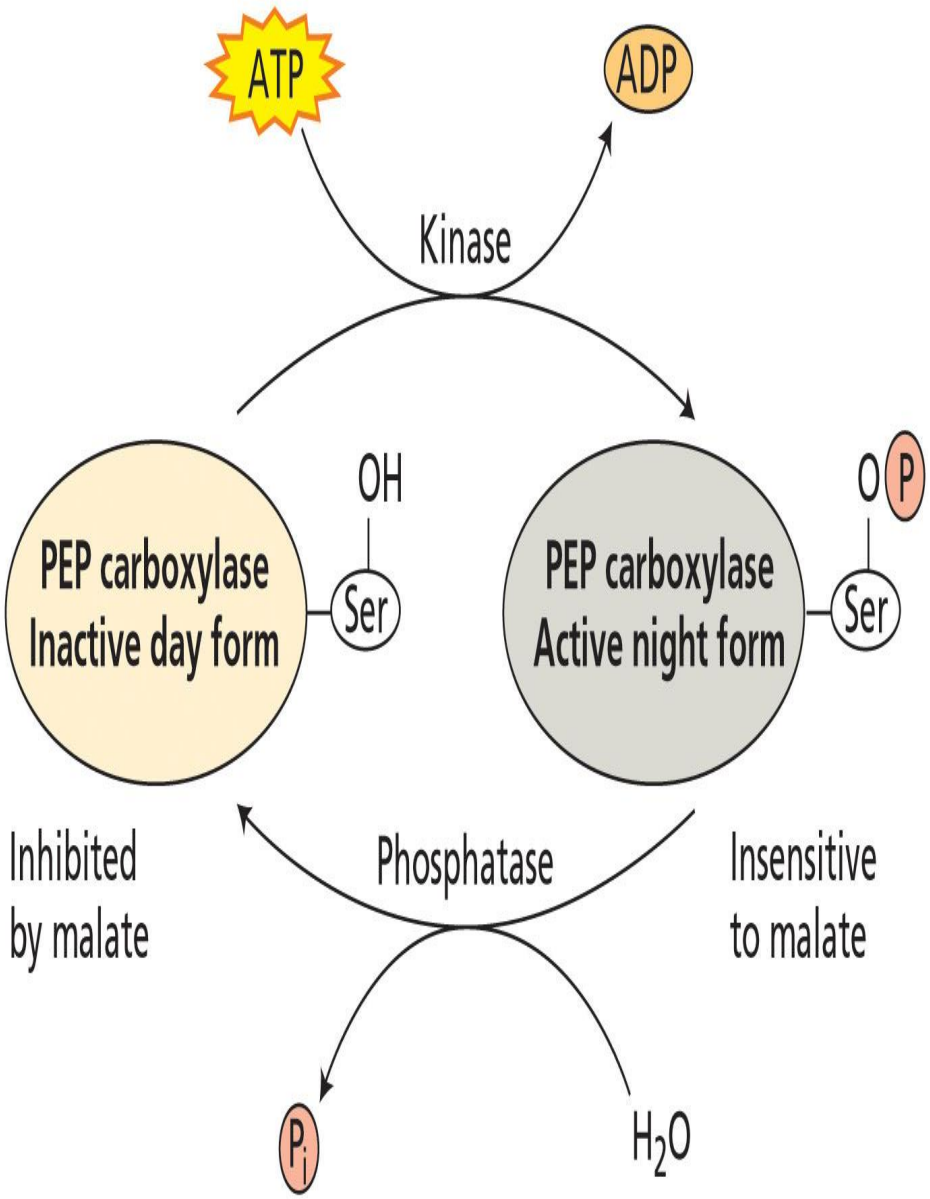
Regulation

Diuranal **regulation is used.**

Phosphorylation of the serine residue of phosphenol-pyruvate (PEP) carboxylase (Ser-OP) yields a form of the enzyme which is active at night

This is relatively insensitive to malic acid



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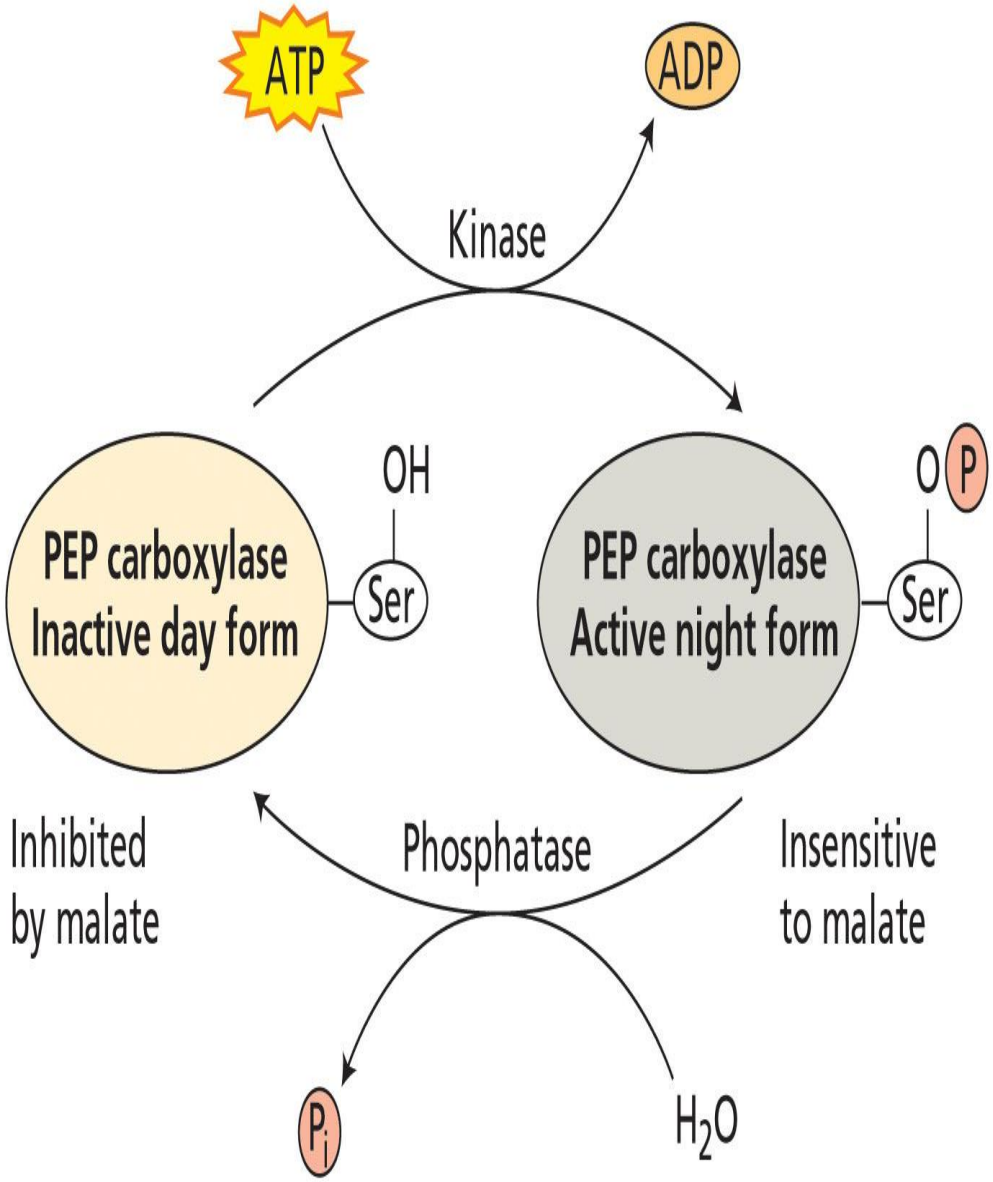
Regulation

During the day:

De-Phosphorylation of the serine (ser-OH) gives a form of the enzyme which is inhibited by malic acid

THIS IS THE OPPOSITE WAY AROUND FOR C_4 PLANTS!



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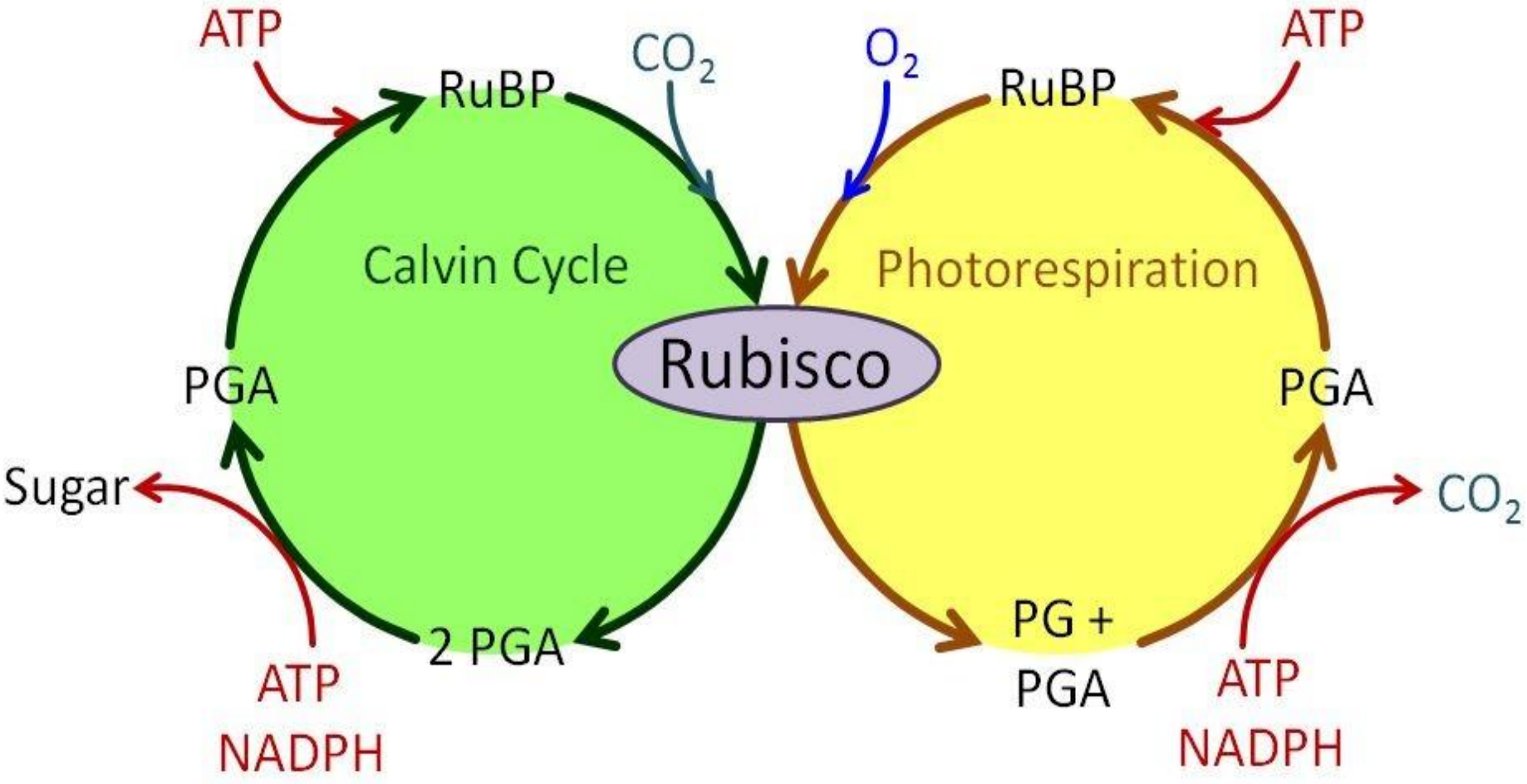
Significance of CAM

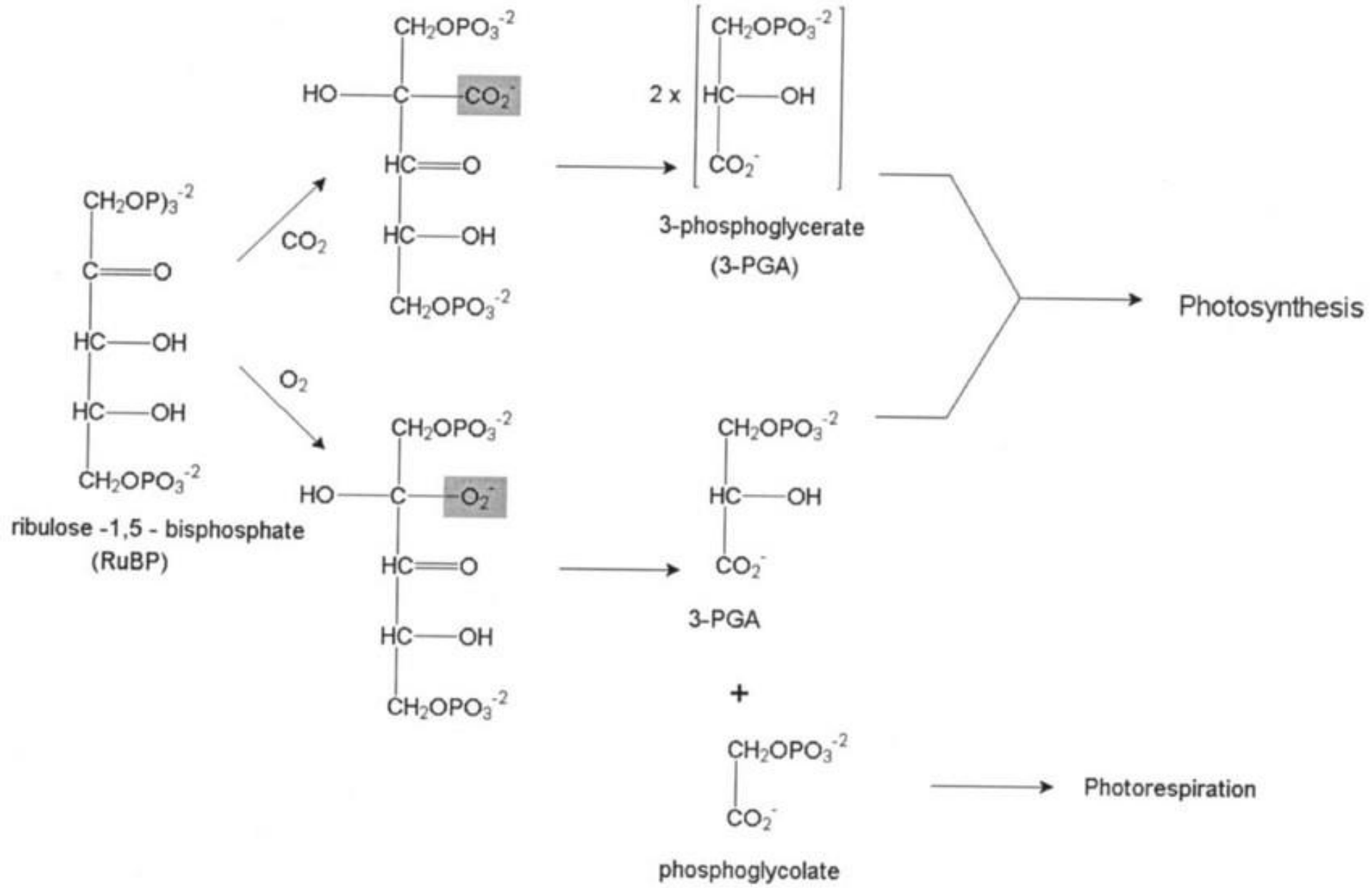
1. CAM plants are true desert plants, growing in shallow,sandy soils with little available water. Nocturnal opening of the stomata allows for CO2 uptake during periods when conditions leading to evaporative water loss are at a minimum.
2. CAM plants are able to retain and reassimilate respired CO2,thus preventing loss of carbon and helping to maintain a favorable dry weight through extended periods of severe drought.
3. Competition for CO2is not a problem at night since Rubisco and the PCR cycle are inoperative in the dark.

Photorespiration

Dual action of RubisCO



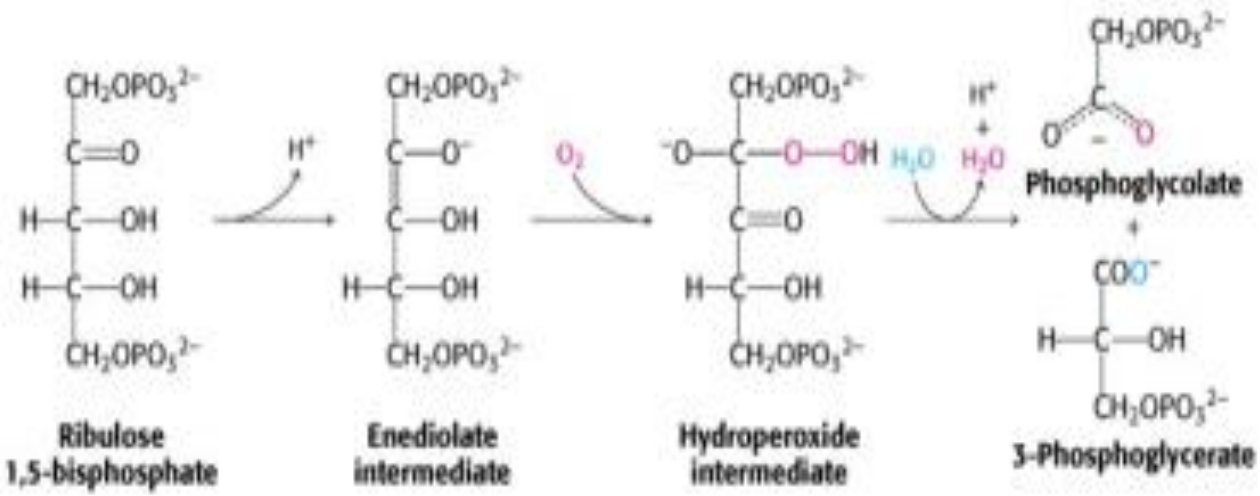
Dual action of RubisCO



Introduction

The photorespiratory pathway involves the activities of at least three different cellular organelles and, because CO2 is evolved, results in a net loss of carbon from the cell.

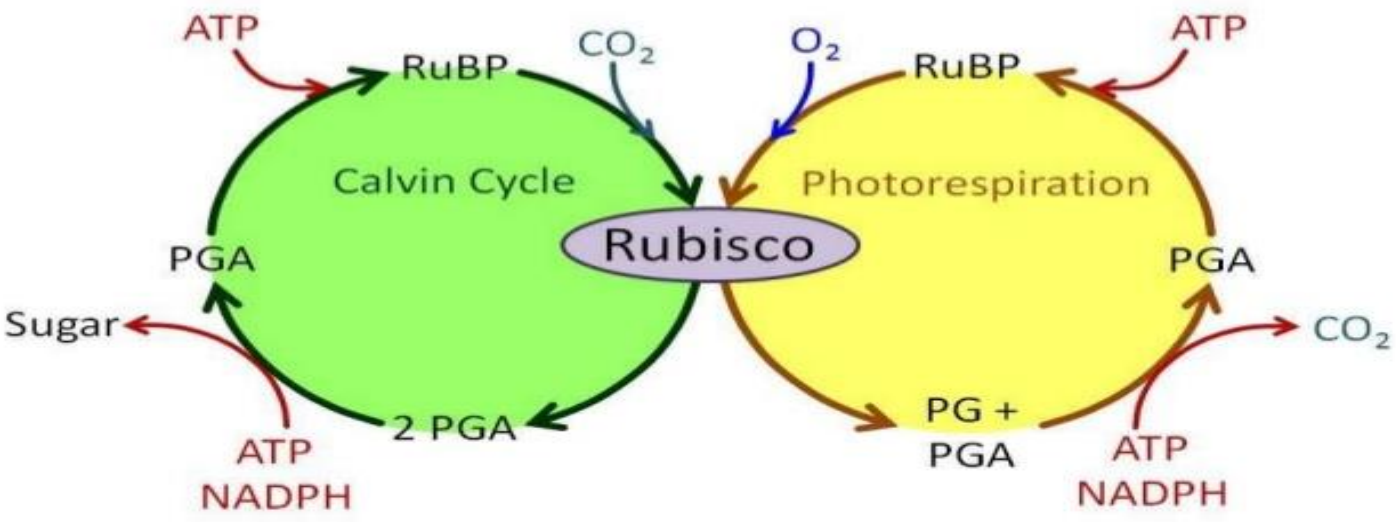
- **Three organelles**
  - **Chloroplast**
  - **Mitochondria**
  - **Peroxisome**
- **Loss of fixed CO2**

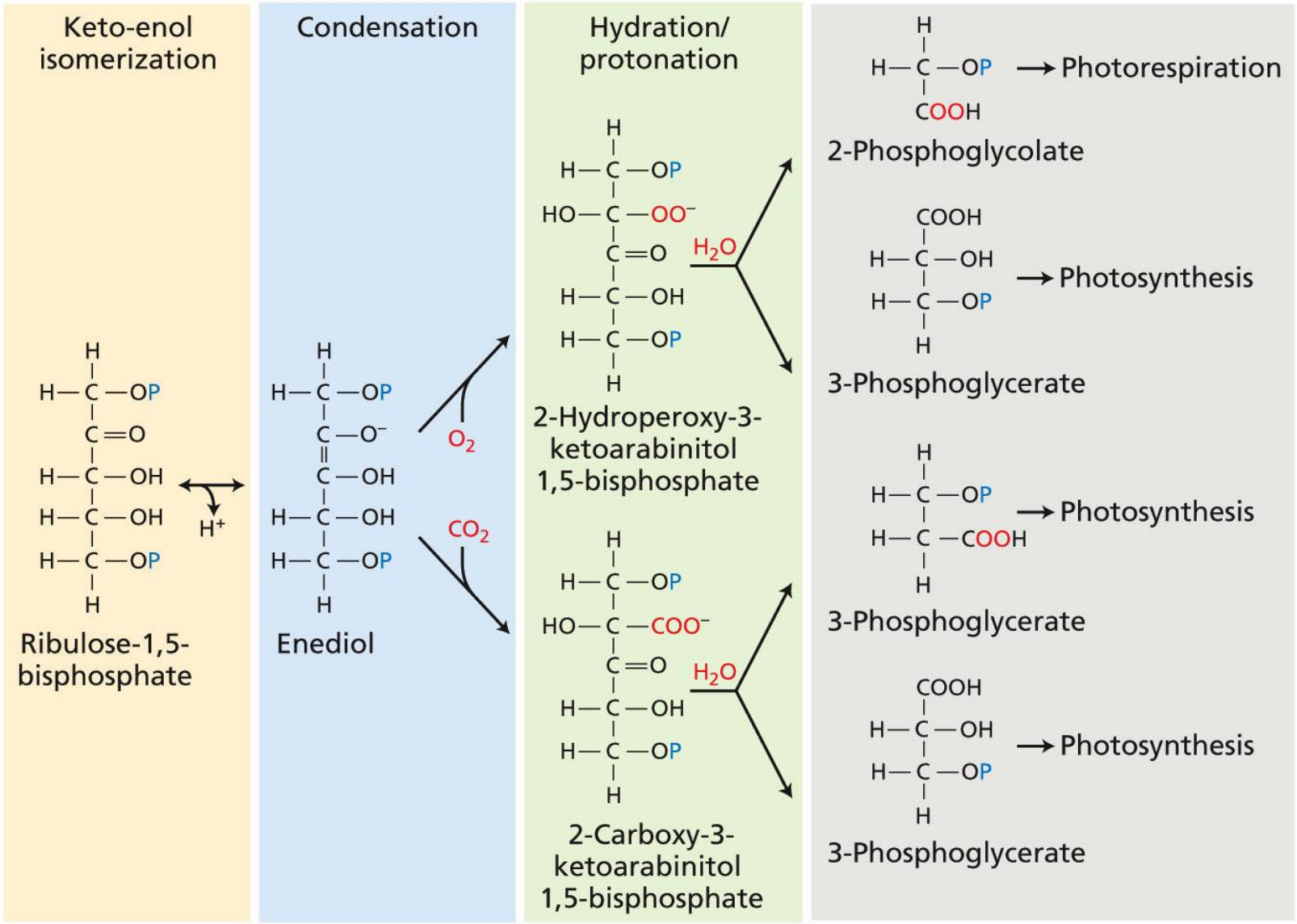


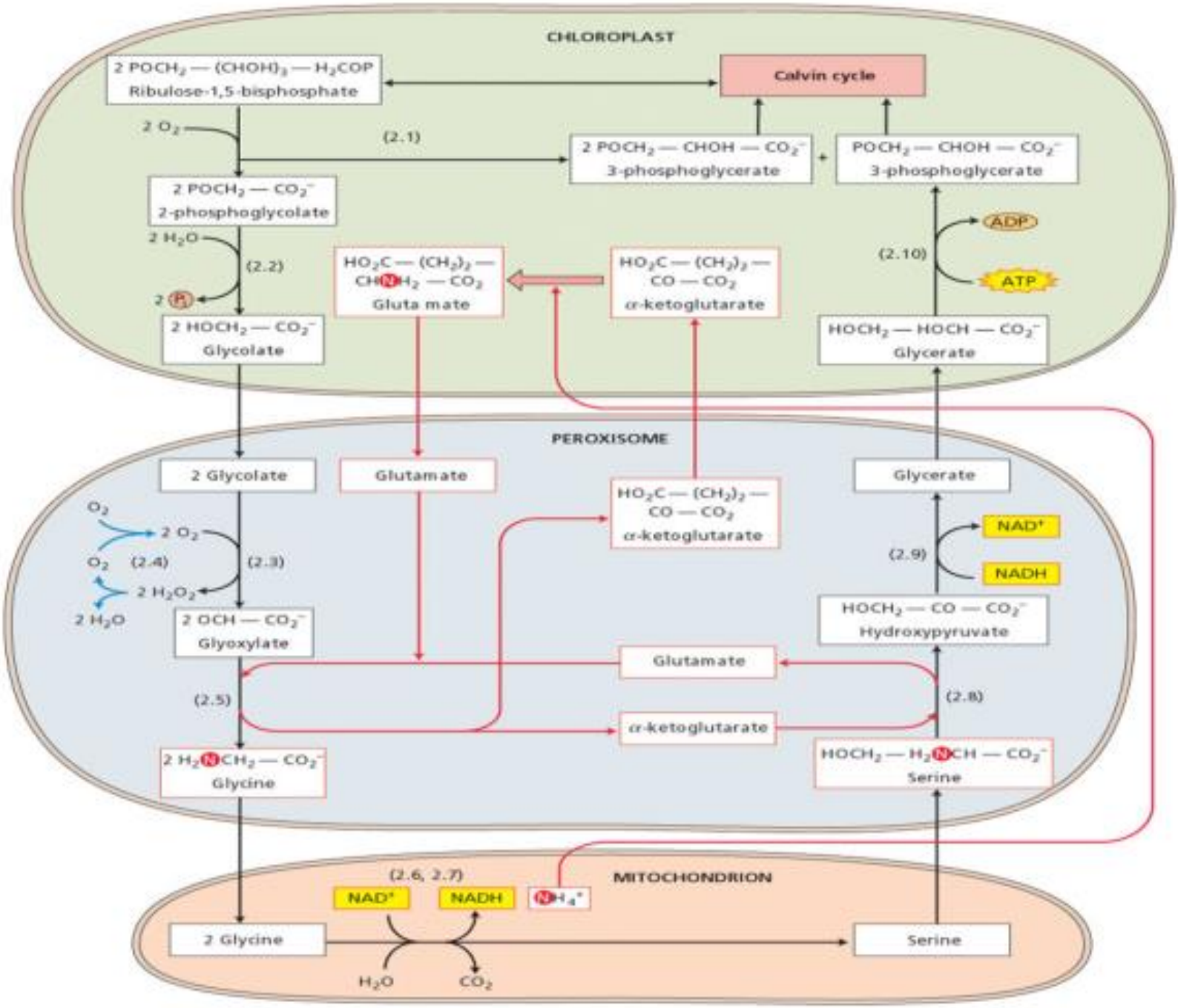
In addition to the carboxylation reaction, Rubisco also catalyzes an oxygenase reaction, hence the name ribulose-1,5 bisphosphate carboxylase-oxygenase.

With the addition of a molecule of oxygen,RuBP is converted into one molecule of 3-PGA and one molecule of phosphoglycolate.

The phosphoglycolate is subsequently metabolized in a series of reactions in the peroxisome and the mitochondrion that result in the release of a molecule of CO2 and recovery of the remaining carbon by the PCR cycle.







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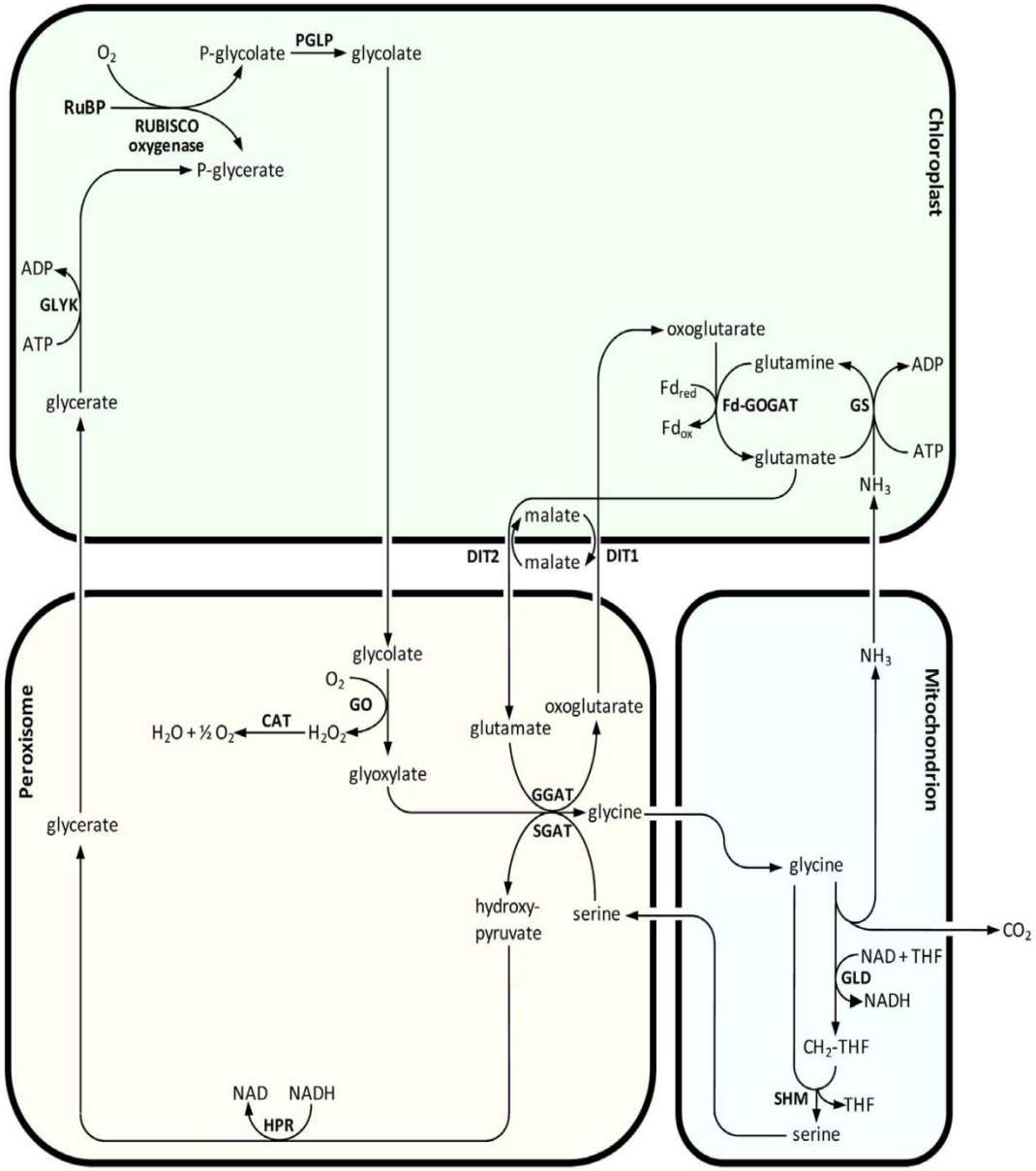
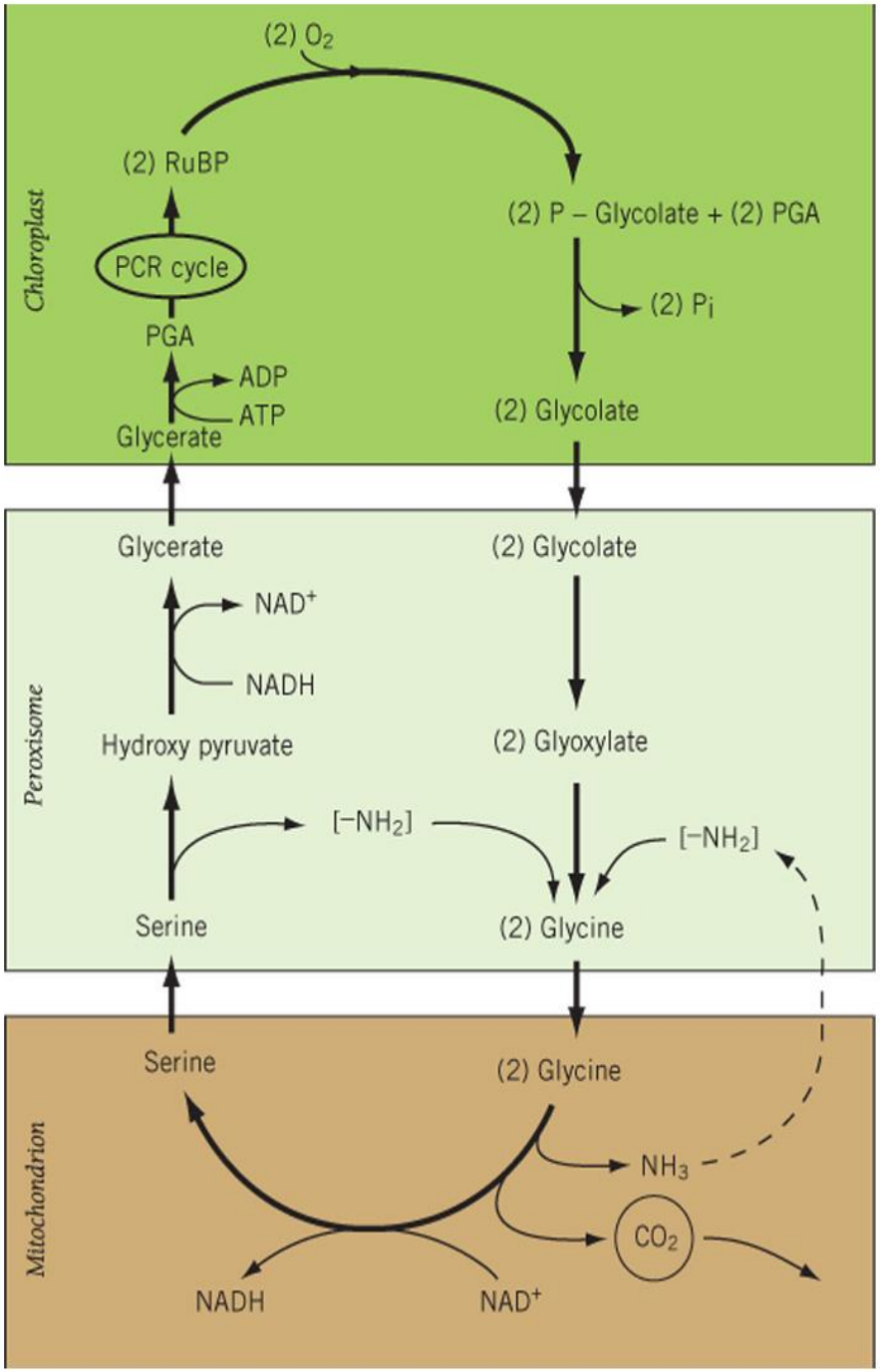
The C2 glycolate cycle, also known as the photo-synthetic carbon oxidation (PCO) cycle, begins with the oxidation of RuBP to 3-PGA and Phospho-glycolate (within chloroplast).

The 3-PGA is available for further metabolism by the PCR cycle, but the P-glycolate is rapidly dephospho-rylated to glycolate in the chloroplast.



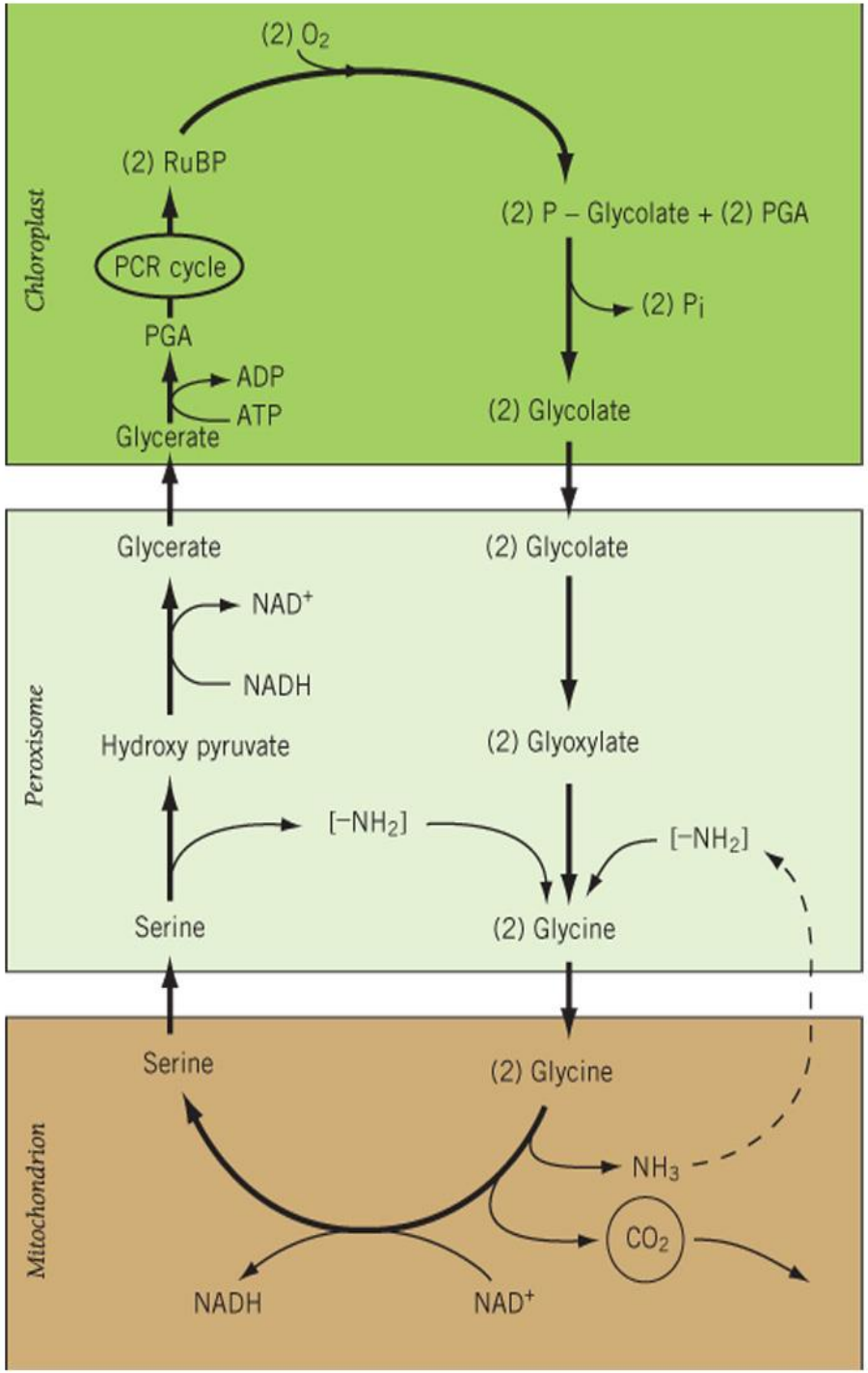
Taken up by the per-oxisome, the glycolate is oxidized to glyoxylate and hydrogen peroxide.

The peroxide is broken down by catalase and the glyoxylate under-goes a transamination reaction to form the amino acid glycine.



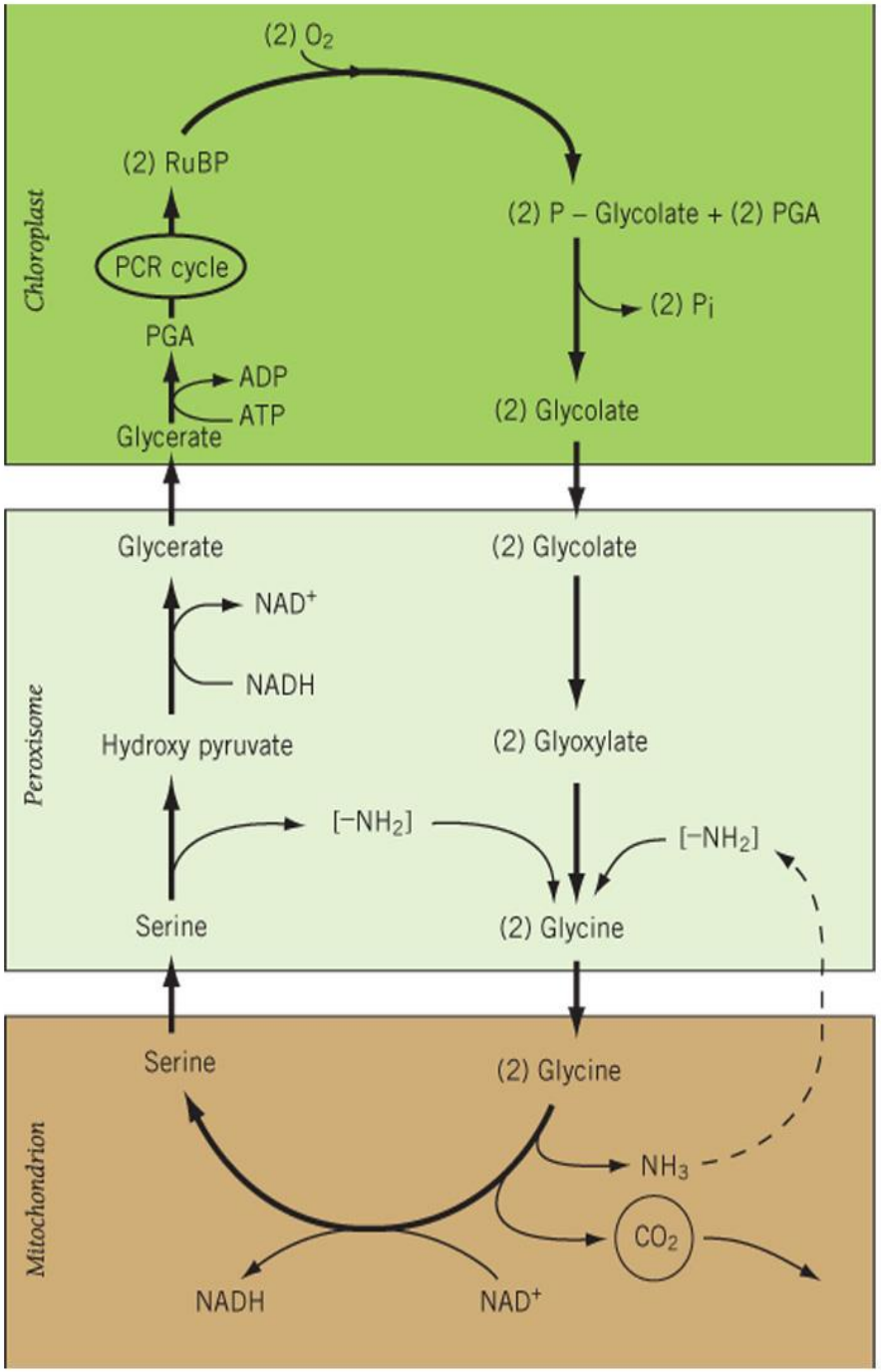
Glycine is then transferred to a mitochondrion where two molecules of glycine (4 carbons) are converted to one molecule of serine (3 carbons) plus one CO2.

The serine then leaves the mitochondrion, returning to a peroxisome where the amino group is given up in a transamination reaction and the product, hydroxypyruvate, is reduced to glycerate.



Finally, glycerate is returned to the chloroplast where it is phosphorylated to 3-PGA.

75% of the carbon lost during the oxygenation of *Rubisco* is recovered during photorespiration and is returned to the Calvin cycle



Significance of Photorespiration

There is also an energy cost associated with photorespiration and the glycolate pathway.

Not only is the amount of ATP and NAD(P)H expended in the glycolate pathway following oxygenation (5 ATP + 3 NADPH) greater than that expended for the reduction of one CO2 in the PCR cycle (3 ATP + 2 NADPH), but there is also a net loss of carbon.

Rubisco evolved at a time when the atmosphere contained large amounts of CO2 but little oxygen.

It is believed that oxygen began to accumulate in the atmosphere primarily due to photosynthetic activity, but by the time the atmospheric content of O2 had increased to significant proportions, the bifunctional nature of the enzyme had been established without recourse.

In a sense, C3 plants were the architect of their own problem —generating the oxygen that functions as a competitive inhibitor of carbon reduction.

By this view, then, the oxygenase function is an evolutionary ‘‘hangover’’ that has no useful role.

Significance of Photorespiration

The glycolate pathway, for example, undoubtedly serves a scavenger function.

For each two turns of the cycle, two molecules of phosphoglycolate are formed by oxygenation. Of these four carbon atoms, one is lost as CO2and three are returned to the chloroplast.

The glycolate pathway thus recovers75 percent of the carbon that would otherwise be lost as glycolate.

There is also the possibility that some of the intermediates, serine and glycine, for example, are of use in other biosynthetic pathways.

Recently, strong experimental evident support that photorespiration could also function as a sort of safety valve; apparently the O2 consumed by photorespiration is sufficient to protect the plant from photooxidative damage by permitting continued operation of the electron transport system.

Overall Significance of photorespiration

1. Carbon dioxide is evolved during the process and it prevents the total depletion of CO_2 in the vicinity of chloroplasts.

2. The process causes oxidation of glycolic acid which arises as an unwanted byproduct of photosynthesis. The glycolic acid after oxidation is converted into carbohydrate but the remainder is converted into CO_2.

3. It is believed that photorespiration was common in earlier days when CO2 content was too low to allow higher rates.

Factors affecting Rate of CO2 Reduction

Light Intensity:

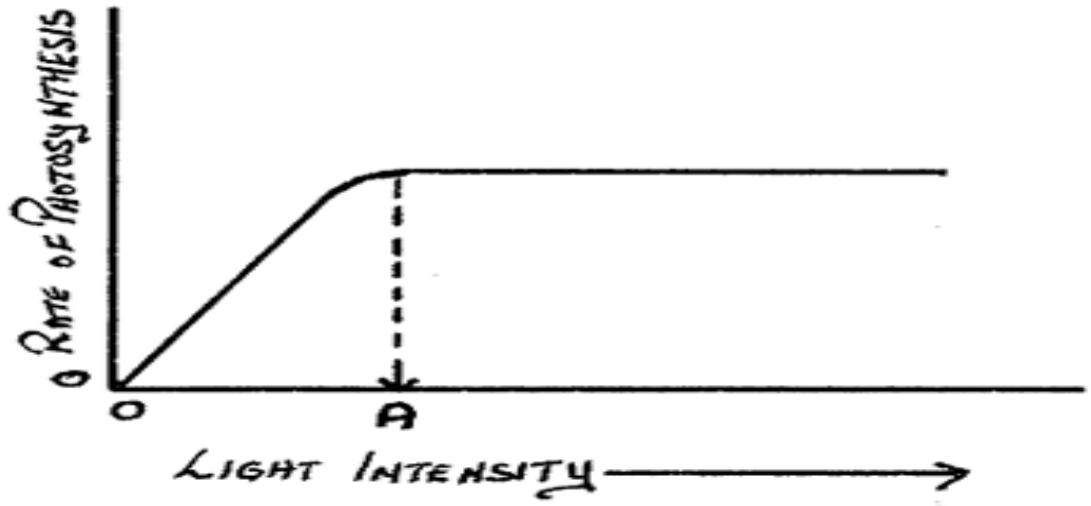
Increase rate of photosynthesis, then levels off (max. rate of photosynthesis)

Higher intensity, excites more electrons in cholorphyll

- **@ same intensity, all available electrons are excited**

Amount of CO_2

Increases rate of photosynthesis to a point, then levels off



Temperature

- Higher temperature accelerates the chemical rxns.
- Peaks at certain temp. because the enzymes becomes ineffective and unstable
- Stomata closes-limiting H_2O loss and CO_2 entry into the leaves

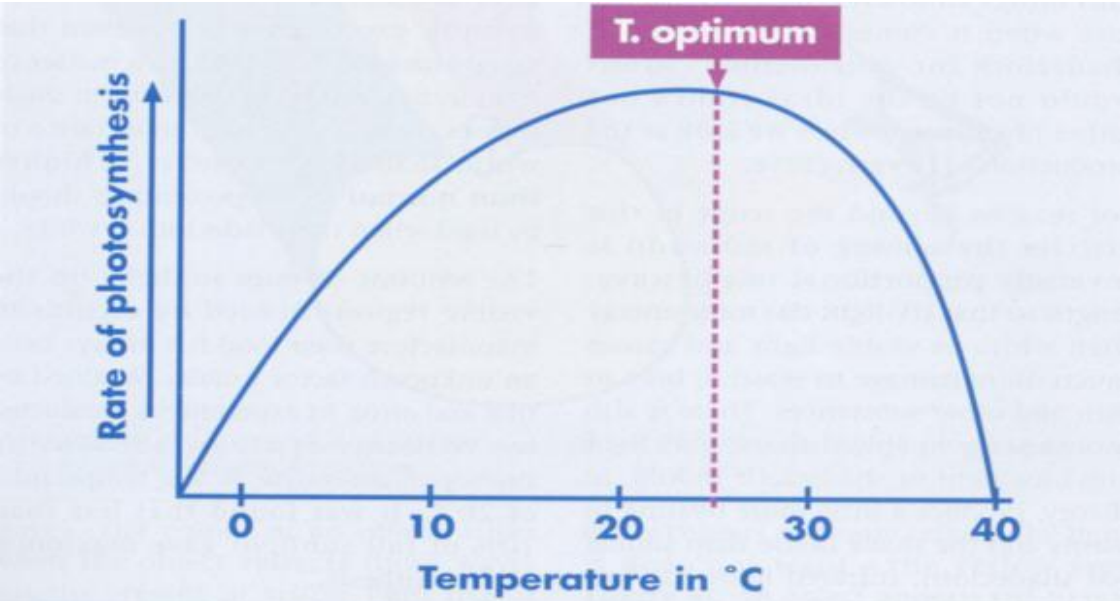


FIGURE 6: Effect of temperature on photosynthesis.

Concentration of O_2

Higher O_2 will decrease the rate of photosynthesis and increase the rate of photorespiration

- **Rubisco will bind with oxygen**

Will send PGA into respiration, instead of finishing photosynthesis

- **Decreasing amount of organic compound produced**