photosynthesis

Part 3

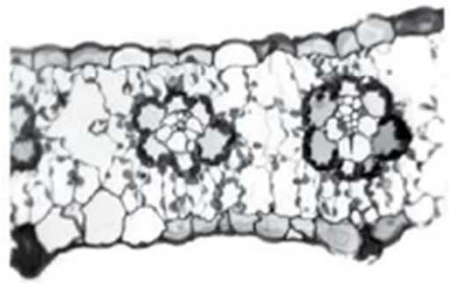
C4 plants utilize two distinct metabolic compartments for fixing CO2

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

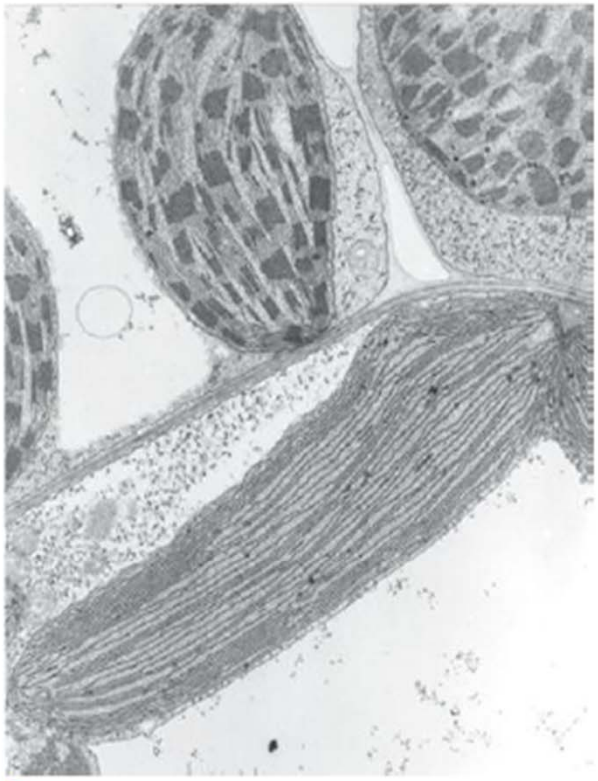
- several plant species form large amounts of four-carbon organic acids as the first products of CO2 fixation. On this basis, plants are classified as C3 or **C4 plants** based on the primary product of carbon fixation in photosynthesis: three-carbon (3-PGA) and four-carbon (oxaloacetic acid, OAA) compounds are the primary products of carbon fixation in C3 and C4 plants, respectively.

- **C4 photosynthesis** biochemical functions are separated between two intracellular compartments, which allow the primary and the secondary carboxylations to occur simultaneously.

- Sugar cane (*Saccharum* sp.), maize (*Zea mays*), and numerous tropical grasses are among the species that exhibit the C4 labeling pattern. The leaves of these and almost all C4 species exhibit an unusual anatomy involving two different types of chloroplast-containing cells: mesophyll cells surround enlarged bundle sheath cells, which in turn are arranged concentrically with respect to vascular tissues.
- Reduced interveinal distance and limited leaf thickness maximize the contact between mesophyll and bundle sheath cells. The 19th-century German botanists who originally described this feature called it **Kranz anatomy** (German: wreath). The biochemical differentiation of mesophyll cells and bundle sheath cells is essential for the effective operation of C4 photosynthesis:



A



B

FIGURE 12.43 *Leaf (Kranz) anatomy of C_4 plants. (A) Electron micrograph showing the leaf anatomy of maize (*Zea mays*). The closely spaced vascular bundles are surrounded by large bundle sheath cells. (B) Electron micrographs comparing the chloroplasts of a bundle sheath cell (bottom) and a mesophyll cell (top) in sorghum. The chloroplast morphologies reflect their biochemical functions. The bundle sheath chloroplasts lack stacked thylakoids and contain little PSII. In contrast, the mesophyll chloroplasts contain all the transmembrane complexes required for the light reactions of photosynthesis but little or no Rubisco.* *Source:* (A, B) Newcomb, University of Wisconsin, Madison; previously unpublished.

- PEP carboxylase (PEPCase) accumulates in the cytosol of mesophyll cells (external compartment), and Rubisco in the chloroplasts of bundle sheath cells (internal compartment). The biphasic C4 system reduces photorespiration dramatically by increasing levels of CO2 near Rubisco to enable Rubisco carboxylation to outcompete the oxygenation reaction. The low photosystem II activity in the bundle sheath also minimizes the Rubisco oxygenation reaction.
- C4 photosynthesis also occurs, however, in numerous organisms devoid of Kranz anatomy, including a number of aquatic and land plants. In some of these cases, photosynthetic enzymes and dimorphic chloroplasts occur in separate cytoplasmic domains within a single photosynthetic cell (Fig.). ×

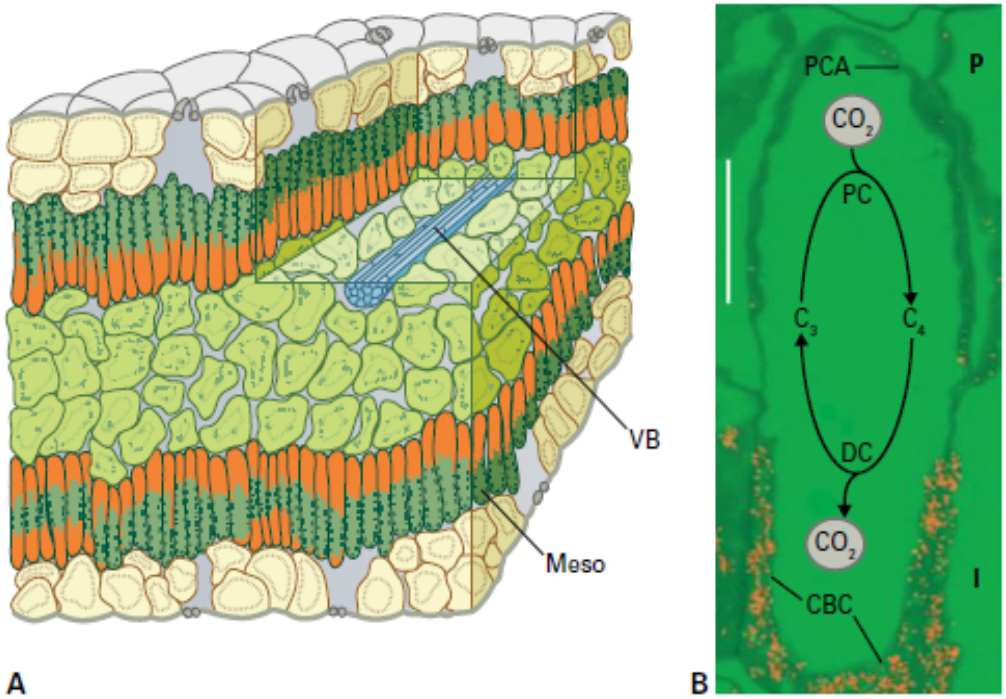


FIGURE 12.44 *Leaf anatomy of single-cell C4 plants. (A) Three-dimensional drawing of the leaf anatomy of* Borszczowia aralocaspica, *a single-cell C_4 plant. Meso, mesophyll; VB, vascular bundles. Note the lack of differentiation in the cells. (B) Immunolocalization of Rubisco at the internal region (I) of a photosynthetic cell from* Borszczowia aralocaspica. *Red chloroplasts containing the Calvin–Benson cycle (CBC) are functionally equivalent to C_4 bundle sheath chloroplasts. Chloroplasts containing PPDK, which are functionally equivalent to C_4 mesophyll chloroplasts, are situated at the peripheral region (P) where they contribute to the photosynthetic carbon assimilation (PCA). PC and DC refer to the initial carboxylation catalyzed by PEPCase and the decarboxylation of C_4 acids, respectively.*

The C4 pathway increases the concentration of CO2 in internal compartments close to vascular tissues

HCO3- (1C)
PEP (3C)
OAA (4C)

OAA (4C)
CO2 (1C)
Pyrovat (3C)
PEP (3C)

- The C4 pathway of CO2 fixation requires a complex interaction between two different compartments and involves the following steps:

$CO_2 + H_2O \rightarrow HCO_3^-$

1 Primary carboxylation: fixation of HCO_3^- by PEPCase in the outer compartment (for example, mesophyll cells) to yield OAA. $HCO_3^- + PEP \rightarrow OAA$

2 Transformation of OAA into another four-carbon acid (malate or aspartate) and transport of the four-carbon acids from the outer to the internal compartment (e.g., bundle sheath cells)

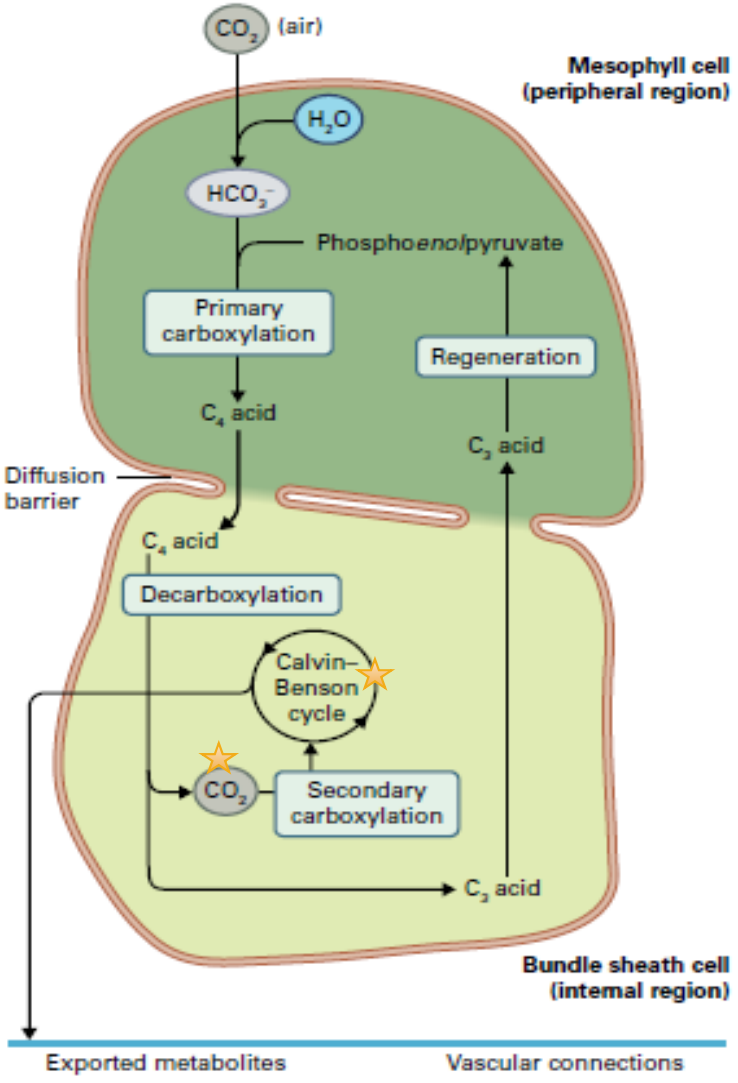
3 Decarboxylation: release of CO_2 from the four-carbon acid.

4 Secondary carboxylation: subsequent refixation of the CO_2 by Rubisco and the Calvin–Benson cycle.

5 Transfer of the three-carbon intermediates (pyruvate or alanine) resulting from decarboxylation of the four-carbon acids back to the outer compartment.

6 Conversion of the three-carbon intermediates to PEP to reinitiate the fixation of HCO_3^-.

FIGURE 12.45 *General aspects of the C_4 pathway. Atmospheric CO_2 enters the peripheral region and is converted to HCO_3^- for reaction with PEP, yielding OAA (primary carboxylation). OAA is transformed to a second C_4 acid (malate or aspartate) that flows across a diffusion barrier to the internal region. There, the C_4 acid is decarboxylated, yielding CO_2 and a C_3 acid (pyruvate or PEP) (decarboxylation). The released CO_2 is fixed by Rubisco in the Calvin–Benson cycle (secondary carboxylation) and converted to carbohydrate for export to other parts of the plant. The remaining C_3 acid is transported back to the external region to regenerate PEP (regeneration).*



- In the context of the two-cell C4 pathway, all carbon fixation begins in the cytosol of mesophyll cells, where carbonic anhydrase catalyzes the conversion of atmospheric CO_2 into HCO_3^- for subsequent carboxylation.

- OAA is generated from HCO_3^- and PEP in the cytosol of the outer compartment (i.e., mesophyll cells), catalyzed by PEPCase. Whereas

- enzymes unique to the Calvin–Benson cycle are located only in chloroplasts of the internal compartment (i.e., bundle sheath cells).

- In accordance with distinct functions, electron micrographs of bundle sheath and mesophyll chloroplasts (Fig. 12.43) show structural differences:

- chloroplasts of (internal) bundle sheath cells lack stacked membranes and exhibit little PSII activity, whereas chloroplasts of (peripheral) mesophyll cells have retained stacked membranes as well as PSII and PSI activities (Fig. 12.43B).

Reaction 12.26: Carbonic anhydrase

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

Reaction 12.27: Phospho*enol*pyruvate carboxylase (PEPCase)

$$PEP + HCO_3^- \leftrightarrow OAA + P_i$$

Three different types of C4 photosynthesis are known

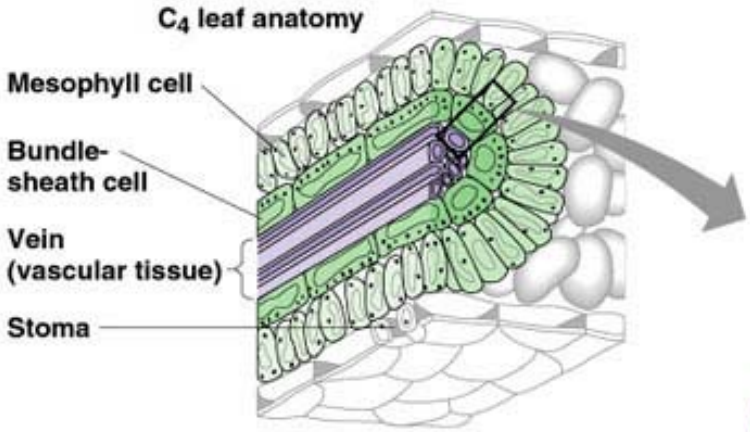
Three types of C4 photosynthesis are known, differing both in the four-carbon acids transported between mesophyll and bundle sheath cells, and the mechanism of decarboxylation in the bundle sheath cells (Table).

TABLE 12.10 *Variations in C_4 photosynthesis.*

C_4 acid transported to bundle sheath cells	C_3 acid transported to mesophyll cells	Decarboxylase	Plant examples
Malate	Pyruvate	NADP-ME	*Zea mays* (maize), *Saccharum officinarum* (sugarcane)
Aspartate	Alanine	NAD-ME	*Panicum miliaceum* (millet)
Aspartate	Alanine, PEP, or pyruvate	PEPCK	*Panicum maximum* (Guinea grass)

The C3 and C4 pathways have different energy costs

- In C3 plants, the Calvin–Benson cycle consumes nine ATP and six NADPH to assimilate three molecules of CO_2 into triose phosphate (3 ATP : 2 NADPH : 1 CO_2).
- In C4 photosynthesis, continuous operation of the C4/C3 shuttle requires restitution of PEP in mesophyll cells, which consumes two additional molecules of ATP in the successive reactions catalyzed by PPDK and adenylate kinase (AMP + ATP → 2 ADP).
- Thus, at least five molecules of ATP and two molecules of NADPH are needed for assimilation of one molecule of CO_2 (5 ATP : 2 NADPH : 1 CO_2).
- C4 photosynthesis evolved to minimize energetically wasteful photorespiration; however, avoidance of photorespiration came with a penalty for photosynthetic efficiency.
- In keeping with an increased metabolic flux across the envelope membranes of C4 chloroplasts, substrate-specific transporters of these membranes are more abundant in C4 than in C3 plants.

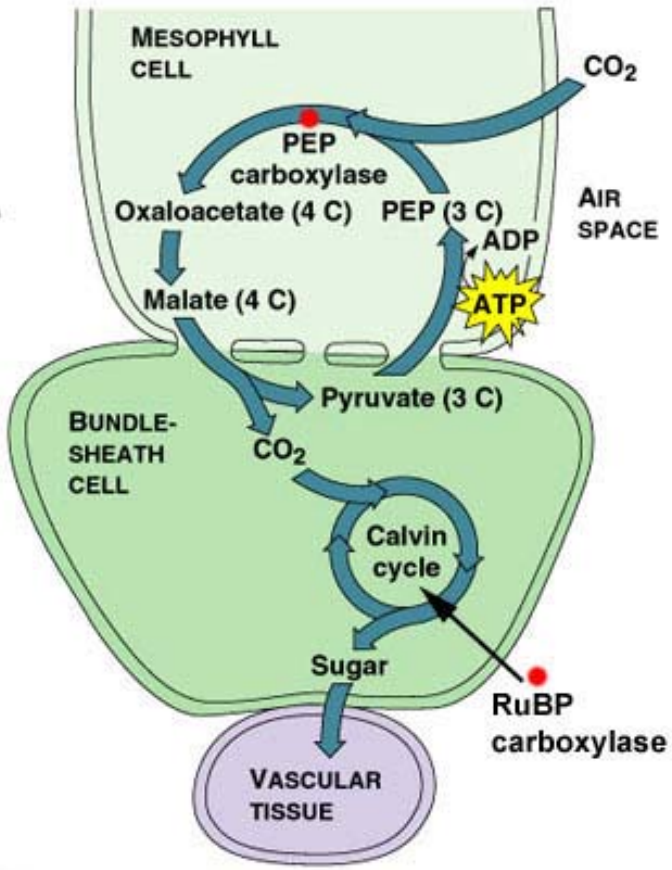


Differences compared to C3 (Calvin) plants

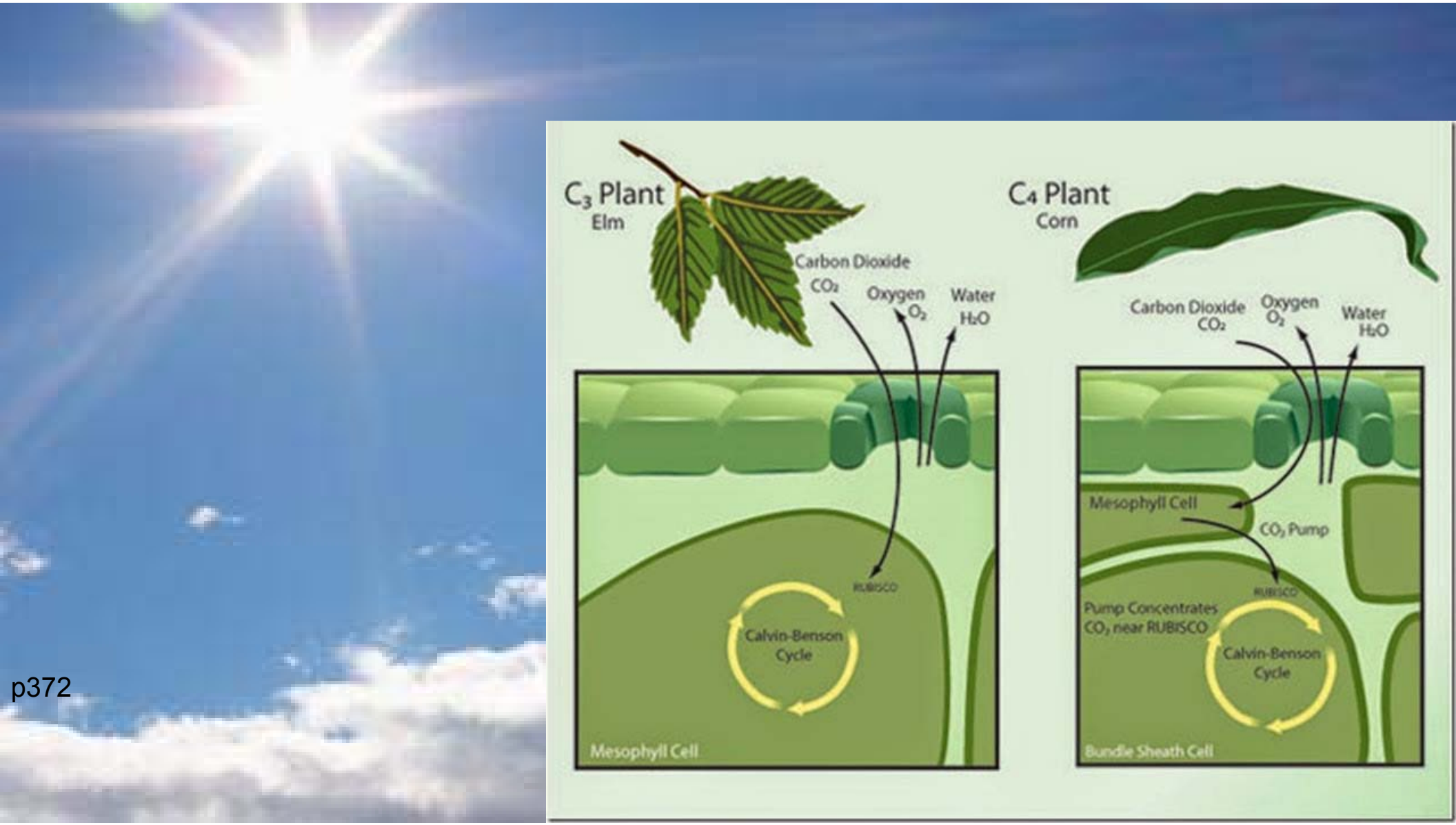
2 cell types: mesophyll & bundle sheath
spatial separation acid & sugar synthesis

2 fixation enzymes: 1) RuBP carboxylase
2) PEP carboxylase

CO_2 is reduced twice



The C_4 pathway


C3 Plant
Elm
Carbon Dioxide
CO2
Oxygen
O2
Water
H2O
RUBISCO
Calvin-Benson Cycle
Mesophyll Cell
C4 Plant
Corn
Carbon Dioxide
CO2
Oxygen
O2
Water
H2O
Mesophyll Cell
CO2 Pump
RUBISCO
Pump Concentrates CO2 near RUBISCO
Calvin-Benson Cycle
Bundle Sheath Cell

p372

Critical enzymes of the C4 pathway are also regulated by light

Light-mediated regulation of enzymes coordinates the activities of mesophyll and bundle sheath cells to ensure that four-carbon acids are available for CO_2 fixation in bundle sheath cells. In C_4 photosynthesis, regulation of Calvin–Benson cycle enzymes by light is similar to C_3 plants.

CAM photosynthesis involves the temporal separation of CO2 capture and carbon fixation

- Another modification of the C3 pathway occurs in plants utilizing Crassulacean acid metabolism (CAM).
- Named after the Crassulaceae family of succulent plants, this pathway has evolved mechanisms for maximizing carbon uptake under environmental conditions that limit productivity, such as high temperature or shortage of water.
- Thus, CAM photosynthesis is commonly associated with plants that inhabit arid environments (e.g., succulents, including cacti and some commercially significant plants, such as pineapple and agave).
- To enhance water conservation, these plants have evolved anatomical and morphological structures, such as thick cuticles, that prevent water loss.
- They have also evolved mechanisms for ensuring a high concentration of CO2 at the active site of Rubisco to minimize photorespiration.

- The biochemical pathway of CO2 fixation in terrestrial CAM plants is similar to the C4 pathway, but instead of a spatial separation of the two carboxylations necessary for CO2 fixation, terrestrial CAM plants use a temporal separation of the initial fixation reaction from the assimilation by Rubisco (Fig.).

- At night, when it is cold and leaf stomata are open, CO2 is fixed initially into OAA and then malate via PEPCase and NADP-malate dehydrogenase. The malic acid is stored in vacuoles, where it can reach high concentrations.

- During the day, stomata close to prevent the loss of water, and malic acid is transported out of the vacuole. At this stage, NADP-ME catalyzes the decarboxylation of malate, yielding CO2 and pyruvate, and the released CO2 is fixed by Rubisco via the Calvin–Benson cycle, while pyruvate is used to form starch. The starch confined to photosynthesizing tissues of terrestrial CAM plants, named **transitory starch**, is broken down at night via glycolysis and serves as a source of PEP for the fixation of CO2 via PEPCase.

- In this circadian rhythm, the opening/closing of stomata, variations of organic acids and storage carbohydrates, and the activities of carboxylation and decarboxylation enzymes modulate the proportion of CO2 taken up via PEPCase at night and Rubisco during the day.

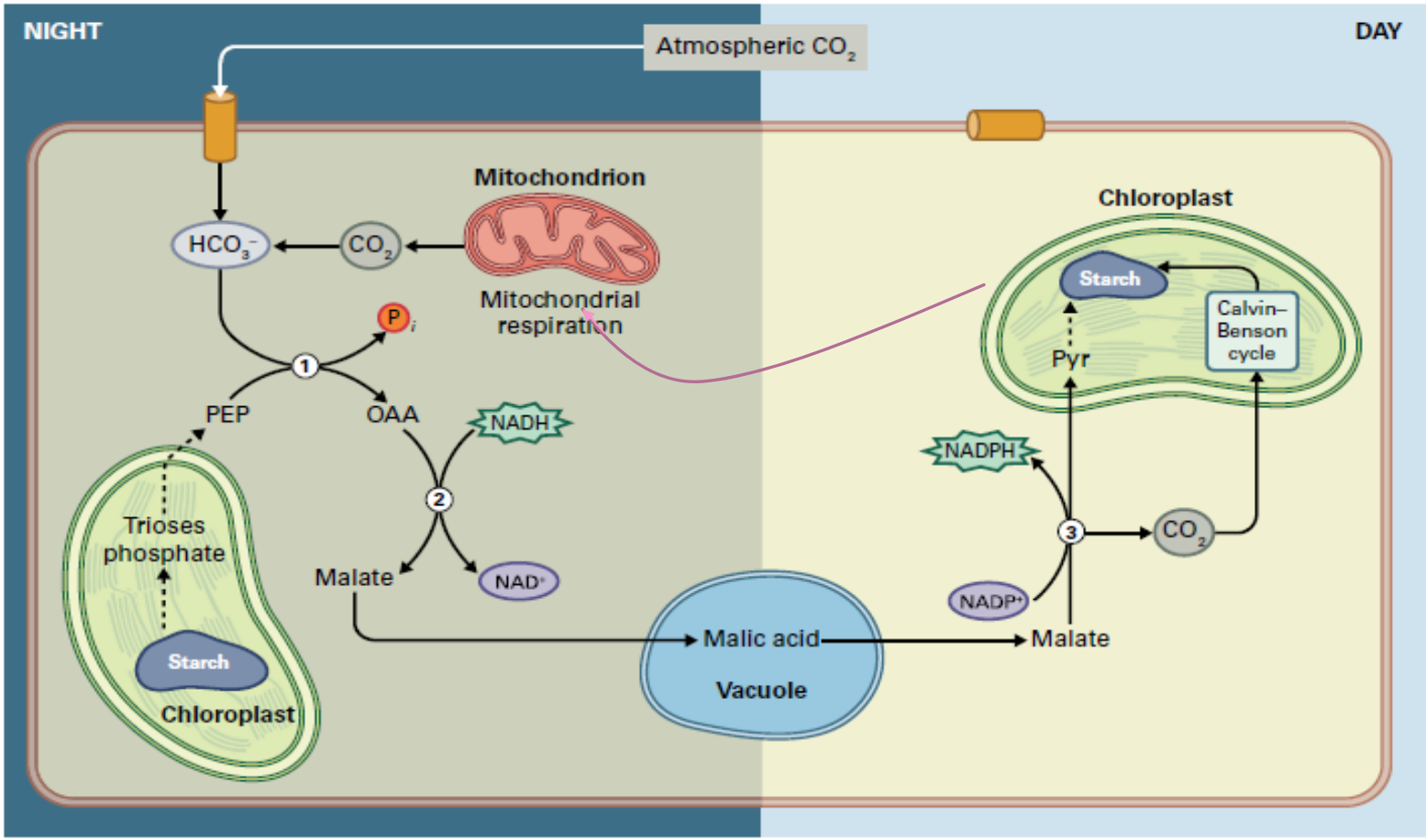
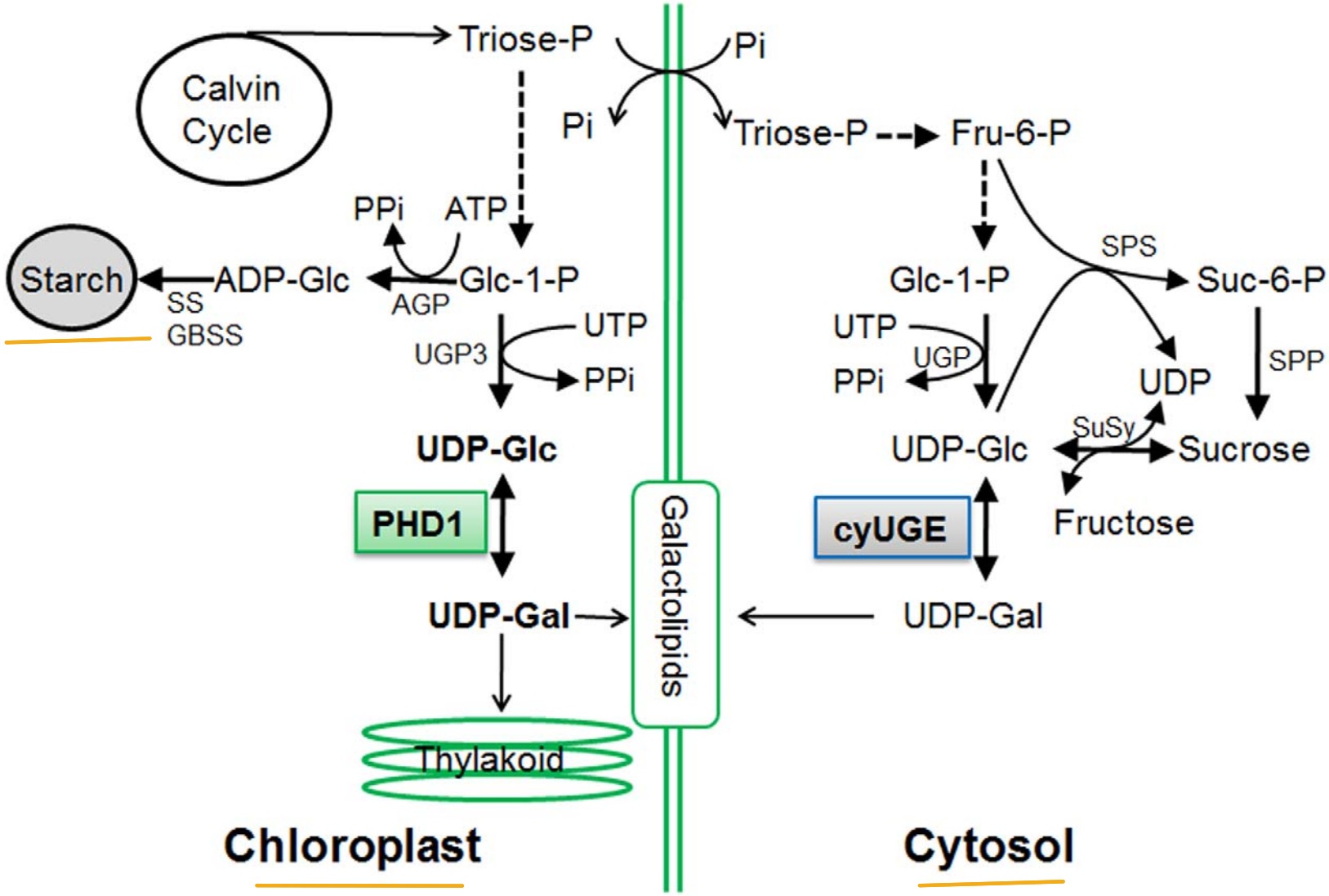


FIGURE 12.49 *Crassulacean acid metabolism (CAM). At night, both atmospheric and respiratory CO_2 provide HCO_3^- for carboxylation of PEP catalyzed by PEPCase (reaction 1). NAD-malate dehydrogenase catalyzes the reduction of the C_4 organic acid—oxaloacetic (OAA)—to malate (reaction 2). Malate is stored in the vacuole overnight. During the day, the stored malate is decarboxylated by NAD(P)-ME yielding NAD(P)H, pyruvate, and CO_2 (reaction 3). In the chloroplast, pyruvate and CO_2 are used for synthesis of carbohydrates via gluconeogenesis and the Calvin–Benson cycle, respectively.*

A notable feature of terrestrial CAM plants is the alternation between stomatal opening and closing. Stomata remain open during the cool and relatively humid night, allowing uptake of atmospheric CO_2 while sustaining minimal water loss. During the heat and dryness of the day, the stomata close, preventing not only water loss but also the entry of atmospheric CO_2. Malate becomes the internal source of CO_2 in leaves. The decarboxylation of malate raises the concentration of CO_2 to high levels because CO_2 cannot escape through the closed stomata. These high concentrations of CO_2 further increase the efficiency of Rubisco as a carboxylase. The Calvin–Benson cycle is regulated as in C3 plants.


Calvin Cycle
Triose-P
Pi
Pi
Triose-P
Fru-6-P
PPi
ATP
Starch
ADP-Glc
Glc-1-P
SS
GBSS
AGP
UTP
UGP3
PPi
SPS
Glc-1-P
Suc-6-P
UTP
UGP
PPi
UDP
SPP
SuSy
UDP-Glc
Sucrose
UDP-Glc
PHD1
cyUGE
Fructose
Galactolipids
UDP-Gal
UDP-Gal
Thylakoid
Chloroplast
Cytosol

Limiting Factors

- Factors affecting the rate of photosynthesis.
- As the rate of photosynthesis increases so will the plants growth rate.
- Three factors limit photosynthesis from going any faster: Light level, carbon dioxide level, and temperature.
- Without enough **light** a plant cannot photosynthesize very fast, even if there is plenty of water and carbon dioxide. Increasing the light intensity will make photosynthesis faster.

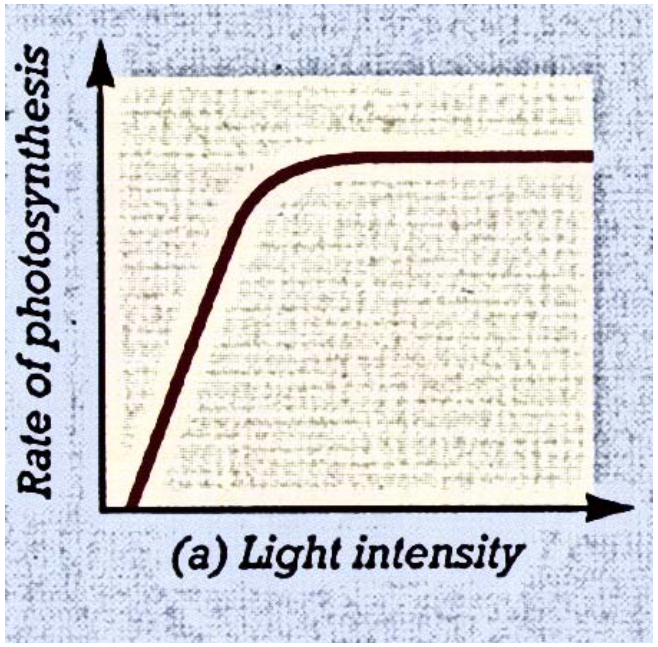
Limiting Factors

- Sometimes photosynthesis is limited by the level of **carbon dioxide**. Even if there is plenty of light a plant cannot photosynthesize if it has run out of carbon dioxide.

- **Temperature** can be a limiting factor too. If it gets too cold the rate of photosynthesis will slow right down; equally, plants cease to be able to photosynthesize if it gets

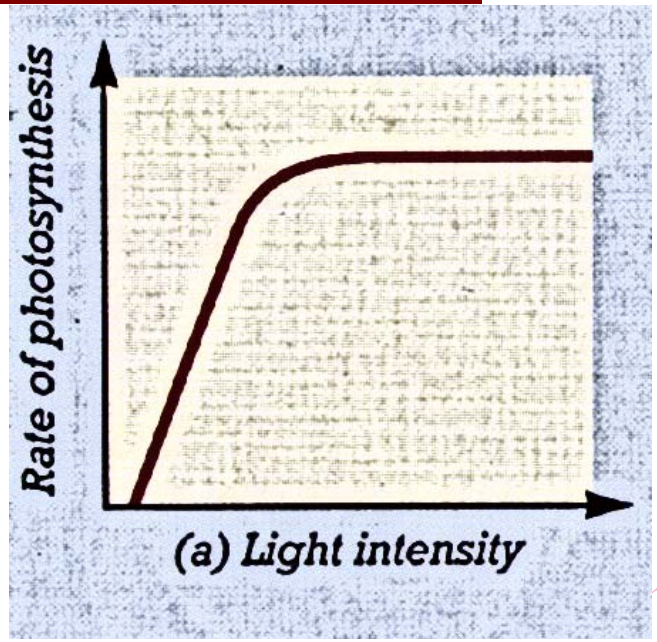
- This plateau represents the **maximum rate of photosynthesis** ---as seen in the diagram.

- Higher light intensity initially causes more electrons in the chlorophyll molecules to become excited *(gain energy)*.



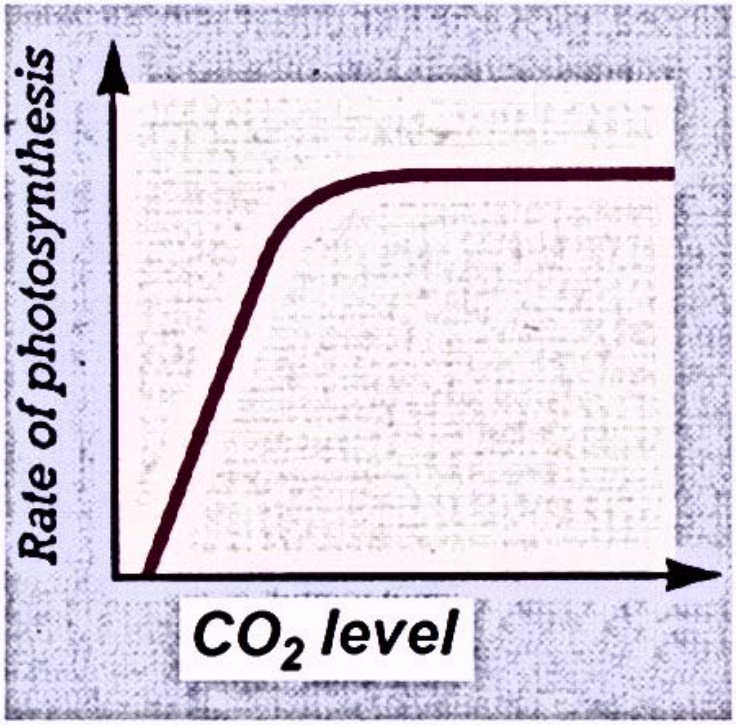
- As more and more electrons are excited, the light reactions occur more rapidly.

- At a certain light intensity, <u>however, all the available electrons are excited and a further increase in light intensity will not increase the rate of photosynthesis.</u>



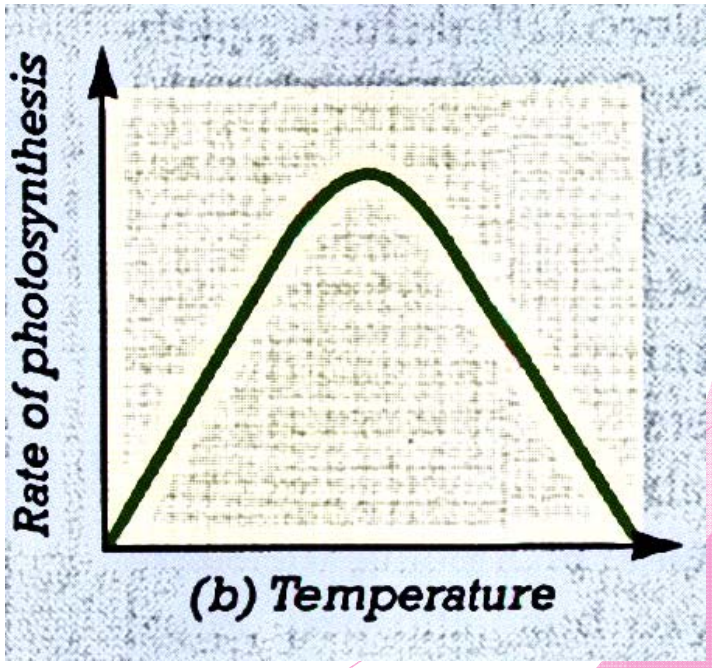
2) Carbon dioxide

Like increasing light intensity, increasing levels of carbon dioxide around the plant stimulates photosynthesis until it reaches a plateau. This graph would resemble that of light intensity.



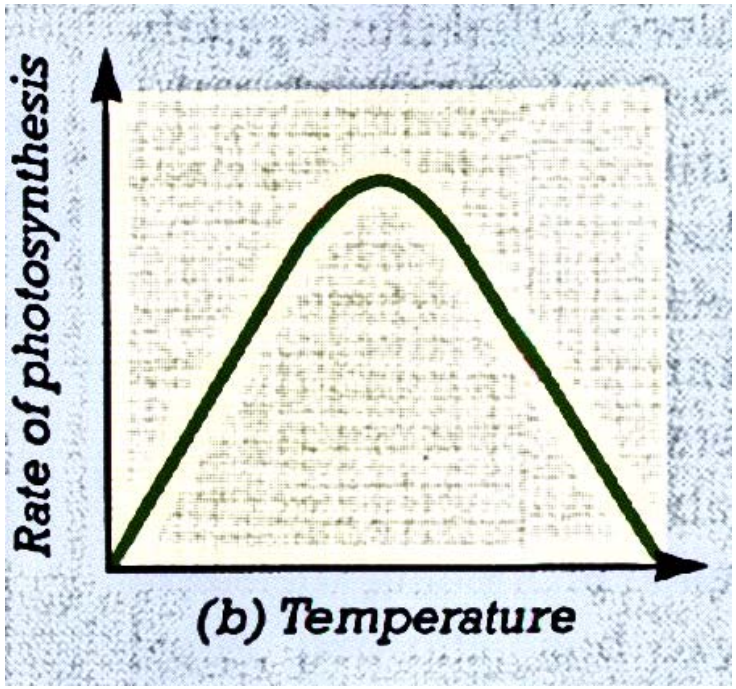
3) Temperature

a) Raising the temperature accelerates various chemical reactions of photosynthesis. As a result, the rate of photosynthesis increases, over a certain range.

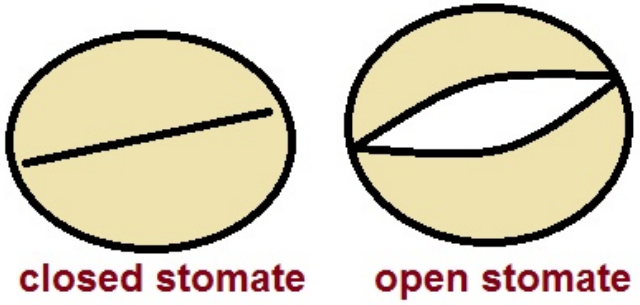


- b) The rate of photosynthesis generally **peaks at a certain temperature**, as seen in the graph.

c) **Above this temperature, the rate decreases**.



d) As the temperature increases, the **stomates begin to close**, to limit water loss. This will have the effect of **stopping the carbon dioxide from entering** the leaf. This will also decrease the rate of photosynthesis. (Also: Enzymes do not function well at too high a temperature.)



4) Water

- A lack of water will also slow the rate of photosynthesis. Stomata can close from water loss.
- Plants such as the cactus have adaptations to prevent water loss in dry, desert climates.

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 10.1

Comparison of Photosynthesis in C_3, C_4, and CAM Plants

	C_3 PLANTS	C_4 PLANTS	CAM PLANTS
Calvin cycle used?	Yes	Yes	Yes
Primary CO_2 acceptor	RuBP	PEP	PEP
CO_2-fixing enzyme	Rubisco	PEP carboxylase	PEP carboxylase
First product of CO_2 fixation	3PG (3-carbon)	Oxaloacetate (4-carbon)	Oxaloacetate (4-carbon)
Affinity of carboxylase for CO_2	Moderate	High	High
Photosynthetic cells of leaf	Mesophyll	Mesophyll and bundle sheath	Mesophyll with large vacuoles
Photorespiration	Extensive	Minimal	Minimal

CARACTERISTICA	MECANISMO DE FIJACIÓN DE CO2		
	C3	C4	CAM
Requerimiento teórico de energía (CO2:ATP:NADPH)	1 : 3 : 2	1 : 5 : 2	1 : 6,5 : 2
Enzima Carboxilante	RuDP carboxilasa	PEP carboxilasa y RuDp carboxilasa	PEP carboxilasa y RuDp carboxilasa
Tasa máxima de fotosíntesis neta (mg de CO2 / dm2 hoja/ hora)	15 - 35	40 - 80	1 - 18
Fotorespiración	Presente	Difícil de detectar	Difícil de detectar
Sensibilidad de la fotosíntesis a cambios de [O2]	si	no	-
Temperatura Optima para: a.- Fijación de CO2 b.- Crecimiento	 15 a 25 ° C 20 a 35 ° C	 30 a 47 ° C 30 a 35 ° C	 ≈ 35 ° C ≈ 35 ° C
Saturación a la luz	En ¼ a ½ de la plana exposición	Si se satura es a plana exposición	
Relación de transpiración (g de agua/ g de MS)	450 - 950	250 - 350	50 - 55
Producción de materia seca (Ton/ha/ año)	22 ± 3,3	38, 6 ± 16,9	Variable

Summary

- Photosynthesis produces organic compounds from inorganic carbon by using the energy of sunlight.
- These redox processes are carried out in plants, algae, and various bacteria.
- In all cases, the photosynthetic reactions may be divided into two phases: the light reactions and the carbon reactions.
- In eukaryotic organisms, photosynthesis takes place in the chloroplast.
- The two phases of photosynthesis occur simultaneously but take place in different regions of the chloroplast
- The light reactions being localized to the thylakoid membranes and the carbon reactions to the stroma

Summary

- The light reactions of photosynthesis involve the photosynthetic pigments, the photosynthetic electron transport chain, and the ATP synthesis machinery
- Light is absorbed by pigments localized in pigment–protein complexes (photosystems) within the thylakoid membrane.
- This light energy can be transferred from antenna pigments to special pigment–protein complexes, known as reaction centers, where the light energy is converted into chemical products (photochemistry)
- Oxygenic photosynthetic organisms contain two reaction centers and two photosystems, PSII and PSI.
- In plants the two photosystems are spatially separated: PSII is localized in appressed thylakoids, and PSI is localized in stroma-exposed thylakoids.

Summary

- During noncyclic electron transfer, the two photosystems cooperate in the transfer of electrons from water to $NADP^+$ via a series of redox reactions
- In addition to O_2, reduced ferredoxin, and NADPH, the noncyclic electron transfer reactions are coupled to the formation of ATP
- In addition to the noncyclic mechanism for ATP synthesis, chloroplasts synthesize ATP via two cyclic pathways that involve only PSI.
- The reduction of CO_2 to carbohydrates requires the reduced ferredoxin, NADPH, and ATP that are synthesized by the photosynthetic light reactions.
- All plants employ the C3 photosynthetic pathway (Calvin–Benson cycle) to fix CO2, using the enzyme Rubisco to convert CO2 and RuBP into the C3 product, 3-PGA.

Summary

- Calvin–Benson cycle occurs in three phases—carboxylation, reduction, and regeneration—and requires three ATP and two NADPH molecules per molecule of CO_2 fixed.
- Reactions of the Calvin–Benson cycle reactions are catalyzed by soluble enzymes localized in the chloroplast stroma.
- Regulation of the cycle is linked to multiple light-dependent mechanisms, including the active removal of Rubisco inhibitors, changes in pH and Mg_{2+}, and disulfide redox transitions catalyzed by the ferredoxin–thioredoxin system.
- Variants of C_3 photosynthesis exist in many plants.

Summary

- In one variation, the C_4 pathway, plants fix CO_2 into a C_4 acid in mesophyll cells, and transport this fixed CO_2 to anatomically distinct bundle sheath cells, where the CO_2 is released, refixed, and assimilated by Rubisco and other enzymes of the Calvin–Benson cycle.
- This sequence of reactions enhances the productivity of C_4 plants by (i) providing a higher concentration of CO_2 for Rubisco in the bundle sheath cell, and (ii) decreasing the oxygenase activity of Rubisco—a reaction that competes with the fixation of CO_2 in C_3 chloroplasts.
- In another variant, CAM metabolism, CO_2 is fixed at night into malate, which is decarboxylated during the day to provide CO_2 for Rubisco.
- CAM photosynthesis aids in the retention of water and enables plant to grow in arid environments.
- Several photosynthetic enzymes in C_4 and CAM plants are regulated to ensure efficient interaction of the CO_2- concentrating mechanisms with the Calvin–Benson cycle.