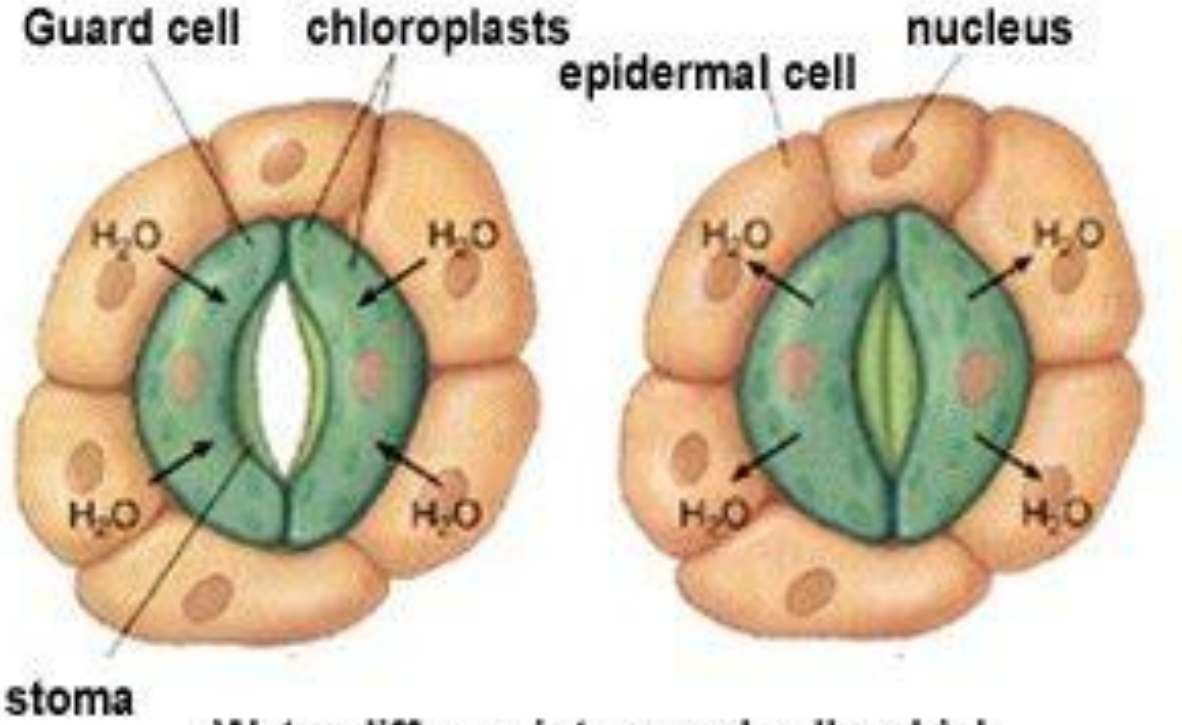
Photorespiration

How Gases Enter and Leave Plants



Water diffuses into guard cells which causes them to open. On hot/dry days, the guard cells have less water, they relax and the stoma close

Other Pathways of Photosynthesis

1. The Calvin Cycle is the MOST Common Pathway for Carbon Fixation. Plant Species that fix Carbon EXCLUSIVELY through the Calvin Cycle are known as C3 PLANTS.

2. Other Plant Species Fix Carbon through alternative Pathways and then Release it to enter the Calvin Cycle.

3. When a plant's Stomata are partly CLOSED, the level of CO_2 FALLS (Used in Calvin Cycle), and the Level of O_2 RISES (as Light reactions Split Water Molecules).

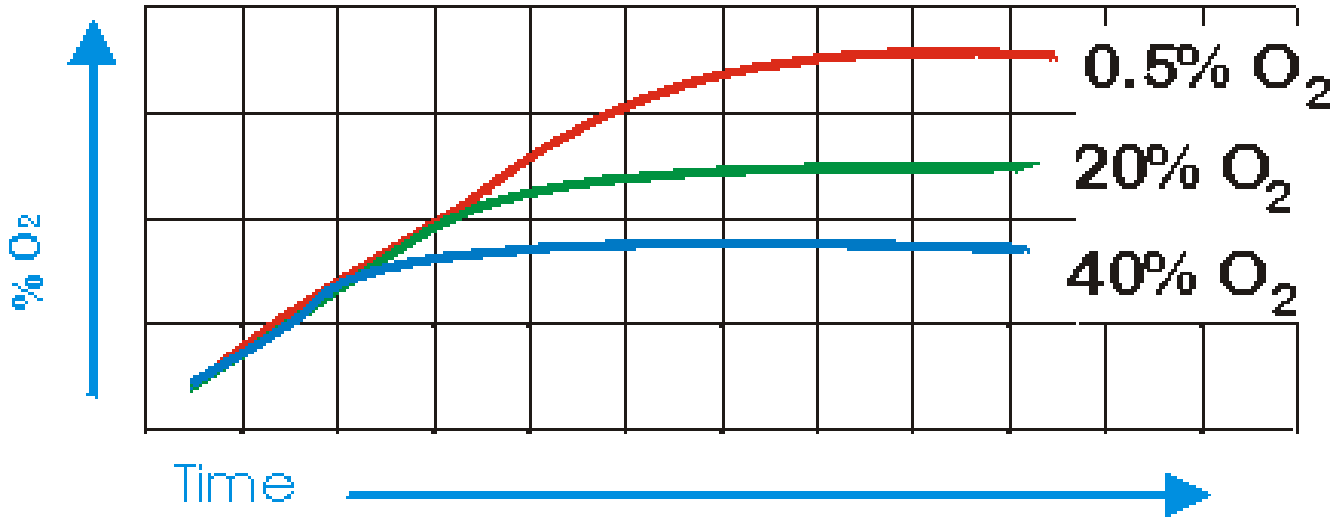
4. A LOW CO_2 and HIGH O_2 Level inhibits Carbon Fixing by the Calvin Cycle. Plants with alternative pathways of Carbon fixing have Evolved ways to deal with this problem.

5. C4 PLANTS - Allows certain plants to fix CO_2 into FOUR-Carbon Compounds. During the Hottest part of the day, C4 plants have their Stomata Partially Closed. C4 plants include corn, sugar cane and crabgrass. Such plants Lose only about Half as much Water as C3 plants when producing the same amount of Carbohydrate.

6. THE CAM PATHWAY - Cactus, pineapples have different adaptations to Hot, Dry Climates. They Fix Carbon through a pathway called CAM. Plants that use the CAM Pathway Open their Stomata at NIGHT and Close during the DAY, the opposite of what other plants do. At NIGHT, CAM Plants take in CO_2 and fix into Organic Compounds. During the DAY, CO_2 is released from these Compounds and enters the Calvin Cycle. Because CAM Plants have their Stomata open at night, they grow very Slowly, But they lose LESS Water than C3 or C4 Plants.

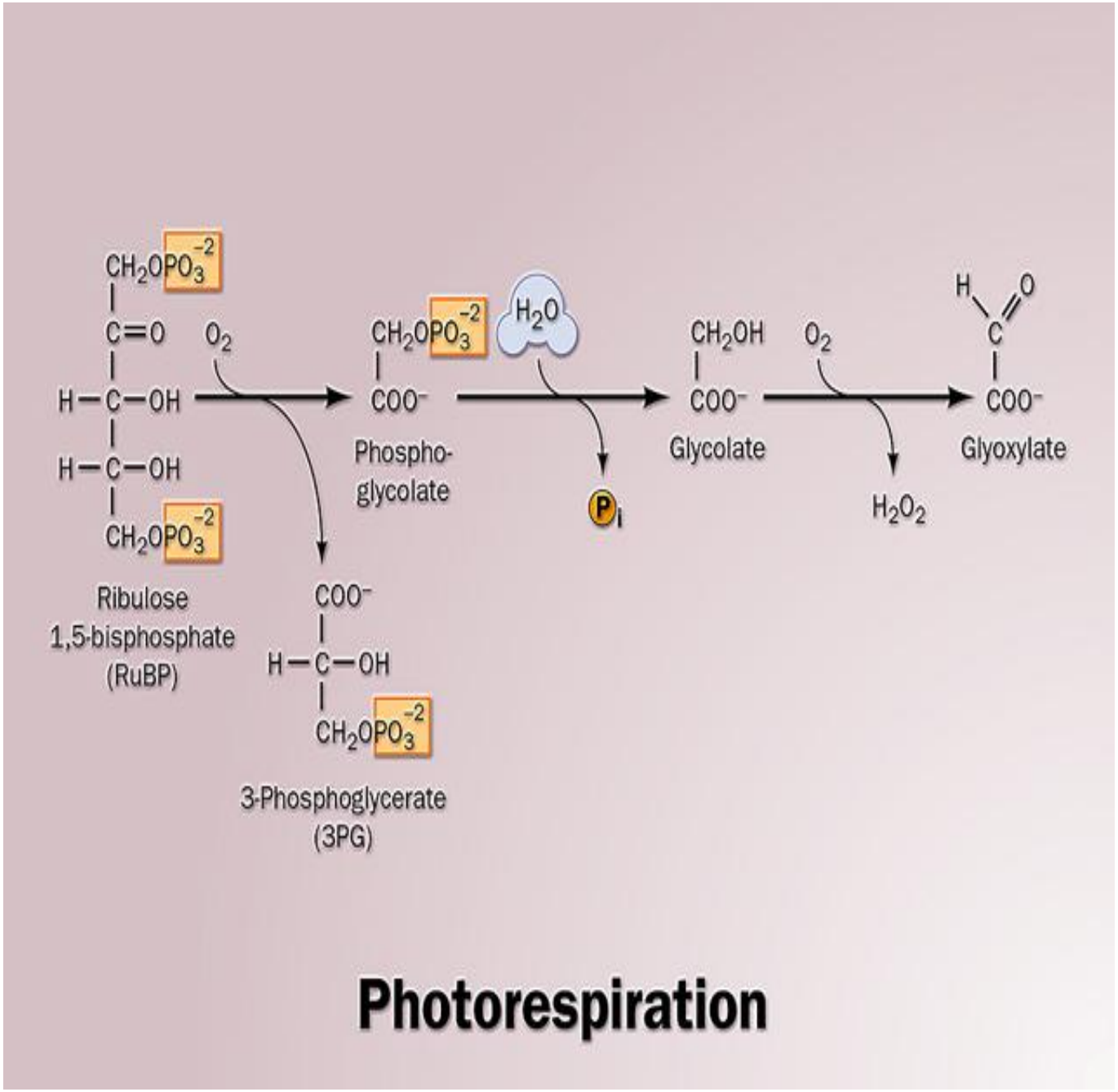
What happens during the process called "photorespiration"?

- O_2 has an inhibitory effect upon photosynthesis. As shown in the following hypothetical experiment, in the presence of elevated O_2 levels, photosynthesis rates are lower.



Light stimulated production of carbon dioxide in the presence of oxygen

- not associated with mitochondrial respiration
- requires light not accompanied by ATP synthesis
- wastes energy (i.e., ATP, NADPH)



The ratio of [CO_2 / O_2] determines the product of the rubisco reaction.

- if [CO_2 / O_2] is high

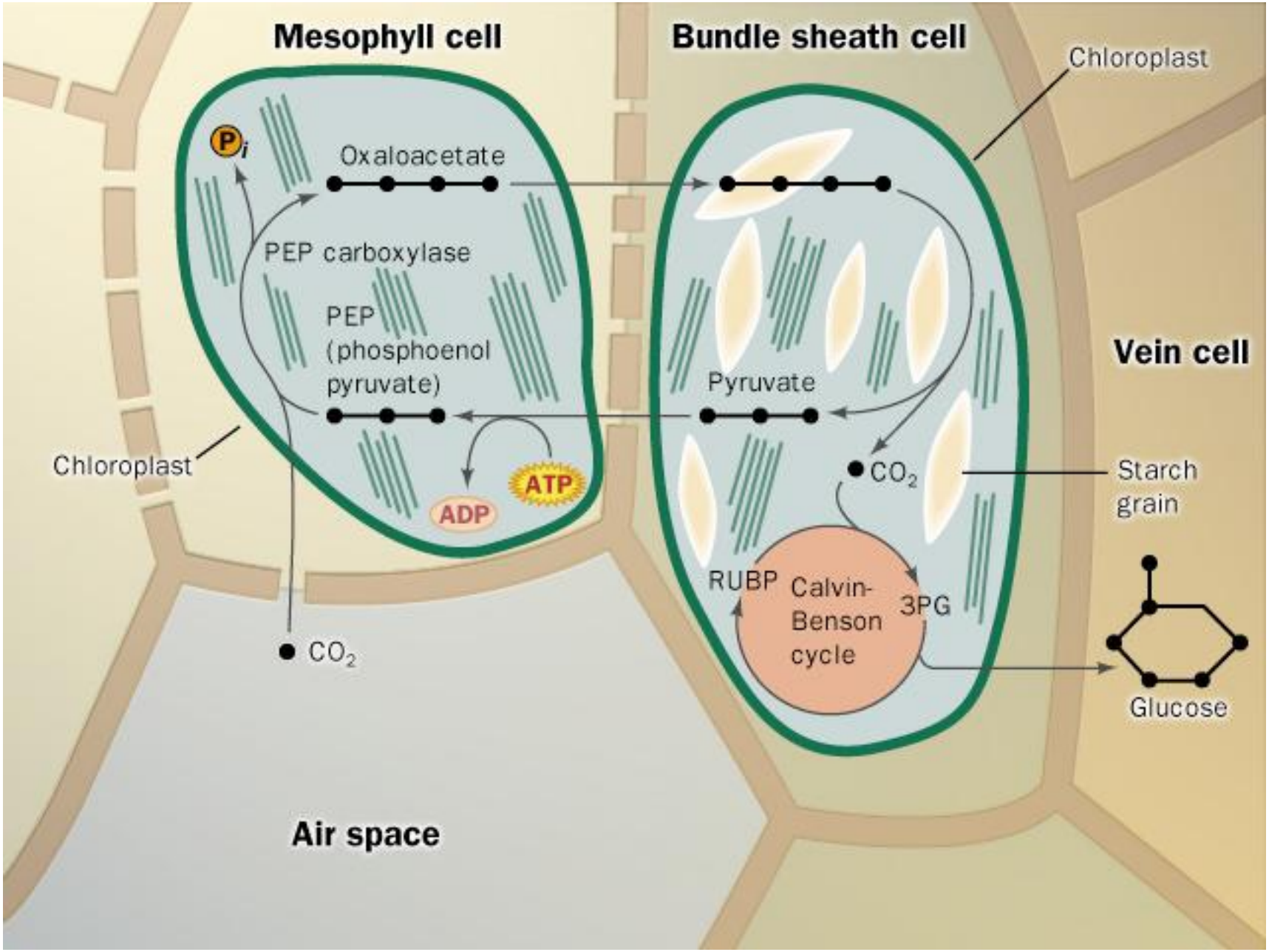
  favors normal Calvin cycle

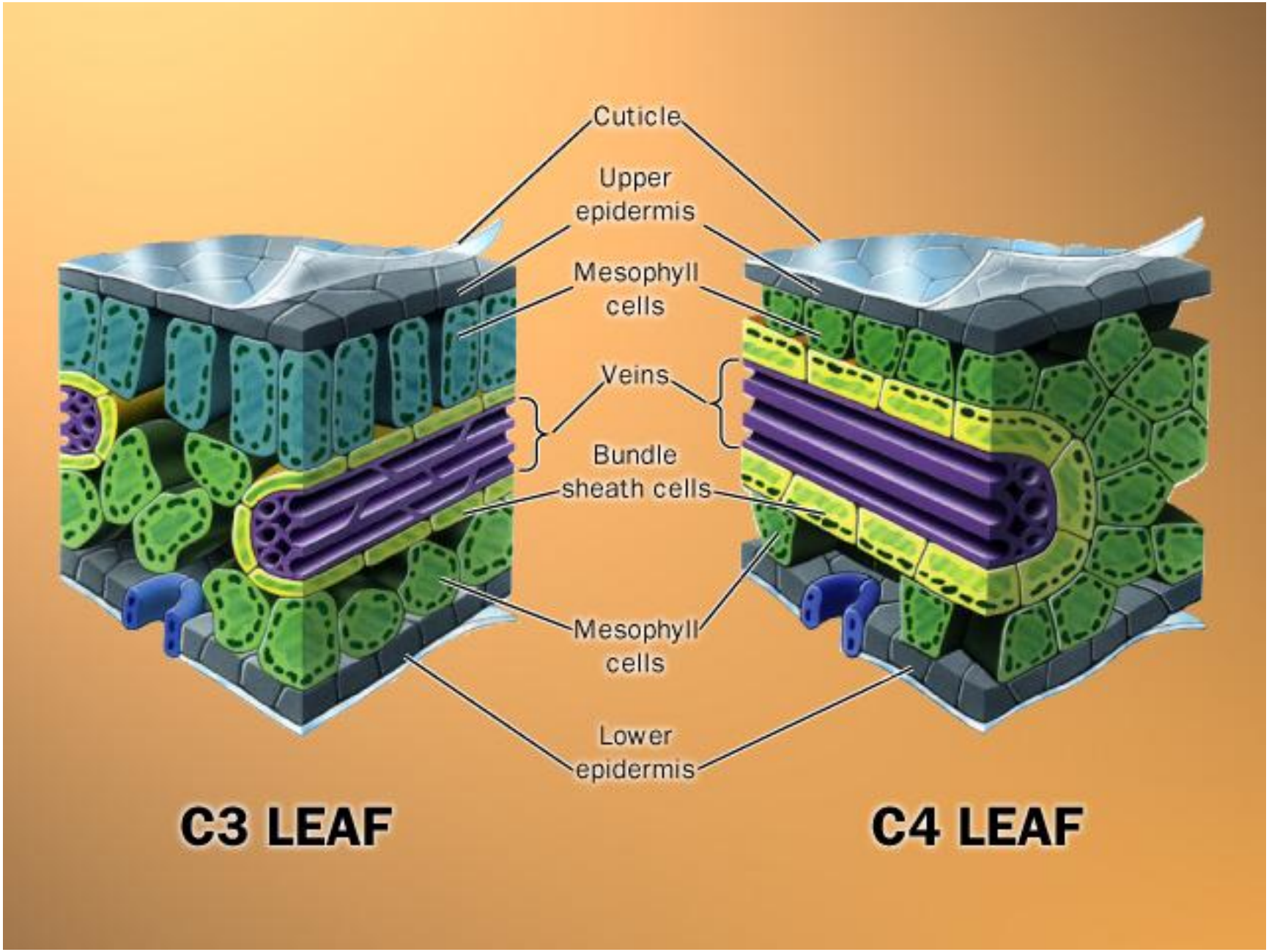
- if [CO_2 / O_2] is low

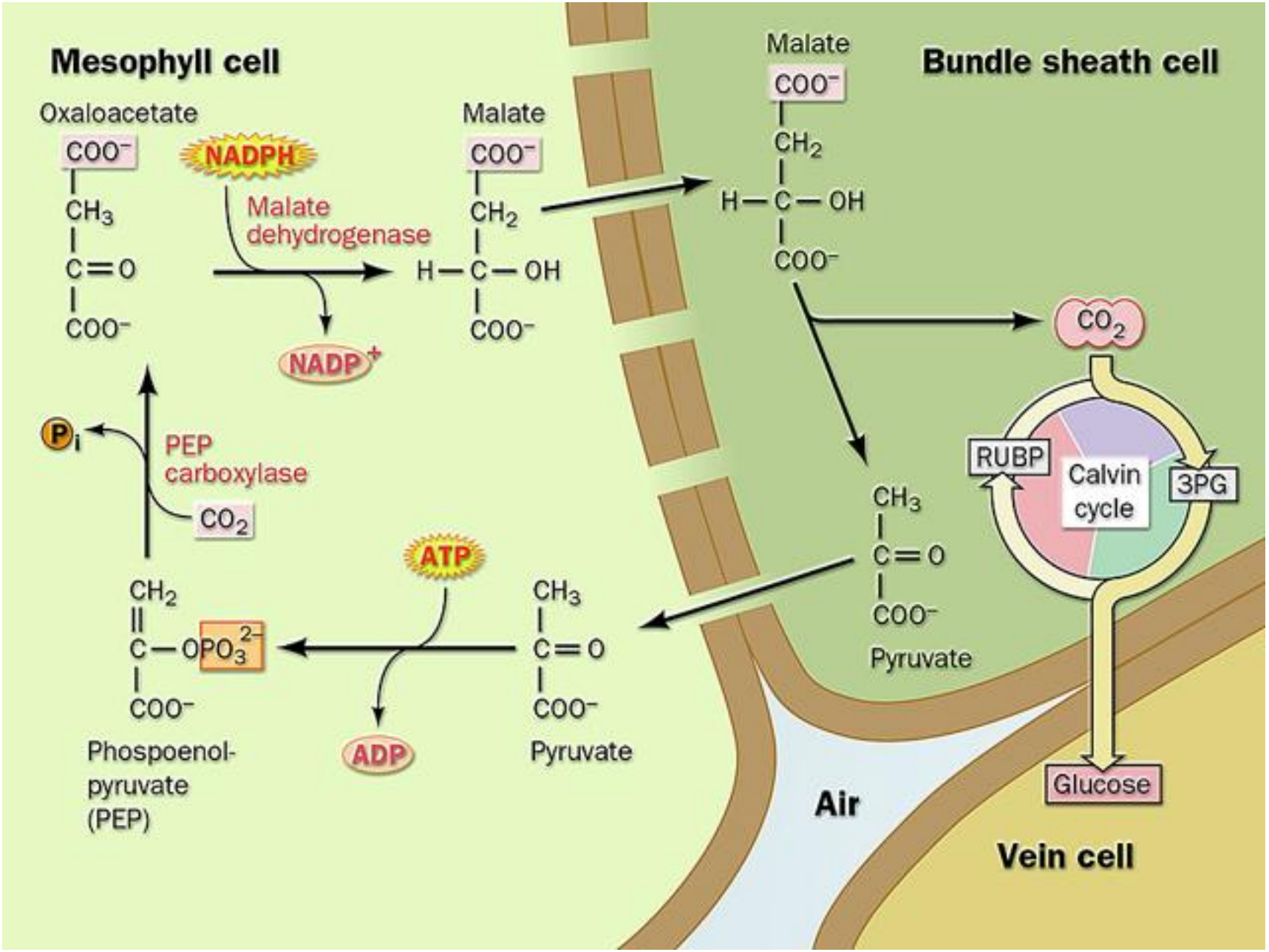
  favors oxygenase activity

The capture of carbon dioxide by PEP is mediated by the enzyme PEP carboxylase, which has a stronger affinity for carbon dioxide than does RuBP carboxylase When carbon dioxide levels decline below the threshold for RuBP carboxylase, RuBP is catalyzed with oxygen instead of carbon dioxide. The product of that reaction forms glycolic acid, a chemical that can be broken down by photorespiration, producing neither NADH nor ATP, in effect dismantling the Calvin Cycle. C-4 plants, which often grow close together, have had to adjust to decreased levels of carbon dioxide by artificially raising the carbon dioxide concentration in certain cells to prevent photorespiration. C-4 plants evolved in the tropics and are adapted to higher temperatures than are the C-3 plants found at higher latitudes. Common C-4 plants include crabgrass, corn, and sugar cane. Note that OAA and Malic Acid also have functions in other processes, thus the chemicals would have been present in all plants, leading scientists to hypothesize that C-4 mechanisms evolved several times independently in response to a similar environmental condition, a type of evolution known as convergent evolution.

- Oxygen is a poison. This may come as a surprise to some, but plants using the typical Calvin cycle method have difficulty fixing carbon dioxide at high temperatures such as might typically be found in the tropics. That's because oxygen competes with carbon dioxide for an active site on the enzyme responsible for bringing carbon dioxide into the Calvin cycle. Plants that use the normal Calvin cycle to fix carbon dioxide are called C-3 plants, C-3 after the three carbon molecule PGA in the Calvin cycle.
- Many plants, called C-4 plants, in warm areas use a different strategy to fix carbon dioxide. This strategy involves first fixing the carbon dioxide in specially arranged mesophyll cells using a separate cycle and then transferring the carbon dioxide to cells where the normal Calvin cycle happens.
- The function of this arrangement is to isolate the cells where the Calvin cycle takes place from the high concentration of oxygen that interfere with the enzyme involved in carbon fixation.

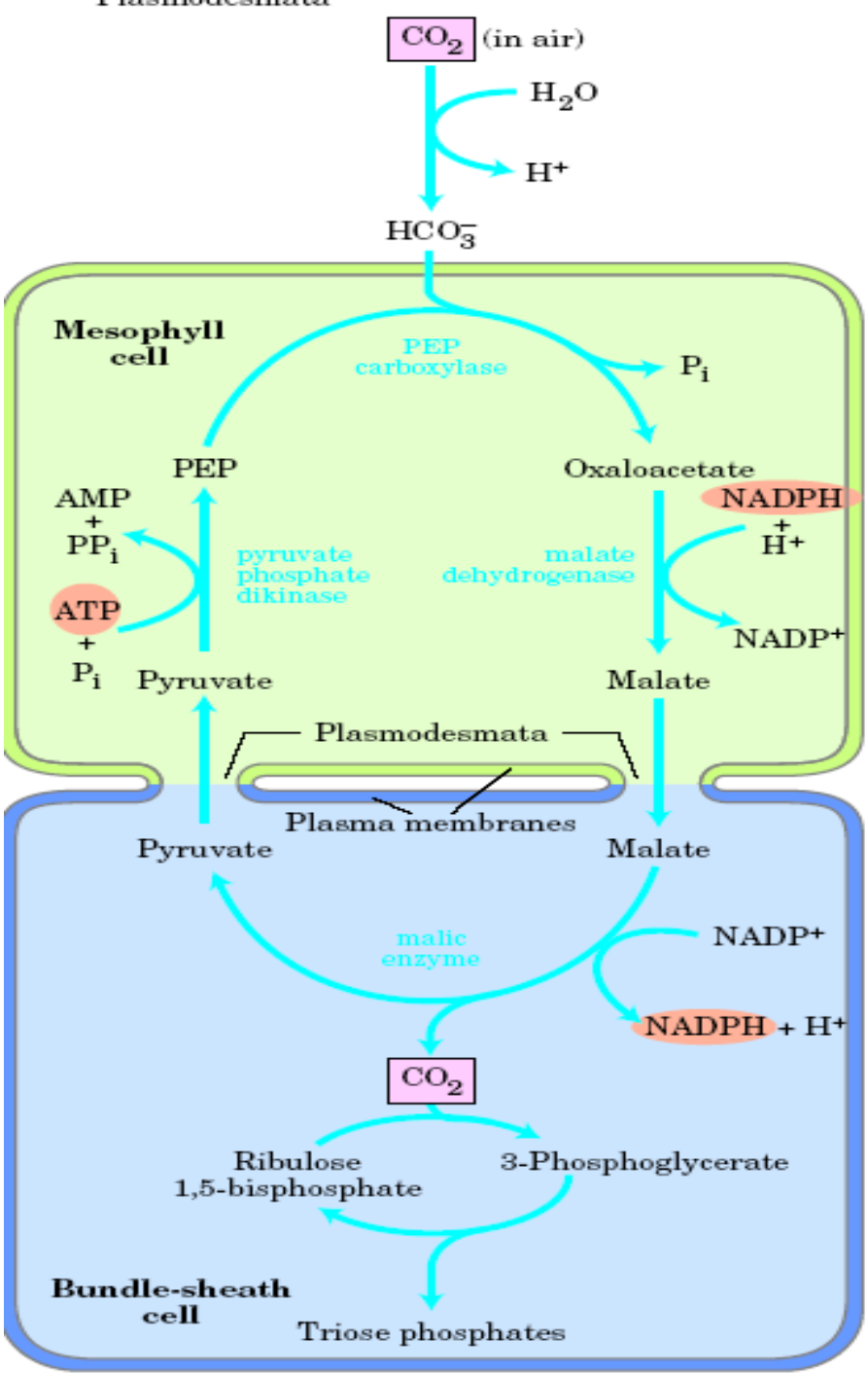

Mesophyll cell
Bundle sheath cell
Chloroplast
Oxaloacetate
PEP carboxylase
PEP
(phosphoenol
pyruvate)
Pyruvate
Vein cell
Chloroplast
ATP
ADP
CO_2
Starch
grain
RUBP
Calvin-
Benson
cycle
3PG
Glucose
CO_2
Air space


Cuticle
Upper epidermis
Mesophyll cells
Veins
Bundle sheath cells
Mesophyll cells
Lower epidermis
C3 LEAF
C4 LEAF


Mesophyll cell
Bundle sheath cell
Oxaloacetate
NADPH
Malate dehydrogenase
Malate
NADP+
Malate
CO2
RUBP
Calvin cycle
3PG
Pi
PEP carboxylase
CO2
ATP
Pyruvate
Phospoenol-pyruvate (PEP)
ADP
Pyruvate
Air
Glucose
Vein cell

Advantages C-3 vs C-4

- In warm climates C-4 plants have an advantage because during the day they can keep their stoma closed thus conserve water.
- The C-4 adaptation allows oxygen to build up to much higher levels without seriously affecting the rate of carbon fixation.
- In cooler climates, C-3 plants have the edge because it takes less energy for them to fix carbon dioxide.


Plasmodesmata
CO_2 (in air)
H_2O
H^+
HCO_3^-
Mesophyll cell
PEP carboxylase
P_i
PEP
Oxaloacetate
NADPH
+
H^+
AMP
+
PP_i
pyruvate phosphate dikinase
malate dehydrogenase
ATP
+
P_i
$NADP^+$
Pyruvate
Malate
Plasmodesmata
Plasma membranes
Pyruvate
Malate
malic enzyme
$NADP^+$
NADPH + H^+
CO_2
Ribulose 1,5-bisphosphate
3-Phosphoglycerate
Bundle-sheath cell
Triose phosphates

The C4 plants, which typically grow at high light intensity and high temperatures, have several important characteristics:

- high photosynthetic rates,
- high growth rates,
- low photorespiration rates,
- low rates of water loss,
- and a specialized leaf structure.

Photosynthesis in the leaves of C4 plants involves two cell types: mesophyll and bundle-sheath cells (Fig. 20–23a). There are three variants of C4 metabolism, worked out in the 1960s by Marshall Hatch and Rodger Slack (Fig. 20–23b).

In plants of tropical origin, the first intermediate into which $^{14}CO_2$ is fixed is oxaloacetate, a four-carbon compound. This reaction, which occurs in the cytosol of leaf mesophyll cells, is catalyzed by **phosphoenolpyruvate carboxylase,** for which the substrate is HCO_3 ,not CO_2. The oxaloacetate thus formed is either reduced to malate at the expense of NADPH) or converted to aspartate by transamination:

Oxaloacetate + α-amino acid ⟶ L-aspartate α-keto acid

The malate or aspartate formed in the mesophyll cells then passes into neighboring bundle-sheath cells through *plasmodesmata*, protein-lined channels that connect two plant cells and provide a path for movement of metabolites and even small proteins between cells.

In the bundle-sheath cells, malate is oxidized and decarboxylated to yield pyruvate and CO_2 by the action of **malic enzyme,** reducing $NADP^+$.

In plants that use aspartate as the CO_2 carrier, aspartate arriving in bundle-sheath cells is transaminated to form oxaloacetate and reduced to malate, then the CO_2 is released by malic enzyme or PEP carboxykinase.

The pyruvate formed by decarboxylation of malate in bundle-sheath cells is transferred back to the mesophyll cells, where it is converted to PEP by an unusual enzymatic reaction catalyzed by **pyruvate phosphate dikinase**

This enzyme is called a dikinase because two different molecules are simultaneously phosphorylated by one molecule of ATP:

pyruvate to PEP, and phosphate to pyrophosphate.

The pyrophosphate is subsequently hydrolyzed to phosphate, so two high-energy phosphate groups of ATP are used in regenerating PEP. The PEP is now ready to receive another molecule of CO_2 in the mesophyll cell.

The PEP carboxylase of mesophyll cells has a high affinity for HCO_3 (which is favored relative to CO_2 in aqueous solution and can fix CO_2 more efficiently than can rubisco).

Unlike rubisco, it does not use O_2 as an alternative substrate, so there is no competition between CO_2 and O_2. The PEP carboxylase reaction, then, serves to fix and concentrate CO_2 in the form of malate.

Release of CO_2 from malate in the bundle-sheath cells yields a sufficiently high local concentration of CO_2 for rubisco to function near its maximal rate, and for suppression of the enzyme's oxygenase activity.

Once CO_2 is fixed into 3-phosphoglycerate in the bundle-sheath cells, the other reactions of the Calvin cycle take place exactly as described earlier.

Thus in C4 plants, mesophyll cells carry out CO_2 assimilation by the C4 pathway and bundle-sheath cells synthesize starch and sucrose by the C3 pathway.

Three enzymes of the C4 pathway are regulated by light, becoming more active in daylight.

Malate dehydrogenase is activated by the thioredoxin-dependent reduction mechanism shown;

PEP carboxylase is activated by phosphorylation of a Ser residue;

and pyruvate phosphate dikinase is activated by dephosphorylation.

In the latter two cases, the details of how light effects phosphorylation or dephosphorylation are not known.

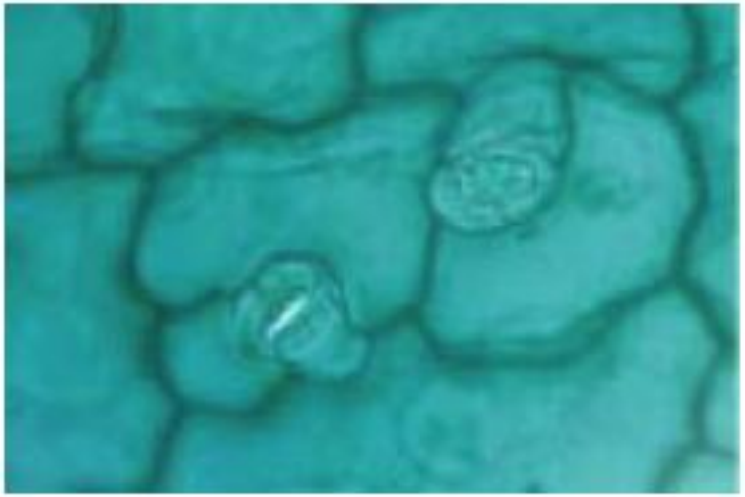
The pathway of CO2 assimilation has a greater energy cost in C4 plants than in C3 plants. For each molecule of CO_2 assimilated in the C4 pathway, a molecule of PEP must be regenerated at the expense of two highenergy phosphate groups of ATP. Thus C4 plants need five ATP molecules to assimilate one molecule of CO_2,

whereas C3 plants need only three (nine per triose phosphate). As the temperature increases (and the affinity of rubisco for CO_2 decreases, as noted above), a point is reached (at about 28 to 30 C) at which the gain in efficiency from the elimination of photorespiration

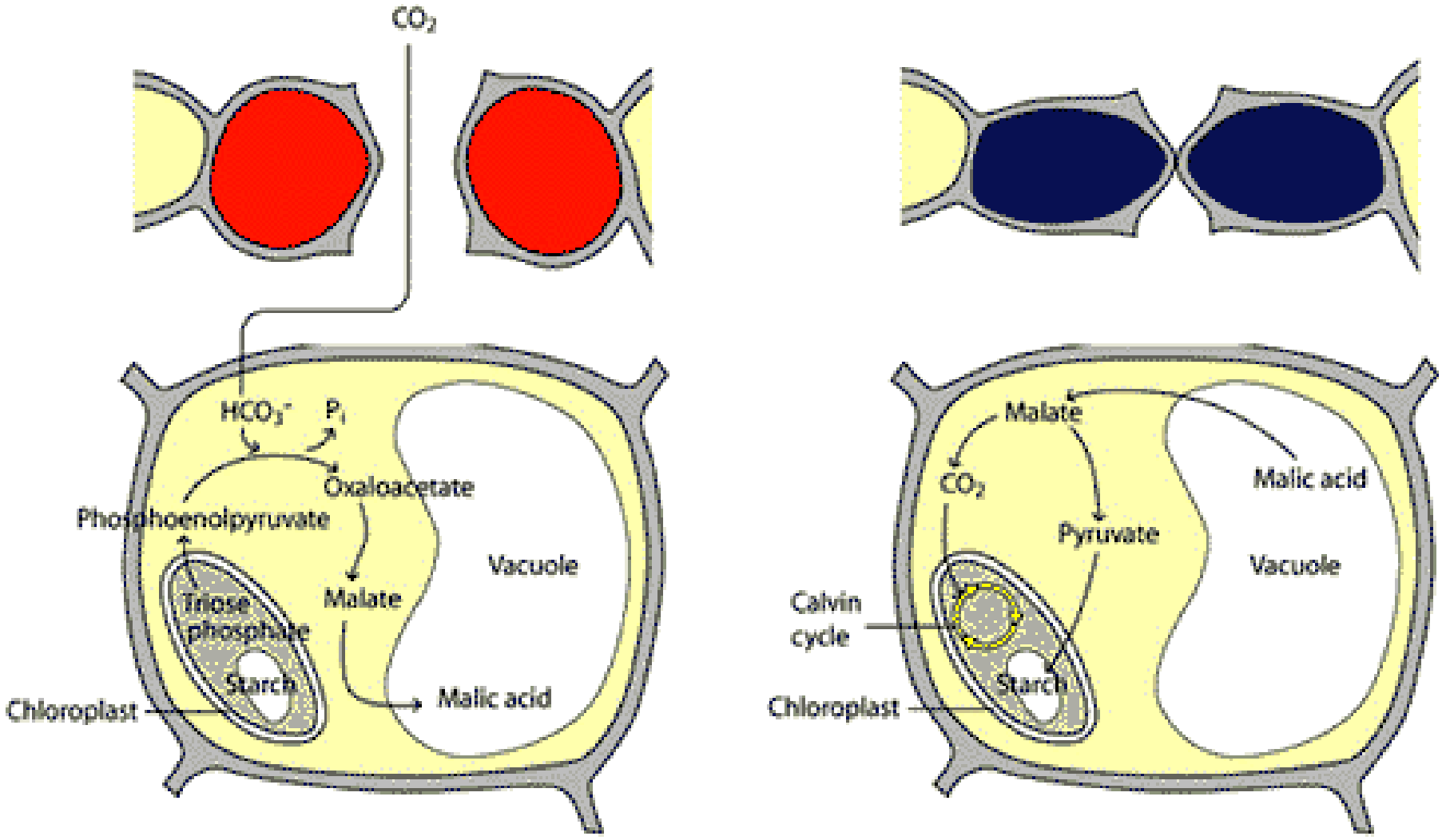
more than compensates for this energetic cost. C4 plants (crabgrass, for example) outgrow most C3 plants during the summer.

Crassulacean Acid Metabolism
Permits Growth in Arid Ecosystems

- *Crassulacean acid metabolism (CAM)* is yet another adaptation to increase the efficiency of the Calvin cycle.
- Crassulacean acid metabolism, named after the genus *Crassulacea* (the succulents), is a response to drought as well as warm conditions.
- In CAM plants, the stomata of the leaves are closed in the heat of the day to prevent water loss . As a consequence, CO_2 cannot be absorbed during the daylight hours when it is needed for glucose synthesis.
- When the stomata open at the cooler temperatures of night, CO_2 is fixed by the C4 pathway into malate, which is stored in vacuoles.
- During the day, malate is decarboxylated and the CO_2 becomes available to the Calvin cycle. In contrast
- with C4 plants, CO_2 accumulation is separated from CO_2 utilization temporally in CAM plants rather than spatially.



Crassulacean Acid Metabolism (CAM)



A Night: stomata open

B Day: stomata closed

Photosynthetic Carbohydrate Synthesis

- The synthesis of carbohydrates in animal cells always employs precursors having at least three carbons, all of which are less oxidized than the carbon in CO_2.
- Plants and photosynthetic microorganisms, by contrast, can synthesize carbohydrates from CO_2 and water, reducing CO_2 at the expense of the energy and reducing power furnished by the ATP and NADPH that are generated by the light-dependent reactions of photosynthesis
- Plants (and other autotrophs) can use CO_2 as the sole source of the carbon atoms required for the biosynthesis of cellulose and starch, lipids and proteins, and the many other organic components of plant cells.
- By contrast, heterotrophs cannot bring about the net reduction
- of CO_2 to achieve a net synthesis of glucose.

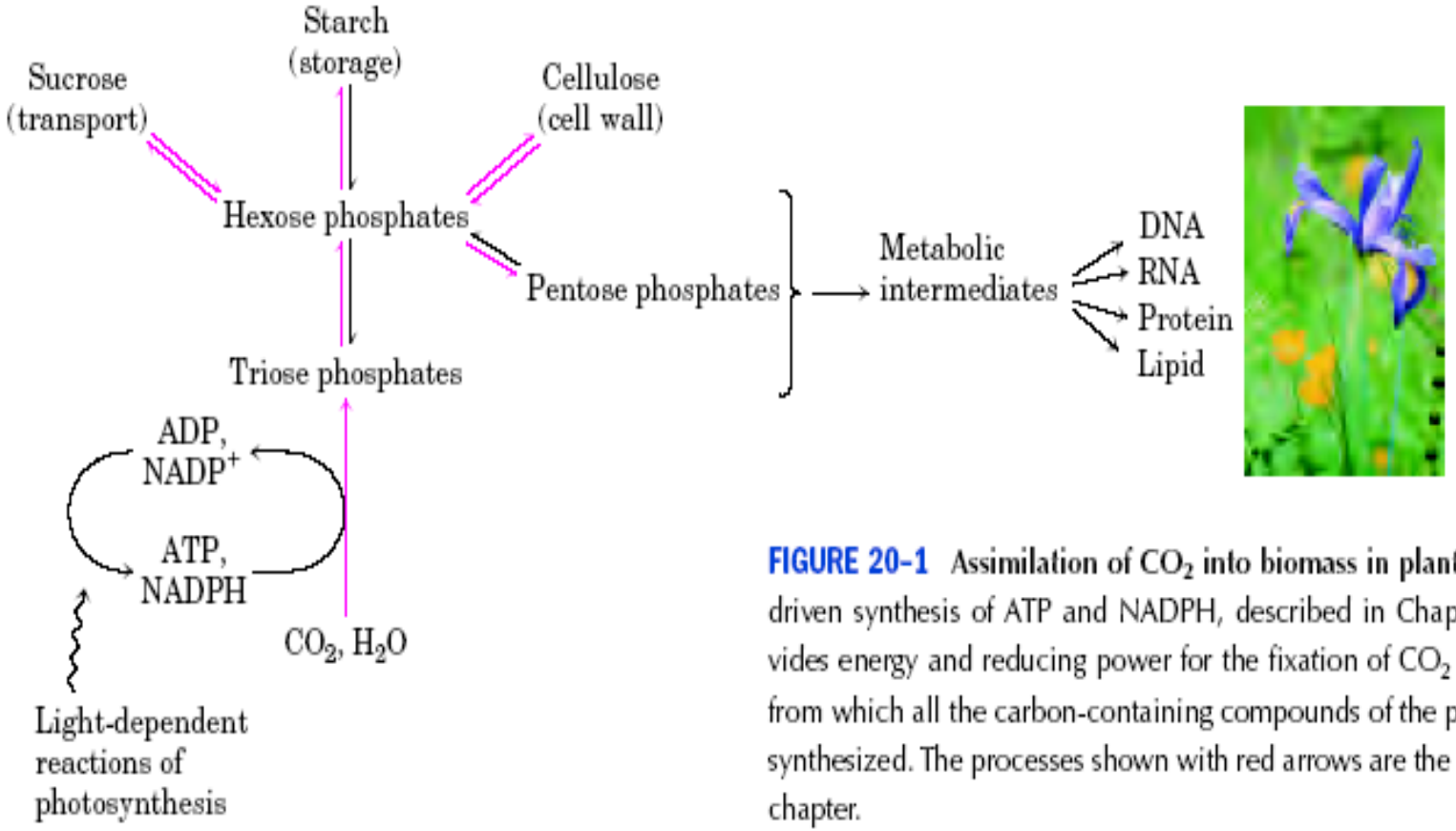
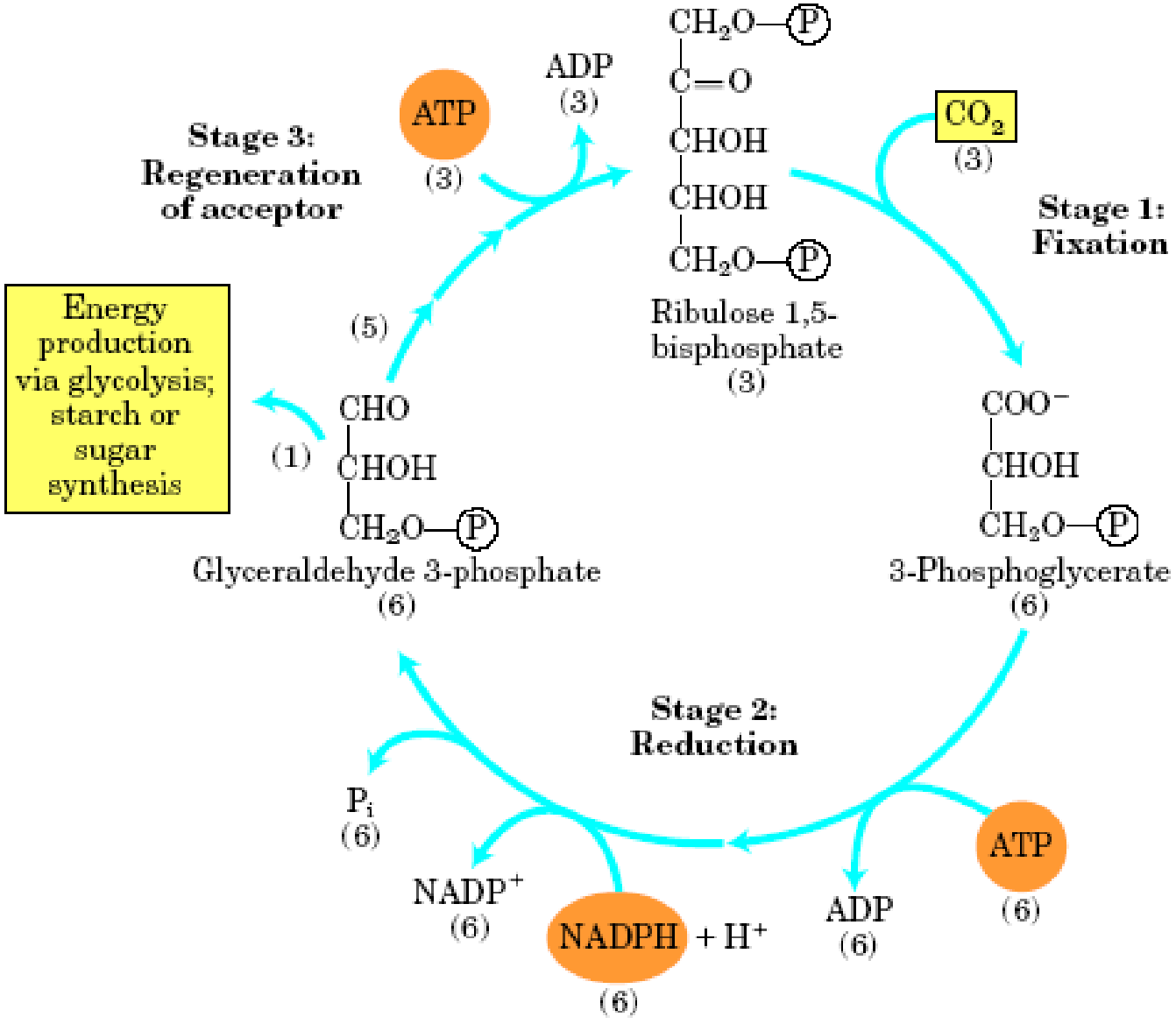
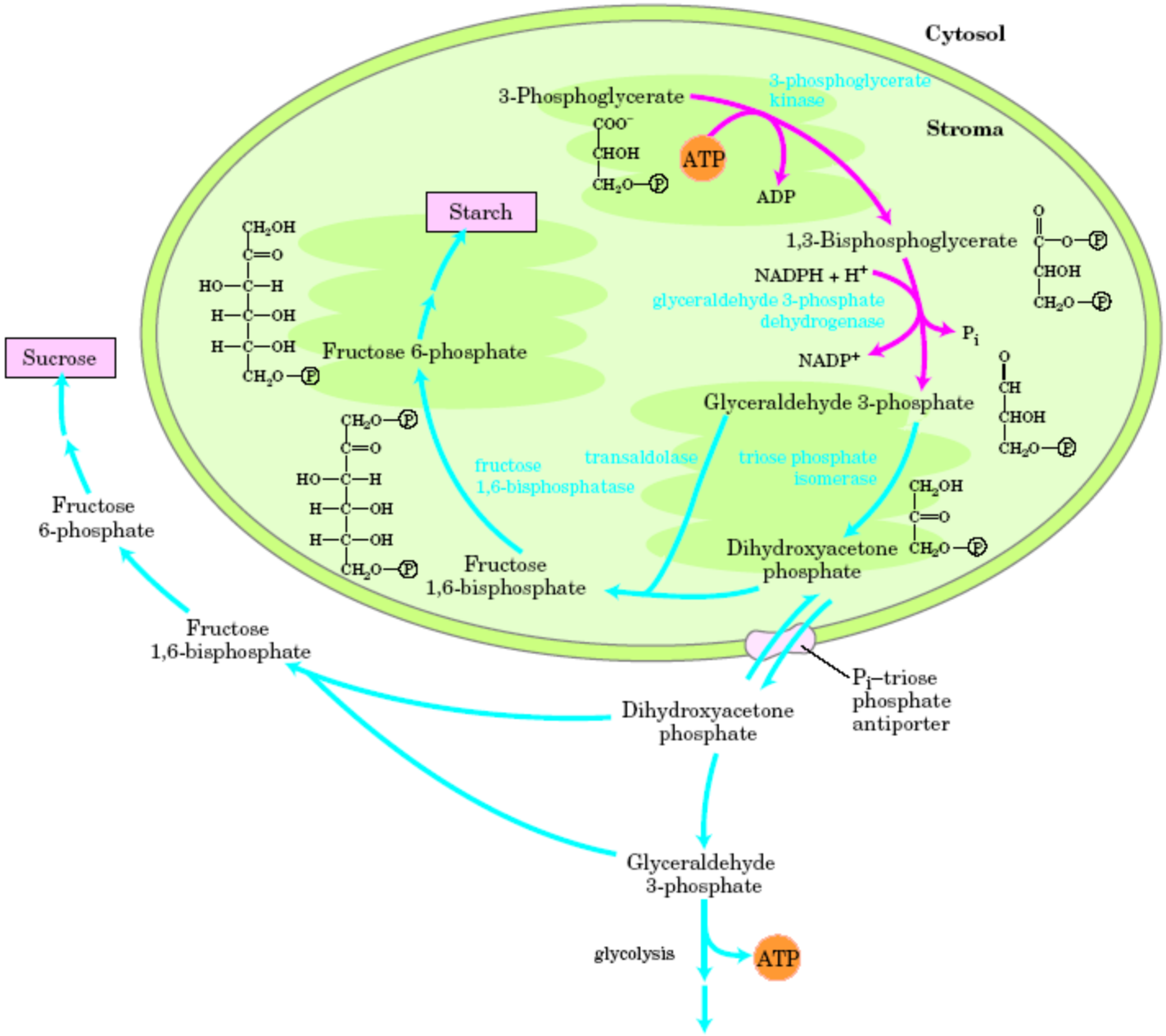


FIGURE 20–1 Assimilation of CO_2 into biomass in plants. The light-driven synthesis of ATP and NADPH, described in Chapter 19, provides energy and reducing power for the fixation of CO_2 into trioses, from which all the carbon-containing compounds of the plant cell are synthesized. The processes shown with red arrows are the focus of this chapter.


Stage 3:
Regeneration
of acceptor
ATP
(3)
ADP
(3)
CH2O—P
C=O
CHOH
CHOH
CH2O—P
Ribulose 1,5-
bisphosphate
(3)
CO2
(3)
Stage 1:
Fixation
Energy
production
via glycolysis;
starch or
sugar
synthesis
(5)
(1)
CHO
CHOH
CH2O—P
Glyceraldehyde 3-phosphate
(6)
COO−
CHOH
CH2O—P
3-Phosphoglycerate
(6)
Stage 2:
Reduction
Pi
(6)
NADP+
(6)
NADPH + H+
(6)
ADP
(6)
ATP
(6)


Cytosol
Stroma
3-Phosphoglycerate
3-phosphoglycerate kinase
ATP
ADP
1,3-Bisphosphoglycerate
NADPH + H+
glyceraldehyde 3-phosphate dehydrogenase
Pi
NADP+
Glyceraldehyde 3-phosphate
transaldolase
triose phosphate isomerase
Dihydroxyacetone phosphate
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
Fructose 6-phosphate
Starch
Sucrose
Fructose 6-phosphate
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
Pi–triose phosphate antiporter
Glyceraldehyde 3-phosphate
glycolysis
ATP

A Transport System Exports Triose Phosphates from the Chloroplast and Imports Phosphate

- The inner chloroplast membrane is impermeable to most phosphorylated compounds, including fructose 6-phosphate, glucose 6-phosphate, and fructose 1,6-bisphosphate.
- It does, however, have a specific antiporter that catalyzes the one-for-one exchange of Pi with a triose phosphate, either dihydroxyacetone phosphate or 3-phosphoglycerate.
- This antiporter simultaneously moves Pi into the chloroplast, where it is used in photophosphorylation, and moves triose phosphate into the cytosol, where it can be used to synthesize sucrose, the form in which the fixed carbon is transported to distant plant tissues.

- Sucrose synthesis in the cytosol and starch synthesis in the chloroplast are the major pathways by which the excess triose phosphate from photosynthesis is “harvested.”
- Sucrose synthesis releases four Pi molecules from the four triose phosphates required to make sucrose.
- For every molecule of triose phosphate removed from the chloroplast, one Pi is transported into the chloroplast, providing the ninth Pi mentioned above, to be used in regenerating ATP.
- If this exchange were blocked, triose phosphate synthesis would quickly deplete the available Pi in the chloroplast, slowing ATP synthesis and suppressing assimilation of CO_2 into starch.

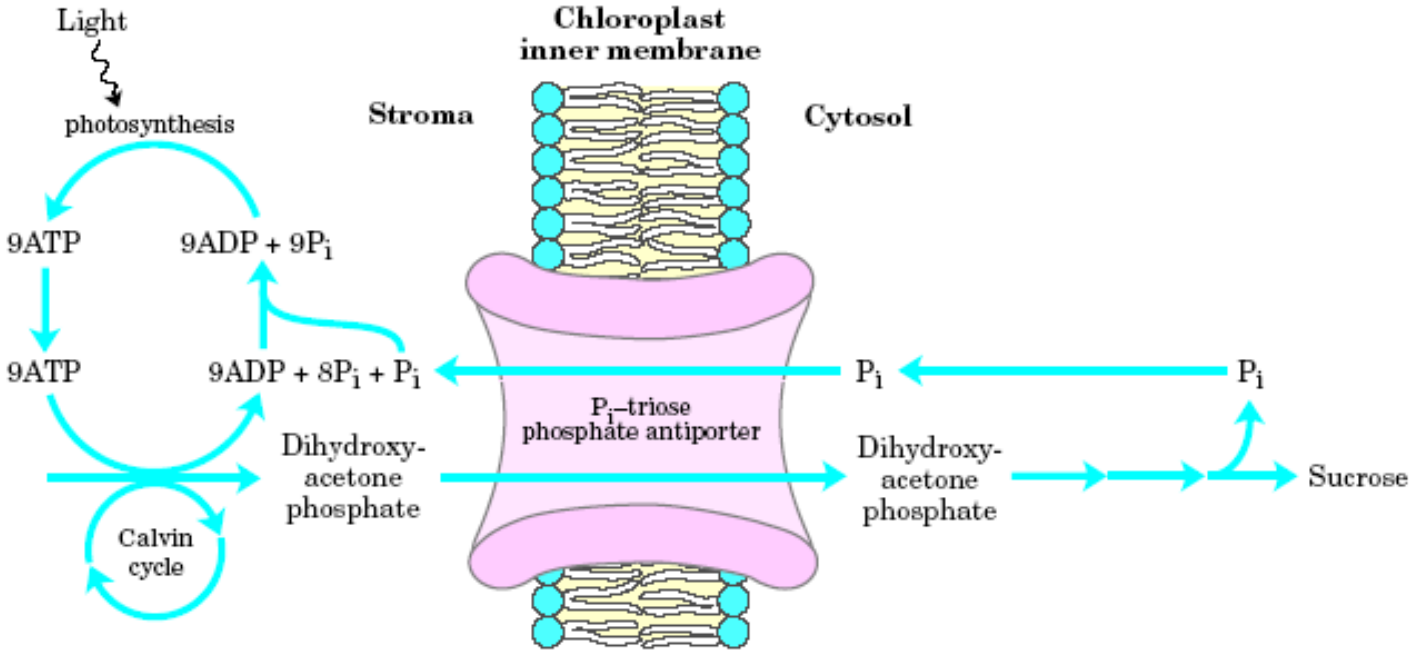
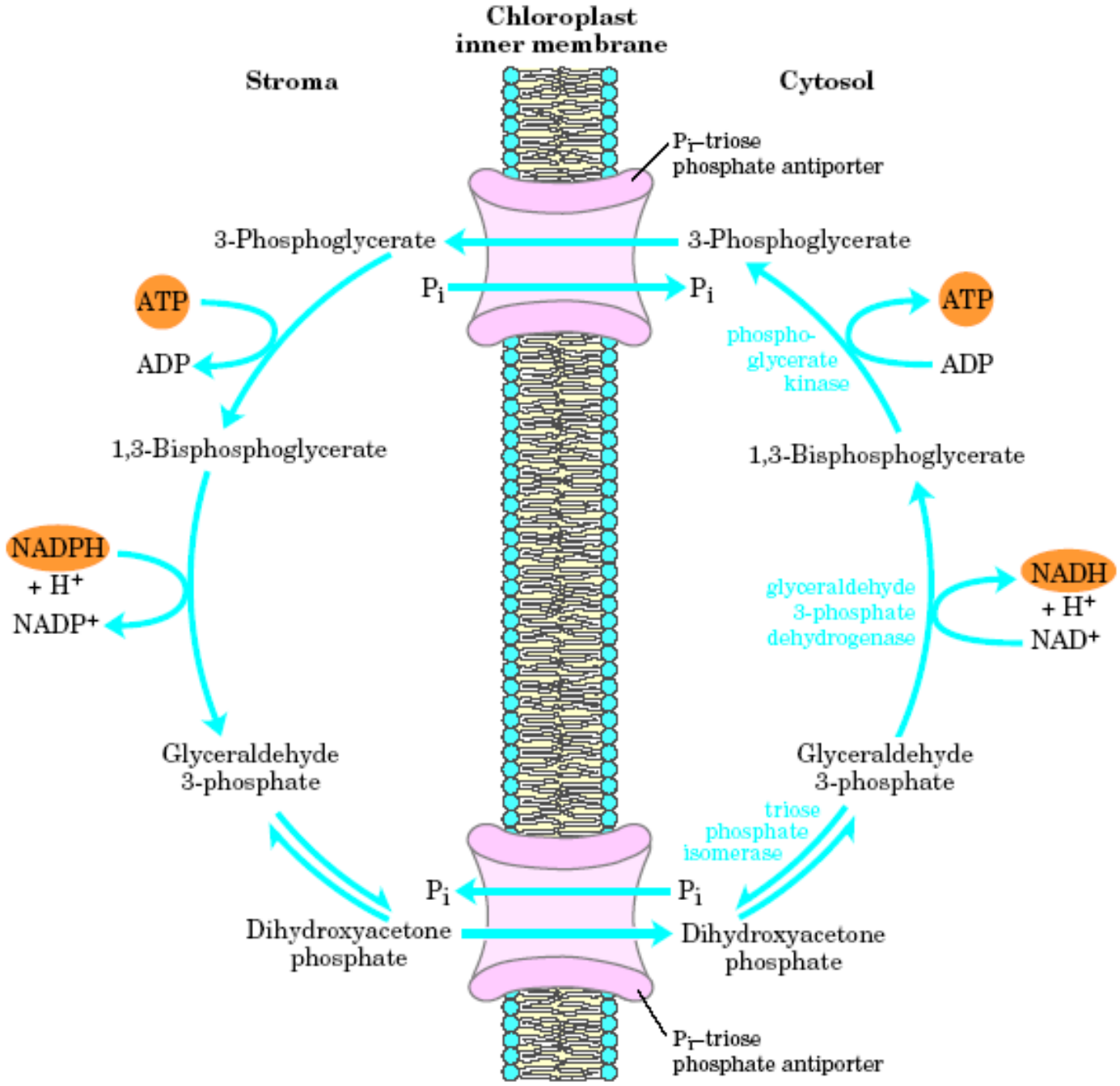


FIGURE 20–15 The P_i–triose phosphate antiport system of the inner chloroplast membrane. This transporter facilitates the exchange of cytosolic P_i for stromal dihydroxyacetone phosphate. The products of photosynthetic carbon assimilation are thus moved into the cytosol where they serve as a starting point for sucrose biosynthesis, and P_i required for photophosphorylation is moved into the stroma. This same antiporter can transport 3-phosphoglycerate and acts in the shuttle for exporting ATP and reducing equivalents (see Fig. 20–16).

- The Pi–triose phosphate antiport system serves one additional function. ATP and reducing power are needed in the cytosol for a variety of synthetic and energy requiring reactions.
- These requirements are met to an as yet undetermined degree by mitochondria, but a second potential source of energy is the ATP and NADPH generated in the chloroplast stroma during the light reactions. However, neither ATP nor NADPH can cross the chloroplast membrane.
- The Pi–triose phosphate antiport system has the indirect effect of moving ATP equivalents and reducing equivalents from the chloroplast to the cytosol.
- Dihydroxyacetone phosphate formed in the stroma is transported to the cytosol, where it is converted by glycolytic enzymes to 3-phosphoglycerate, generating ATP and NADH. 3- Phosphoglycerate reenters the chloroplast, completing the cycle.


Chloroplast
inner membrane
Stroma
Cytosol
Pi–triose
phosphate antiporter
3-Phosphoglycerate
3-Phosphoglycerate
Pi
Pi
ATP
ADP
phospho-
glycerate
kinase
ATP
ADP
1,3-Bisphosphoglycerate
1,3-Bisphosphoglycerate
NADPH
+ H+
NADP+
glyceraldehyde
3-phosphate
dehydrogenase
NADH
+ H+
NAD+
Glyceraldehyde
3-phosphate
Glyceraldehyde
3-phosphate
triose
phosphate
isomerase
Pi
Pi
Dihydroxyacetone
phosphate
Dihydroxyacetone
phosphate
Pi–triose
phosphate antiporter

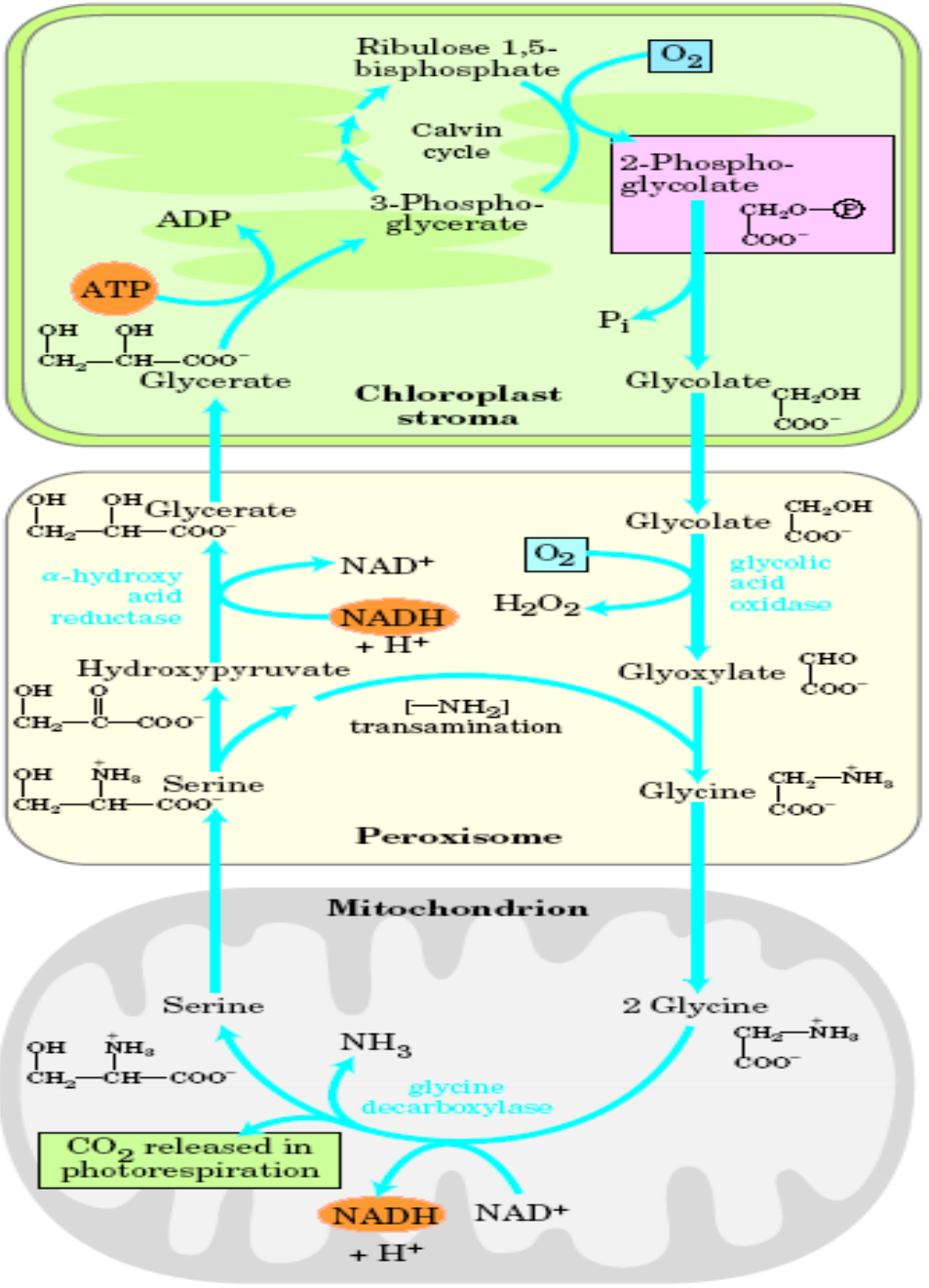


FIGURE 20-21 Glycolate pathway. This pathway, which salvages 2-phosphoglycolate (shaded pink) by its conversion to serine and eventually 3-phosphoglycerate, involves three cellular compartments. Glycolate formed by dephosphorylation of 2-phosphoglycolate in chloroplasts is oxidized to glyoxylate in peroxisomes and then transaminated to glycine. In mitochondria, two glycine molecules condense to form serine and the CO_2 released during photorespiration (shaded green). This reaction is catalyzed by glycine decarboxylase, an enzyme present at very high levels in the mitochondria of C_3 plants (see text). The serine is converted to hydroxypyruvate and then to glycerate in peroxisomes; glycerate reenters the chloroplasts to be phosphorylated, rejoining the Calvin cycle. Oxygen (shaded blue) is consumed at two steps during photorespiration.

Salvage of Phosphoglycolate Is Costly

- The **glycolate pathway** converts two molecules of 2-phosphoglycolate to a molecule of serine (three carbons) and a molecule of CO2 (Fig. 20–21).
- In the chloroplast, a phosphatase converts 2-phosphoglycolate to glycolate, which is exported to the peroxisome. There, glycolate is oxidized by molecular oxygen, and the resulting aldehyde (glyoxylate) undergoes transamination to glycine.
- The hydrogen peroxide formed as a side product of glycolate oxidation is rendered harmless by peroxidases in the peroxisome.
- Glycine passes from the peroxisome to the mitochondrial matrix, where it undergoes oxidative decarboxylation by the glycine decarboxylase complex, an enzyme similar in structure and mechanism to two mitochondrial complexes we have already encountered: the pyruvate dehydrogenase complex and the –ketoglutarate dehydrogenase complex (Chapter 16).
- The **glycine decarboxylase complex** oxidizes glycine to CO2 and NH3, with the concomitant reduction of NAD to NADH and transfer of the remaining carbon from glycine to the cofactor tetrahydrofolate (Fig. 20–22).
- The one-carbon unit carried on tetrahydrofolate is then transferred to a second glycine by serine hydroxymethyltransferase, producing serine. The net reaction catalyzed by the glycine decarboxylase complex and serine hydroxymethyltransferase is

$$2\ \text{Glycine} + \text{NAD}^+ + \text{H}_2\text{O} \longrightarrow \text{serine} + \text{CO}_2 + \text{NH}_3 + \text{NADH} + \text{H}^+$$

- The **glycine decarboxylase complex** oxidizes glycine to CO2 and NH3, with the concomitant reduction of NAD to NADH and transfer of the remaining carbon from glycine to the cofactor tetrahydrofolate.
- The one-carbon unit carried on tetrahydrofolate is then transferred to a second glycine by serine hydroxymethyltransferase, producing serine. The net reaction catalyzed by the glycine decarboxylase complex and serine hydroxymethyltransferase is

- The serine is converted to hydroxypyruvate, to glycerate, and finally to 3-phosphoglycerate, which is used to regenerate ribulose 1,5-bisphosphate, completing the long, expensive cycle .
- In bright sunlight, the flux through the glycolate salvage pathway can be very high, producing about five times more CO_2 than is typically produced by all the oxidations of the citric acid cycle.
- To generate this large flux, mitochondria contain prodigious amounts of the glycine decarboxylase complex: the four proteins of the complex make up *half* of all the protein in the mitochondrial matrix in the leaves of pea and spinach plants!
- In nonphotosynthetic parts of a plant, such as potato tubers, mitochondria have very low concentrations of the glycine decarboxylase complex.

- The combined activity of the rubisco oxygenase and the glycolate salvage pathway consumes O2 and produces CO2—hence the name **photorespiration.**
- This pathway is perhaps better called the **oxidative photosynthetic carbon cycle** or **C2 cycle**, names that do not invite comparison with respiration in mitochondria.
- Unlike mitochondrial respiration, “photorespiration” does not conserve energy and may actually inhibit net biomass formation as much as 50%.
- This inefficiency has led to evolutionary adaptations in the carbon-assimilation processes, particularly in plants that have evolved in warm climates.

Biosynthesis of Starch and Sucrose

- During active photosynthesis in bright light, a plant leaf produces more carbohydrate (as triose phosphates) than it needs for generating energy or synthesizing precursors.

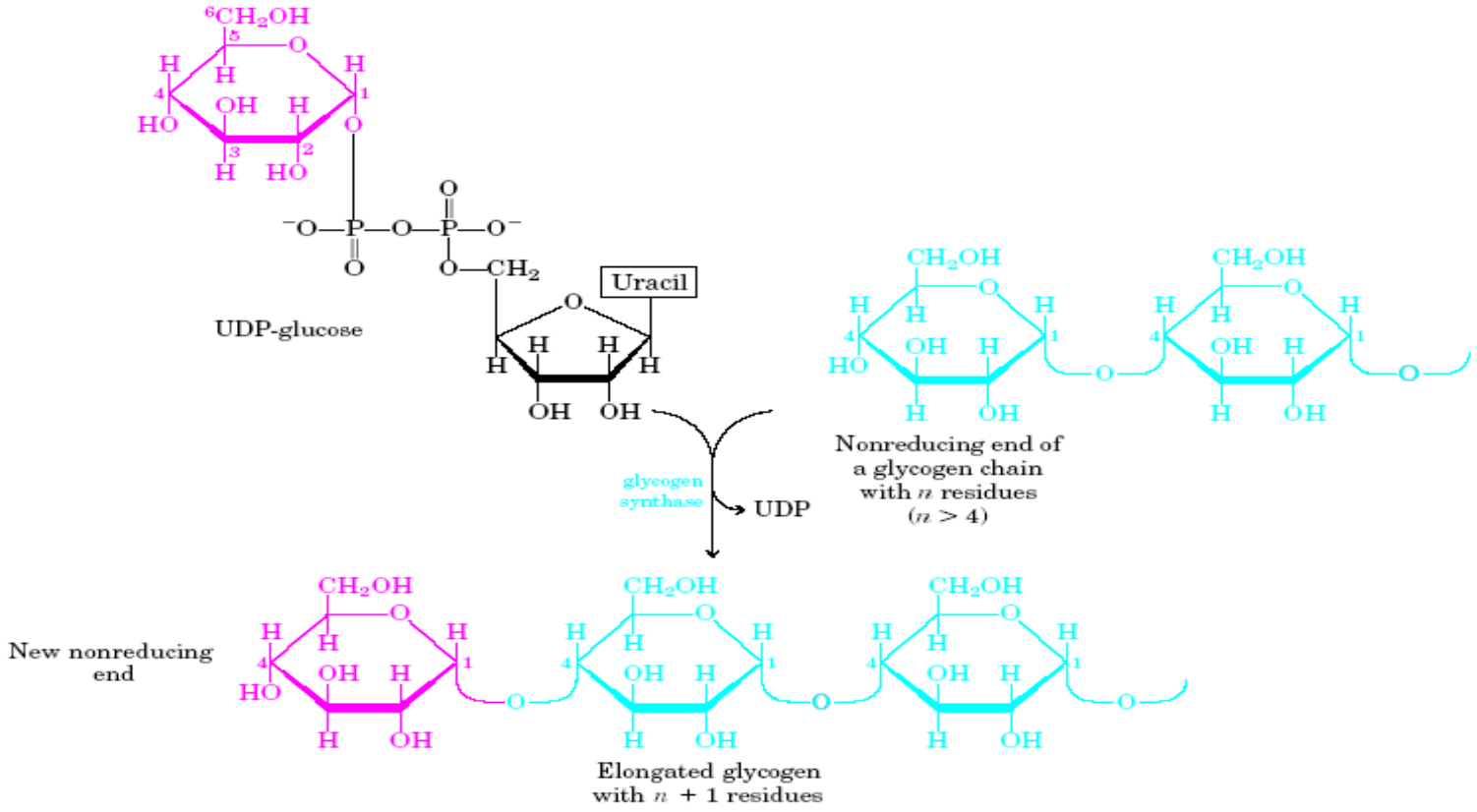
- The excess is converted to sucrose and transported to other parts of the plant, to be used as fuel or stored. In most plants, starch is the main storage form, but in a few plants, such as sugar beet and sugarcane, sucrose is the primary storage form.

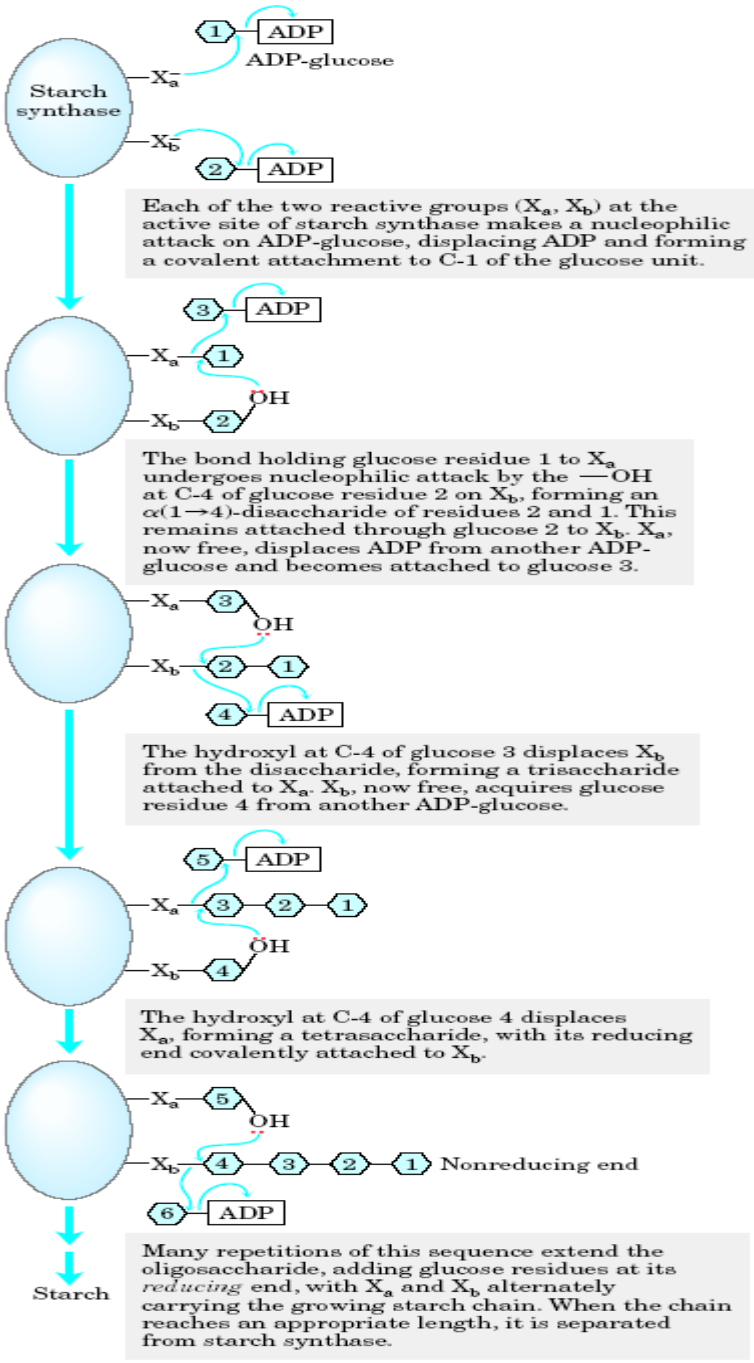
- The synthesis of sucrose and starch occurs in different cellular compartments (cytosol and plastids, respectively), and these processes are coordinated by a variety of regulatory mechanisms that respond to changes in light level and photosynthetic rate.

ADP-Glucose Is the Substrate for Starch Synthesis in Plant Plastids and for Glycogen Synthesis in Bacteria

- Starch, like glycogen, is a high molecular weight polymer of D-glucose in (α1 → 4) linkage. It is synthesized in chloroplasts for temporary storage as one of the stable end products of photosynthesis, and for long-term storage it is synthesized in the amyloplasts of the nonphotosynthetic parts of plants—seeds, roots, and tubers (underground stems).

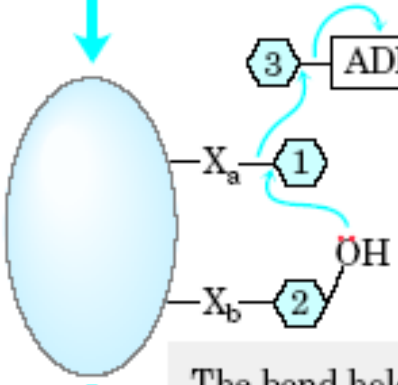
- The mechanism of glucose activation in starch synthesis is similar to that in glycogen synthesis.
- An activated **nucleotide sugar,** in this case **ADP-glucose,** is formed by condensation of glucose 1-phosphate with ATP in a reaction made essentially irreversible by the presence in plastids of inorganic pyrophosphatase
- **Starch synthase** then transfers glucose residues from ADP-glucose to preexisting starch molecules. Although it has generally been assumed that glucose is added to the *nonreducing* end of starch, as in glycogen synthesis



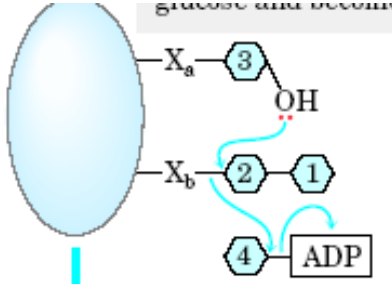


Although it has generally been assumed that glucose is added to the *nonreducing* end of starch, as in glycogen synthesis
, evidence now suggests that starch synthase has two equivalent active sites that alternate in inserting a glucosyl residue onto the *reducing* end of the
growing chain.
This end remains covalently attached to the enzyme, first at one active site, then at the other . Attachment to one active site effectively activates the reducing end of the growing chain for nucleophilic displacement of the enzyme by the attacking C-4 hydroxyl of a glucosyl moiety bound to the other active site, forming the ($\alpha1\rightarrow4$) linkage characteristic of starch.

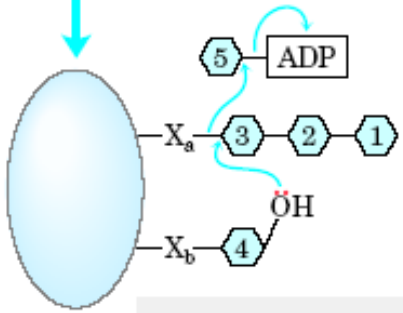
Each of the two reactive groups (X_a, X_b) at the active site of starch synthase makes a nucleophilic attack on ADP-glucose, displacing ADP and forming a covalent attachment to C-1 of the glucose unit.



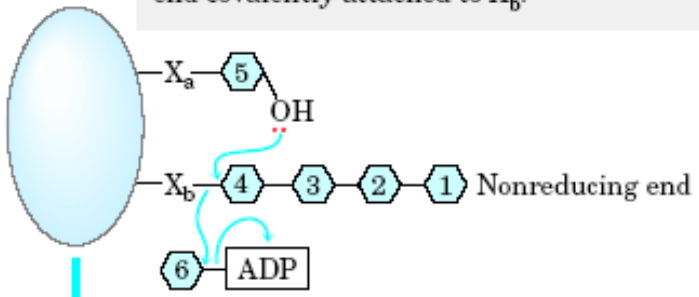
The bond holding glucose residue 1 to X_a undergoes nucleophilic attack by the —OH at C-4 of glucose residue 2 on X_b, forming an $\alpha(1\rightarrow4)$-disaccharide of residues 2 and 1. This remains attached through glucose 2 to X_b. X_a, now free, displaces ADP from another ADP-glucose and becomes attached to glucose 3.



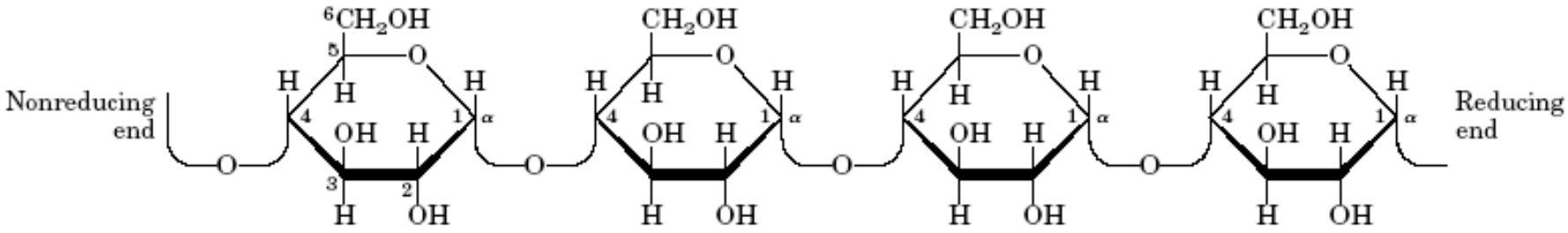
The hydroxyl at C-4 of glucose 3 displaces X_b from the disaccharide, forming a trisaccharide attached to X_a. X_b, now free, acquires glucose residue 4 from another ADP-glucose.



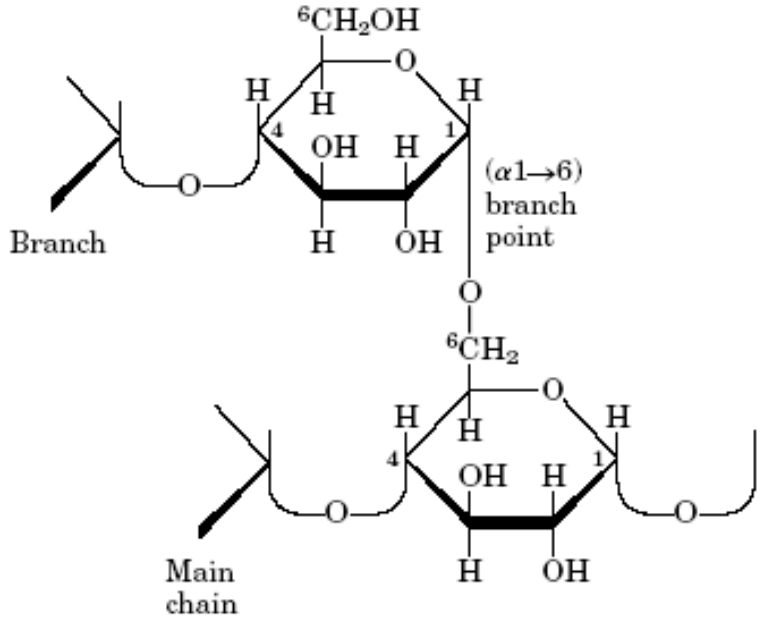
The hydroxyl at C-4 of glucose 4 displaces X_a, forming a tetrasaccharide, with its reducing end covalently attached to X_b.



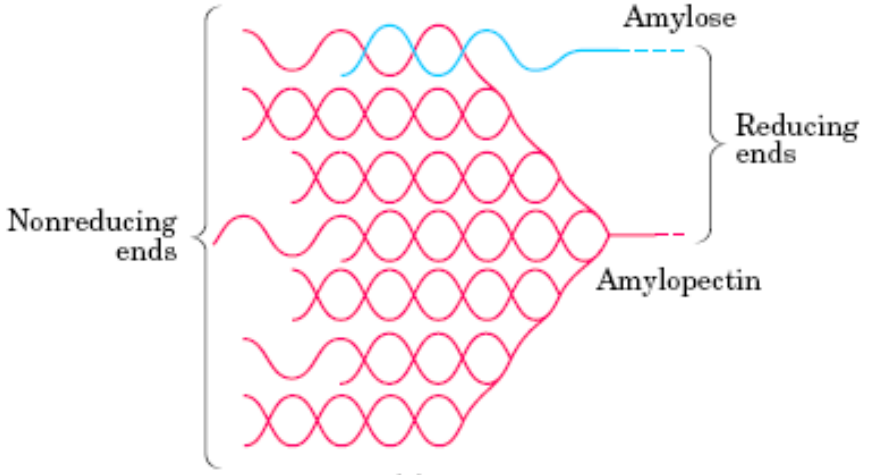
Many repetitions of this sequence extend the oligosaccharide, adding glucose residues at its *reducing* end, with X_a and X_b alternately carrying the growing starch chain. When the chain reaches an appropriate length, it is separated from starch synthase.


Nonreducing end
Reducing end

(a) amylose


Branch
(α1→6) branch point
Main chain

(b)


Amylose
Reducing ends
Nonreducing ends
Amylopectin

(c)

- The amylose of starch is unbranched, but amylopectin has numerous ($\alpha 1 \rightarrow 6$)-linked branches
- Chloroplasts contain a branching enzyme,similar to glycogen-branching enzyme, that introduces the ($\alpha 1 \rightarrow 6$) branches of amylopectin.
- Taking into account the hydrolysis by inorganic pyrophosphatase of the PPi produced during ADP-glucose
- synthesis, the overall reaction for starch formation from glucose 1-phosphate is
- Starch synthesis is regulated at the level of ADP-glucose formation.

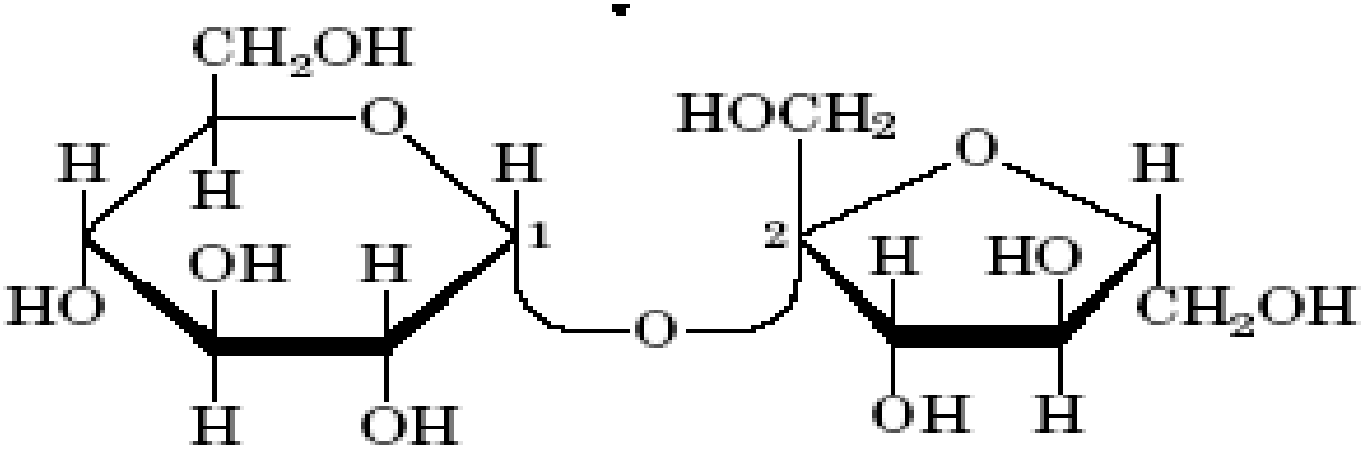
$$\text{Starch}_n + \text{glucose 1-phosphate} + \text{ATP} \longrightarrow \text{starch}_{n+1} + \text{ADP} + 2\text{P}_\text{i}$$

$$\Delta G'^\circ = -50 \text{ kJ/mol}$$

Sucrose Synthesis

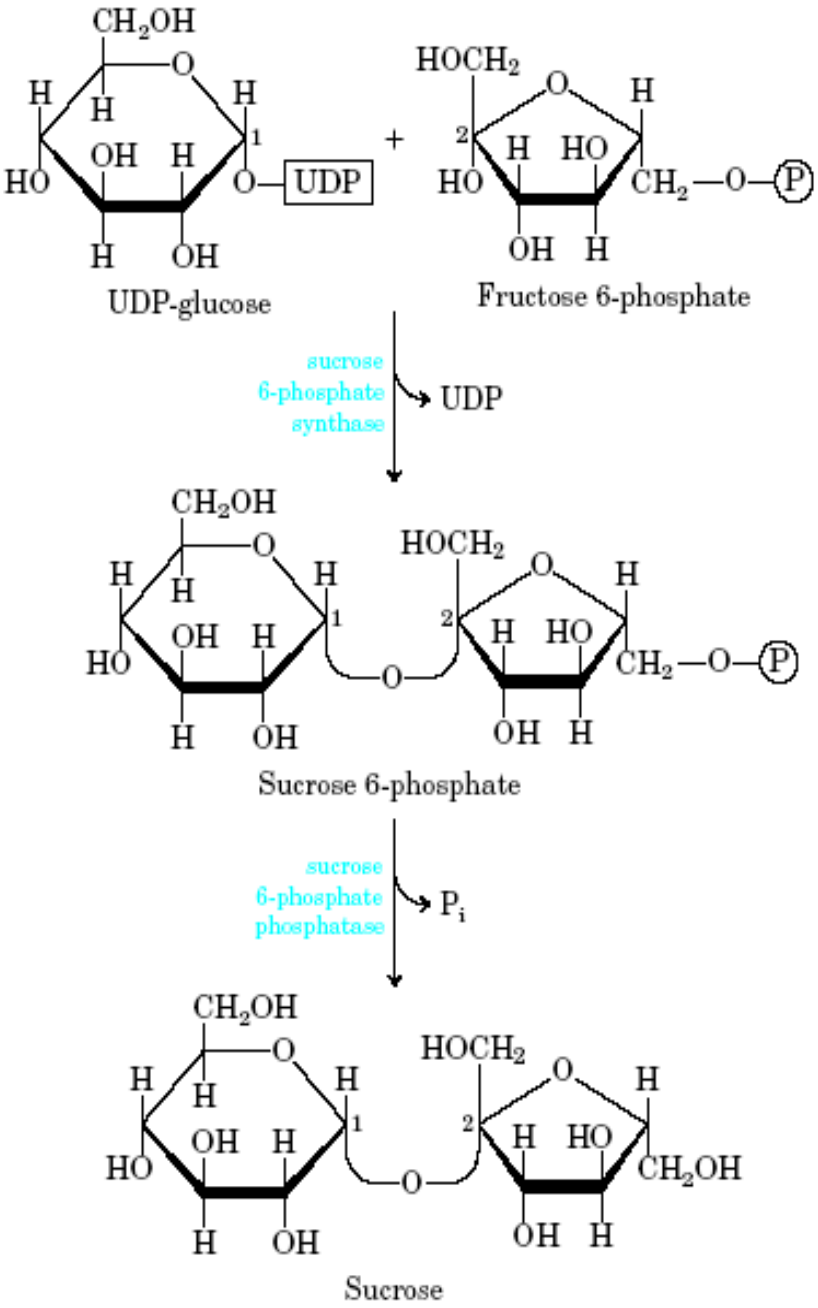
UDP-Glucose Is the Substrate for Sucrose Synthesis in the Cytosol of Leaf Cells

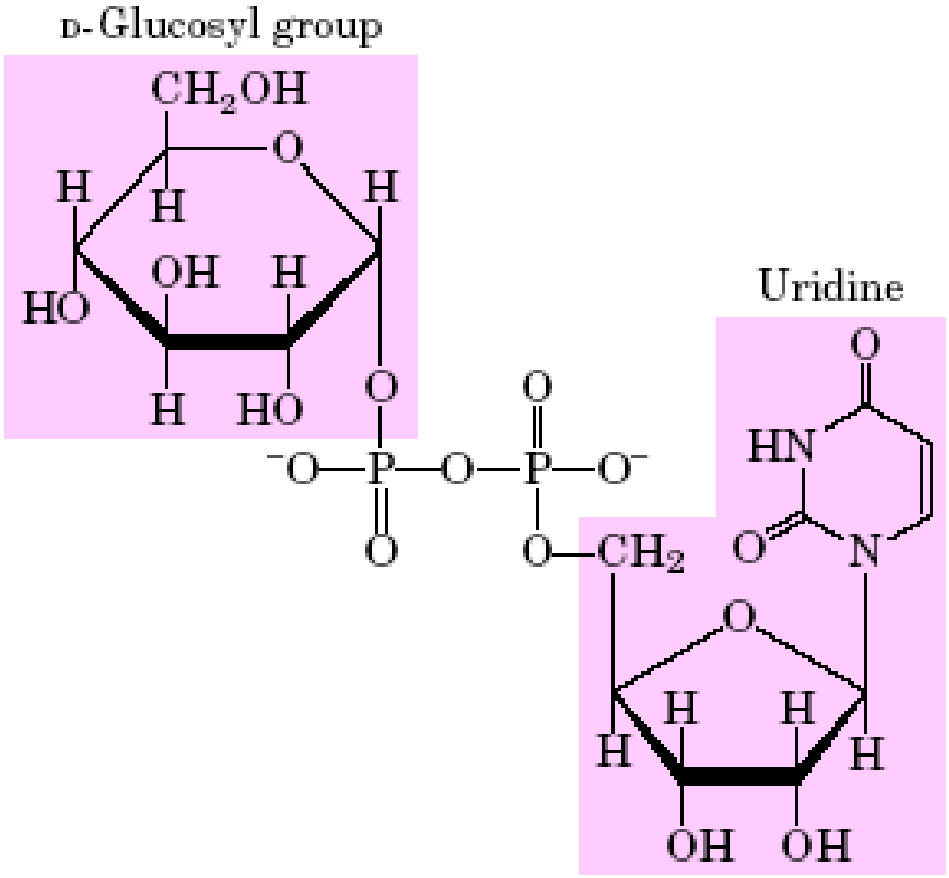
- Most of the triose phosphate generated by CO_2 fixation in plants is converted to sucrose (Fig. 20–25) or starch.
- sucrose may have been selected as the transport form of carbon because of its unusual linkage between the anomeric C-1 of glucose and the anomeric C-2 of fructose. This bond is not hydrolyzed by amylases or other common carbohydrate-cleaving enzymes, and the unavailability of the anomeric carbons prevents sucrose from reacting nonenzymatically (as does glucose) with amino acids and proteins.



Sucrose

- Sucrose is synthesized in the cytosol, beginning with dihydroxyacetone phosphate and glyceraldehyde 3-phosphate exported from the chloroplast.
- After condensation of two triose phosphates to form fructose 1,6-bisphosphate (catalyzed by aldolase), hydrolysis by fructose 1,6-bisphosphatase yields fructose 6-phosphate.
- **Sucrose 6-phosphate synthase** then catalyzes the reaction of fructose 6-phosphate with **UDP-glucose** to form **sucrose 6-phosphate** (Fig. 20–25).
- Finally, **sucrose 6-phosphate phosphatase** removes the phosphate group, making sucrose available for export to other tissues. The reaction catalyzed by sucrose 6-phosphate synthase is a low-energy process (Δ *G* - 5.7 kJ/mol), but the hydrolysis of sucrose 6-phosphate to sucrose is sufficiently exergonic (Δ*G* - 16.5 kJ/mol) to make the overall synthesis of sucrose essentially irreversible. Sucrose synthesis is regulated and closely
- coordinated with starch synthesis.





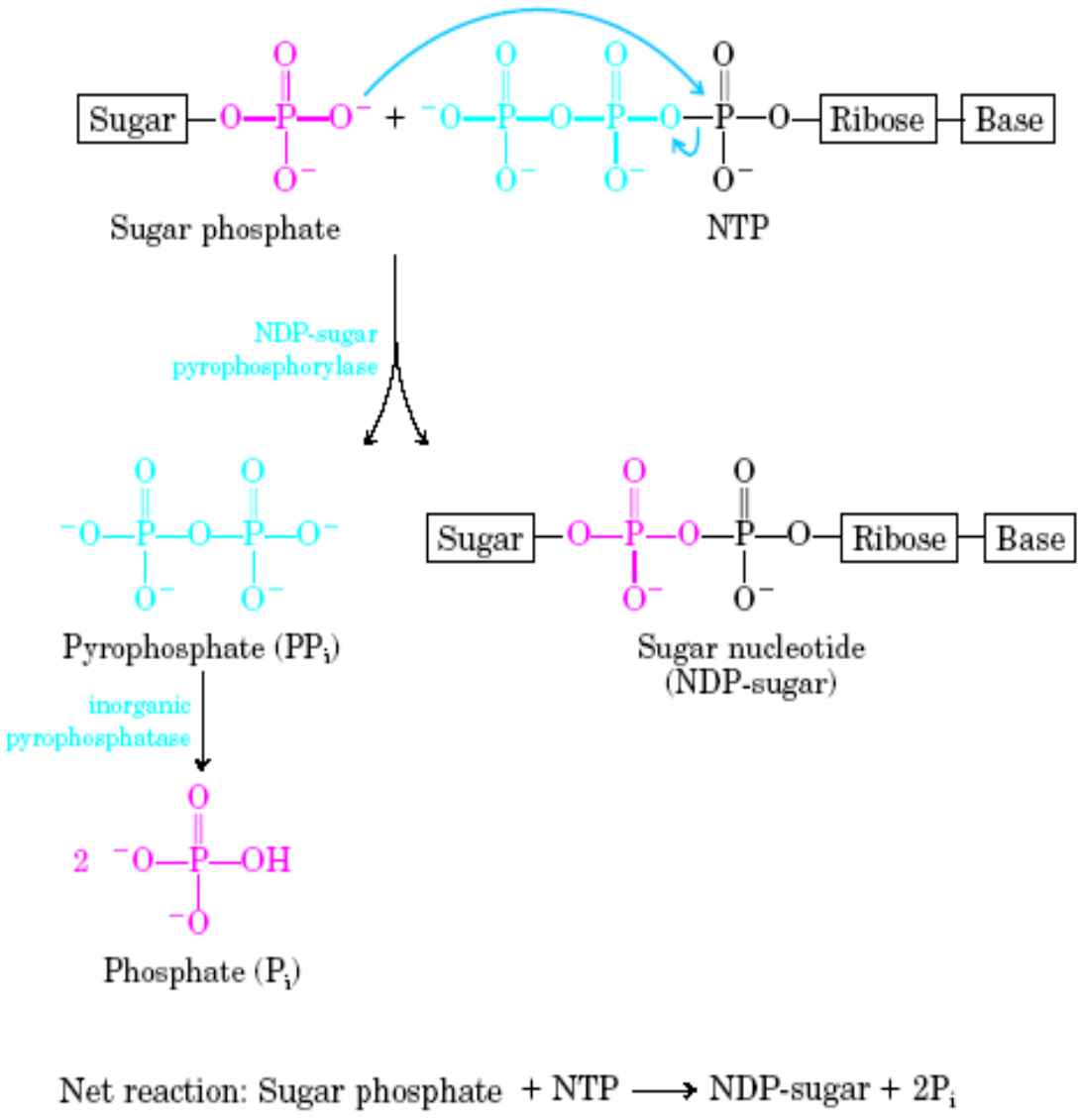
UDP-glucose

(a sugar nucleotide)

- One remarkable difference between the cells of plants and animals is the absence in the plant cell cytosol of the enzyme **inorganic pyrophosphatase**, which catalyzes the reaction;

$$PP_i + H_2O \longrightarrow 2P_i \qquad \Delta G'^\circ = -19.2 \text{ kJ/mol}$$

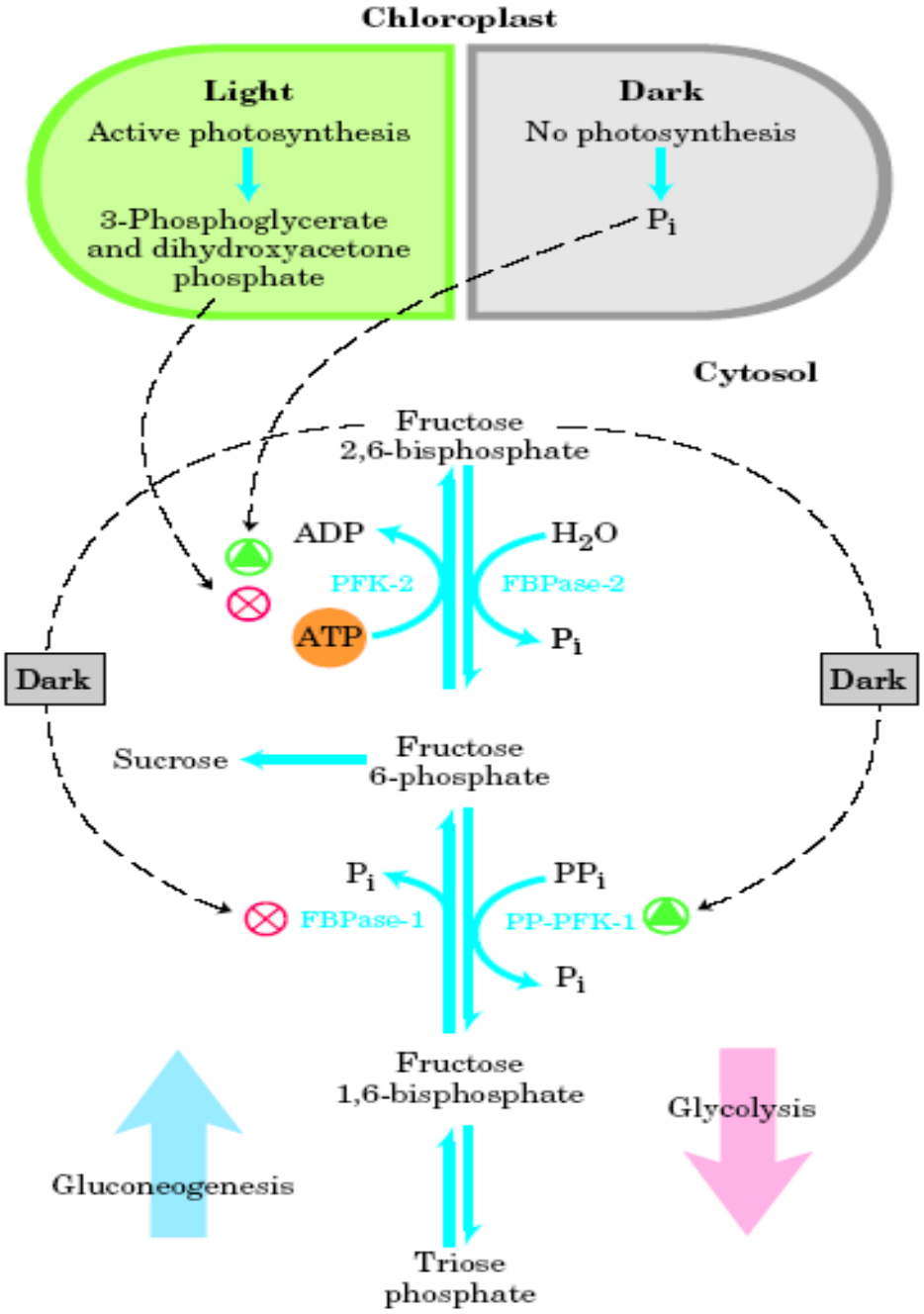
- In plants, this enzyme is present in plastids but absent from the cytosol.
- As a result, the cytosol of leaf cells contains a substantial concentration of PPi—enough (~0.3 mM) to make reactions such as that catalyzed by UDP-glucose pyrophosphorylase readily reversible.
- The cytosolic isozyme of hosphofructokinase in plants uses PPi, not ATP, as the phosphoryl donor.



Conversion of Triose Phosphates to Sucrose and Starch Is Tightly Regulated

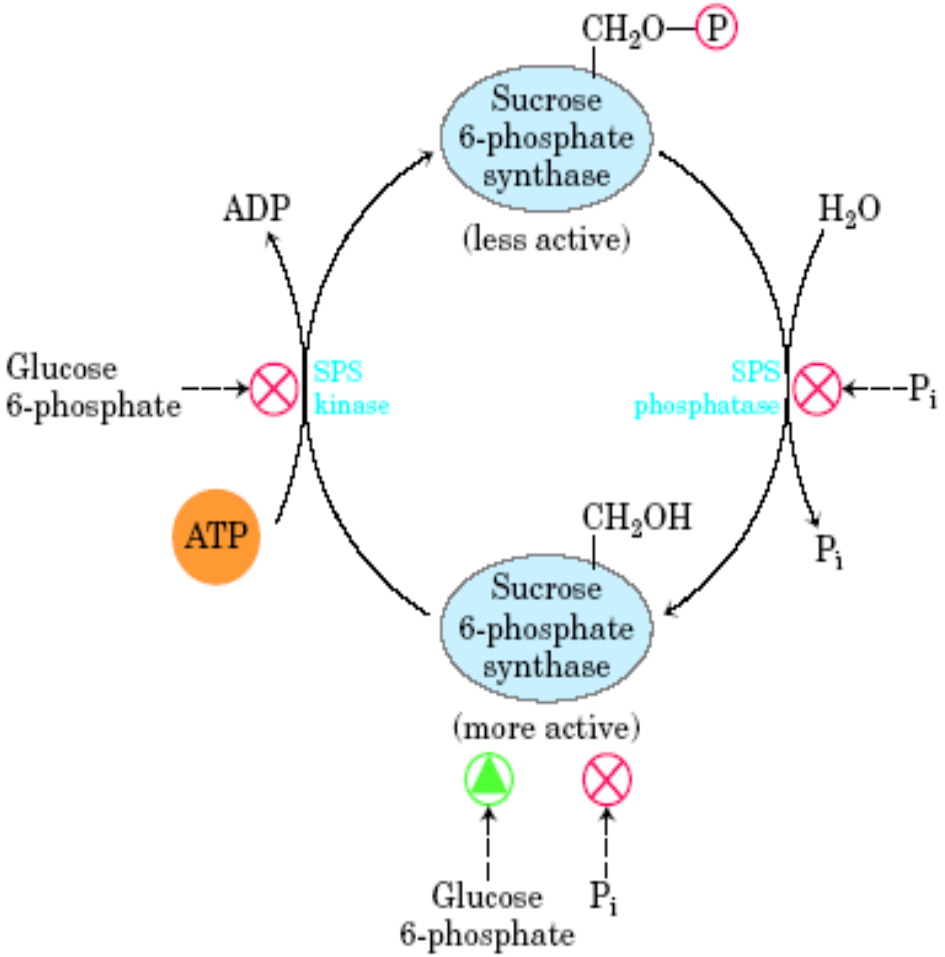
- Triose phosphates produced by the Calvin cycle in bright sunlight, as we have noted, may be stored temporarily in the chloroplast as starch, or converted to sucrose and exported to nonphotosynthetic parts of the plant, or both.
- The balance between the two processes is tightly regulated, and both must be coordinated with the rate of carbon fixation.
- Five-sixths of the triose phosphate formed in the Calvin cycle must be recycled to ribulose 1,5-bisphosphate .
- if more than one-sixth of the triose phosphate is drawn out of the cycle to make sucrose and starch, the cycle will slow or stop.
- However, *insufficient* conversion of triose phosphate to starch or sucrose would tie up phosphate, leaving a chloroplast deficient in Pi, which is also essential for operation of the Calvin cycle.

- The flow of triose phosphates into sucrose is regulated by the activity of fructose 1,6-bisphosphatase (FBPase-1) and the enzyme that effectively reverses its action, PPi-dependent phosphofructokinase (PP-PFK-1).
- These enzymes are therefore critical points for determining the fate of triose phosphates produced by photosynthesis.
- Both enzymes are regulated by **fructose 2,6-bisphosphate (F2,6BP),** which inhibits FBPase-1 and stimulates PP-PFK-1.
- In vascular plants, the concentration of F2,6BP varies inversely with the rate of photosynthesis .

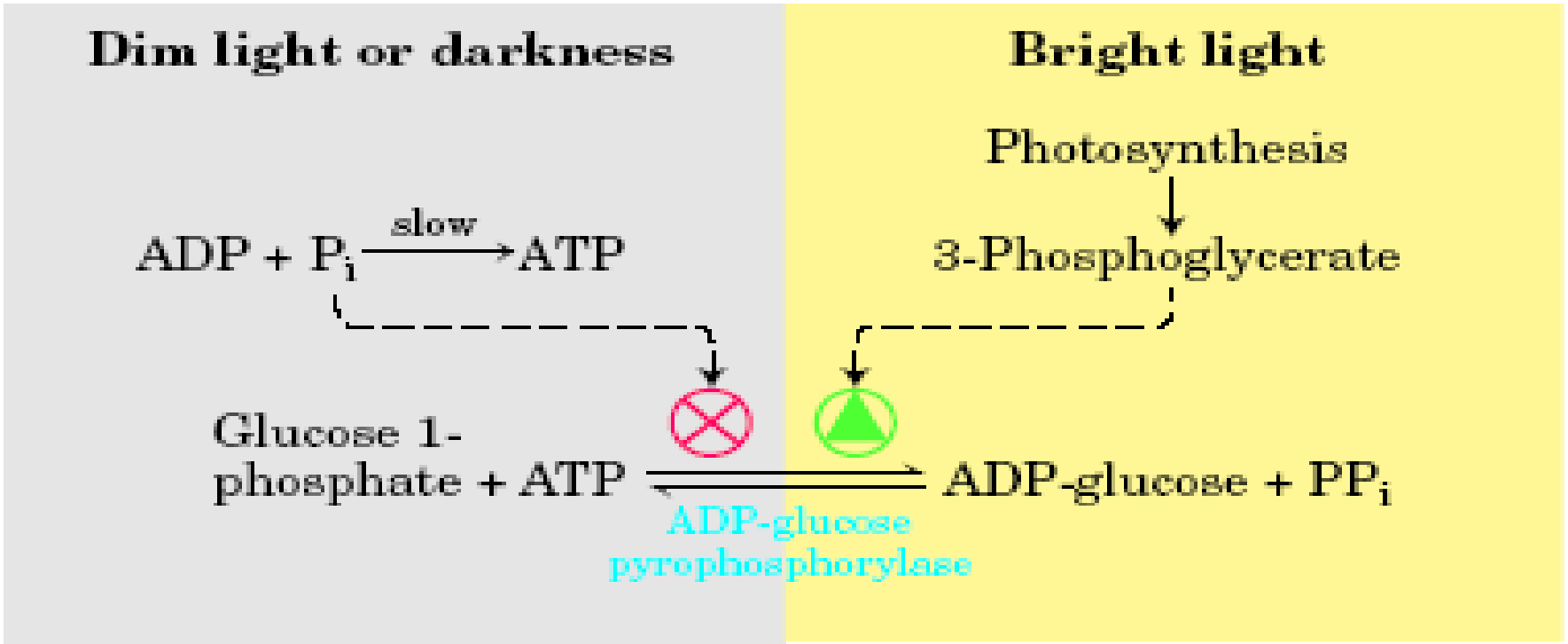

Chloroplast
Light
Active photosynthesis
3-Phosphoglycerate and dihydroxyacetone phosphate
Dark
No photosynthesis
Pi
Cytosol
Fructose 2,6-bisphosphate
ADP
H2O
PFK-2
FBPase-2
ATP
Pi
Dark
Dark
Sucrose
Fructose 6-phosphate
Pi
PPi
FBPase-1
PP-PFK-1
Pi
Fructose 1,6-bisphosphate
Glycolysis
Gluconeogenesis
Triose phosphate

- Phosphofructokinase-2, favors greater flux of triose phosphate into fructose 6-phosphate formation and sucrose synthesis.
- With this regulatory system, sucrose synthesis occurs when the level of triose phosphate produced by the Calvin cycle exceeds that needed to maintain the operation of the cycle.

- Sucrose synthesis is also regulated at the level of sucrose 6-phosphate synthase, which is allosterically activated by glucose 6-phosphate and inhibited by Pi.
- This enzyme is further regulated by phosphorylation and dephosphorylation; a protein kinase phosphorylates the enzyme on a specific Ser residue, making it less active, and a phosphatase reverses this inactivation by removing the phosphate.



- The key regulatory enzyme in starch synthesis is **ADP-glucose pyrophosphorylase**
- it is activated by 3-phosphoglycerate (which accumulates during active photosynthesis) and inhibited by Pi (which accumulates when light-driven condensation of ADP and Pi slows).
- When sucrose synthesis slows, 3-phosphoglycerate formed by CO_2 fixation accumulates, activating this enzyme and stimulating the synthesis of starch.



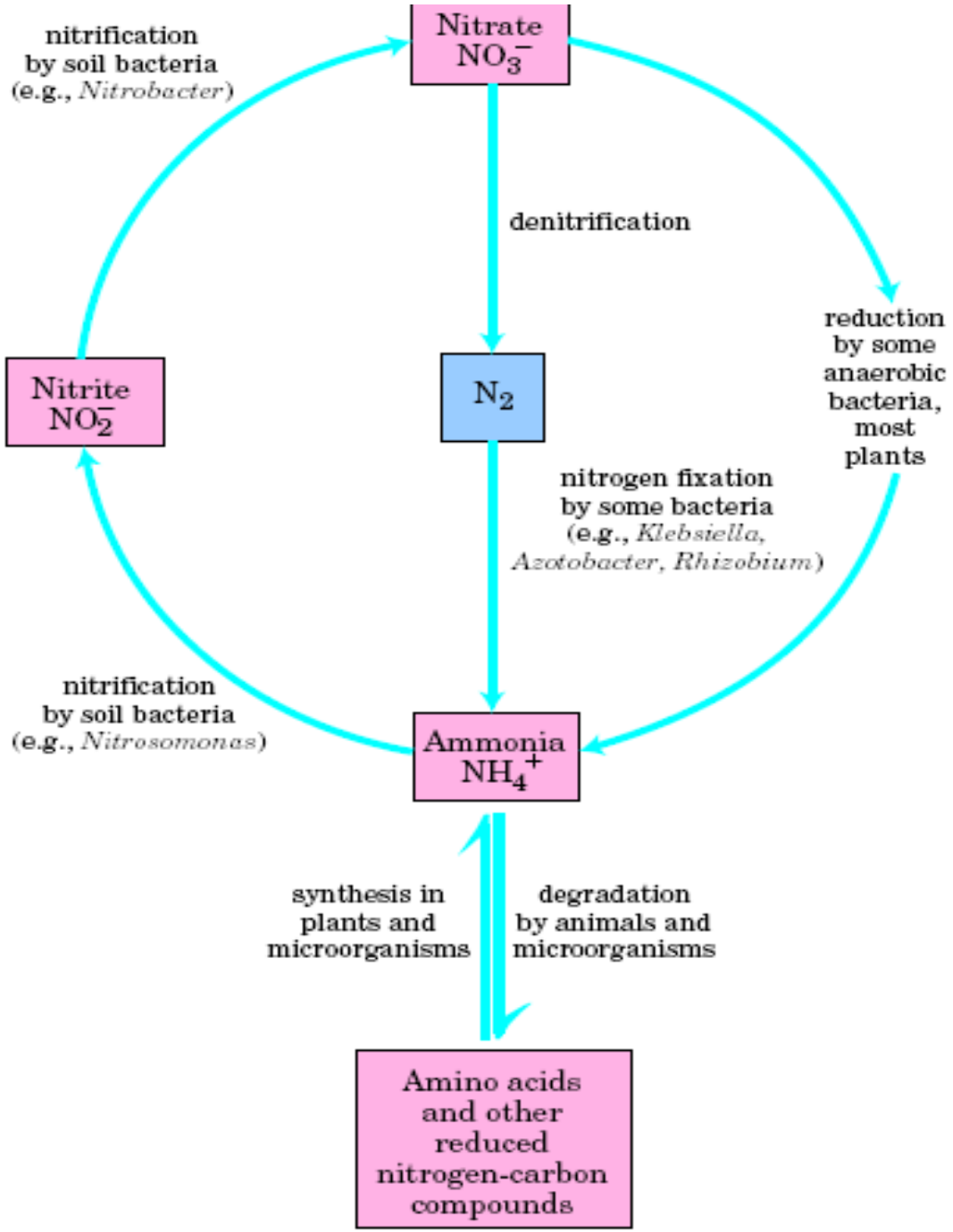
Nitrogen Metabolism

- The biosynthetic pathways leading to amino acids and nucleotides share a requirement for nitrogen.
- Because soluble, biologically useful nitrogen compounds are generally scarce in natural environments, most organisms maintain strict economy in their use of ammonia, amino acids, and nucleotides.
- Free amino acids, purines, and pyrimidines formed during metabolic turnover of proteins and nucleic acids are often salvaged and reused.

Nitrogen Cycle Maintains a Pool of Biologically Available Nitrogen

- The most important source of nitrogen is air, which is four-fifths molecular nitrogen (N_2). However, relatively few species can convert atmospheric nitrogen into forms useful to living organisms.
- In the biosphere, the metabolic processes of different species function interdependently to salvage and reuse biologically available nitrogen in a vast **nitrogen cycle.**

The first
step in the cycle is
fixation (reduction) of
atmospheric
nitrogen by nitrogen-
fixing bacteria to yield
ammonia
(NH3 or NH4).



- The first step in the cycle is **fixation** (reduction) of atmospheric nitrogen by nitrogen-fixing bacteria to yield ammonia (NH_3 or NH_4).
- Although ammonia can be used by most living organisms, soil bacteria that derive their energy by oxidizing ammonia to nitrite (NO_2) and ultimately nitrate (NO_3) are so abundant and active that nearly all ammonia reaching the soil is oxidized to nitrate.
- This process is known as **nitrification.**

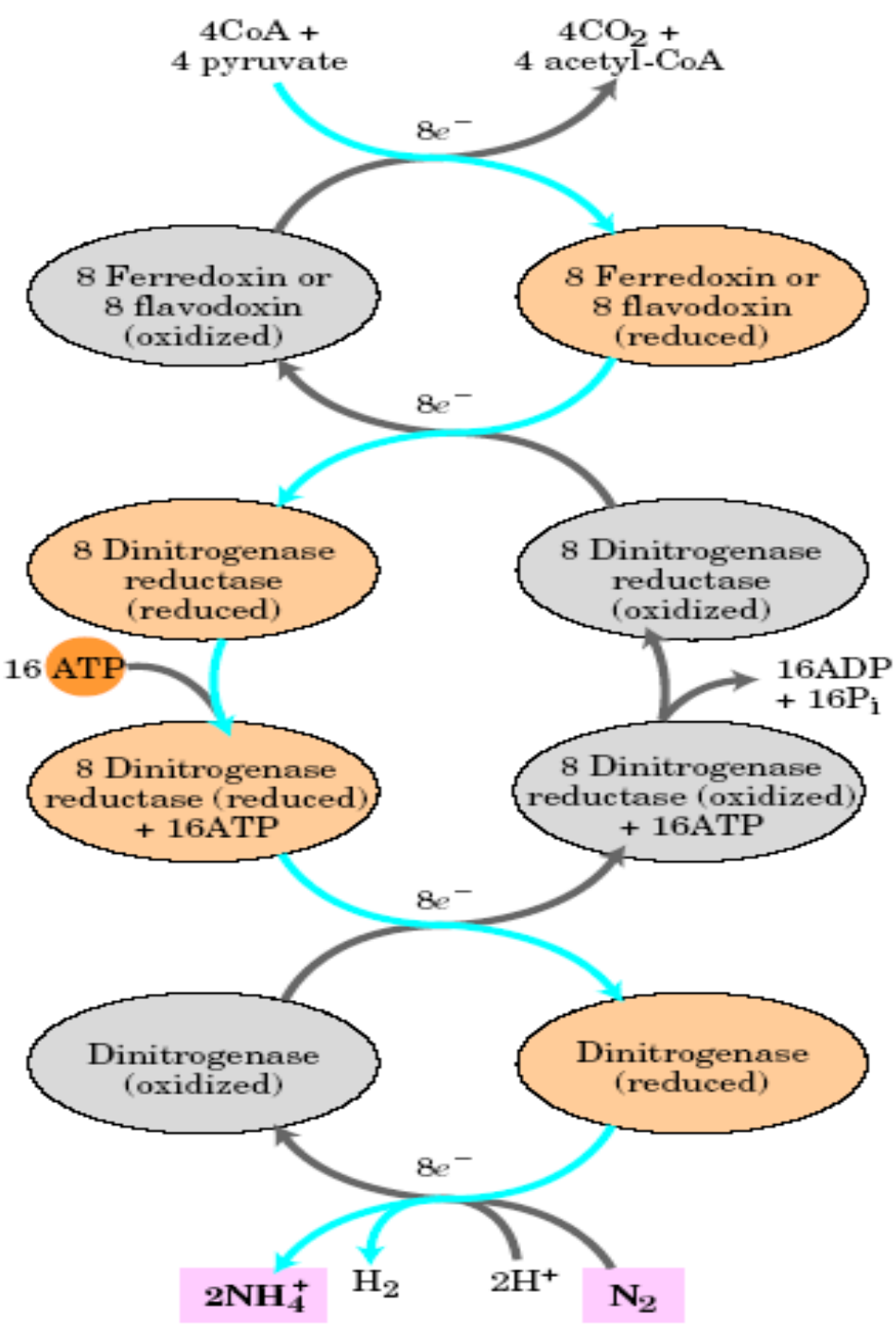
- Plants and many bacteria can take up and readily reduce nitrate and nitrite through the action of nitrate and nitrite reductases.
- The ammonia so formed is incorporated into amino acids by plants. Animals then use plants as a source of amino acids, both nonessential and essential, to build their proteins.
- When organisms die, microbial degradation of their proteins returns ammonia to the soil, where nitrifying bacteria again convert it to nitrite and nitrate.
- A balance is maintained between fixed nitrogen and atmospheric nitrogen by bacteria that convert nitrate to N_2 under anaerobic conditions, a process called **denitrification** .
- These soil bacteria use NO_3 rather than O_2 as the ultimate electron acceptor in a series of reactions that (like oxidative phosphorylation) generates a transmembrane proton gradient, which is used to synthesize ATP.

Nitrogen Is Fixed by Enzymes of the Nitrogenase Complex

- Only certain prokaryotes can fix atmospheric nitrogen.
- These include the cyanobacteria of soils and fresh and salt waters, other kinds of free-living soil bacteria such as *Azotobacter* species, and the nitrogen-fixing bacteria that live as **symbionts** in the root nodules of leguminous plants.
- The first important product of nitrogen fixation is ammonia, which can be used by all organisms either directly or after its conversion to other soluble compounds such as nitrites, nitrates, or amino acids.
- The reduction of nitrogen to ammonia is an exergonic reaction:

$$N_2 + 3H_2 \longrightarrow 2NH_3 \qquad \Delta G'^\circ = -33.5 \text{ kJ/mol}$$

- Biological nitrogen fixation is carried out by a highly conserved complex of proteins called the **nitrogenase complex**, the crucial components of which are **dinitrogenase reductase** and **dinitrogenase**
- Dinitrogenase reductase (*M*r 60,000) is a dimer of two identical subunits. It contains a single 4Fe- 4S redox center, bound between the subunits, and can be oxidized and reduced by one electron.
- It also has two binding sites for ATP/ADP (one site on each subunit). Dinitrogenase (*M*r 240,000), a tetramer with two copies of two different subunits, contains both iron and molybdenum; its redox centers have a total of 2 Mo, 32 Fe, and 30 S per tetramer.
- About half of the iron and sulfur is present as two bridged pairs of 4Fe-4S centers called P clusters; the remainder is present as part of a novel iron-molybdenum cofactor.
- A form of nitrogenase that contains vanadium rather than molybdenum has been discovered, and some bacterial species can produce both types of nitrogenase systems.
- The vanadium-containing enzyme may be the primary nitrogen-fixing system under some environmental conditions, but it is not yet as well characterized as the molybdenum-dependent enzyme.


4CoA +
4 pyruvate
4CO2 +
4 acetyl-CoA
8e−
8 Ferredoxin or
8 flavodoxin
(oxidized)
8 Ferredoxin or
8 flavodoxin
(reduced)
8e−
8 Dinitrogenase
reductase
(reduced)
8 Dinitrogenase
reductase
(oxidized)
16 ATP
16ADP
+ 16Pi
8 Dinitrogenase
reductase (reduced)
+ 16ATP
8 Dinitrogenase
reductase (oxidized)
+ 16ATP
8e−
Dinitrogenase
(oxidized)
Dinitrogenase
(reduced)
8e−
2NH4+
H2
2H+
N2

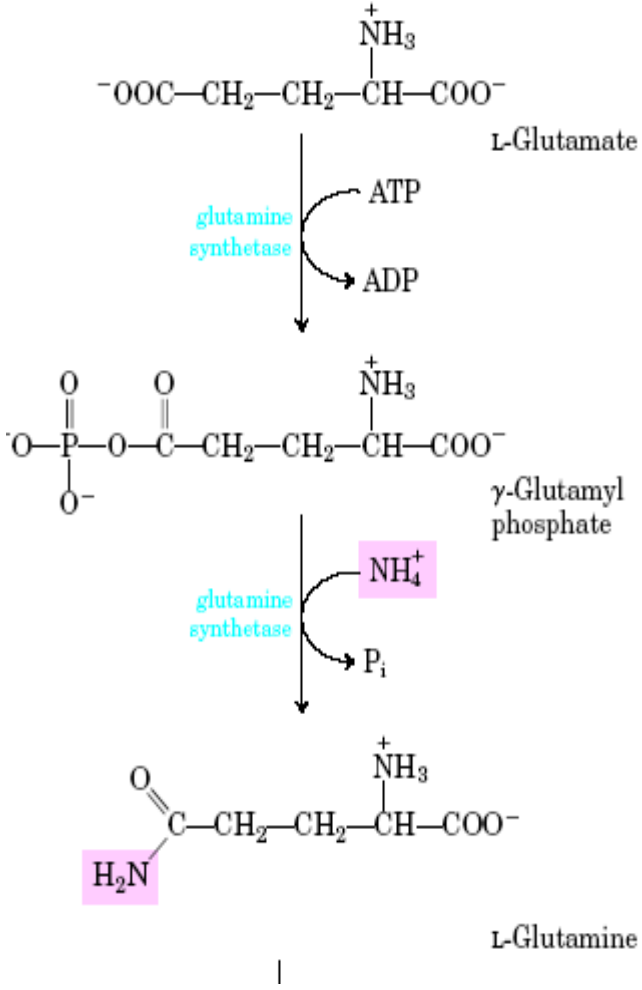
Nitrogen-fixing nodules. Root nodules of bird's-foot trefoil, a legume. The flower of this common plant is shown in the inset.

- Nitrogen fixation is the subject of intense study, because of its immense practical importance.
- Industrial production of ammonia for use in fertilizers requires a large and expensive input of energy, and this has spurred a drive to develop recombinant or transgenic organisms that can fix nitrogen.
- Recombinant DNA techniques are being used to transfer the DNA that encodes the enzymes of nitrogen fixation into non-nitrogen-fixing bacteria and plants.

Ammonia Is Incorporated into Biomolecules through Glutamate and Glutamine

- Reduced nitrogen in the form of NH_4 is assimilated into amino acids and then into other nitrogen-containing biomolecules.
- Two amino acids, **glutamate** and **glutamine,** provide the critical entry point.
- these same two amino acids play central roles in the catabolism of ammonia and amino groups in amino acid oxidation.
- Glutamate is the source of amino groups for most other amino acids, through transamination reactions.

- The biosynthetic pathways to glutamate and glutamine are simple, and all or some of the steps occur in most organisms.
- The most important pathway for the assimilation of NH4 into glutamate requires two reactions.
- First, **glutamine synthetase** catalyzes the reaction of glutamate and NH_4 to yield glutamine.
- This reaction takes place in two steps, with enzyme-bound γ-glutamyl phosphate as an intermediate



(1) Glutamate + ATP ⟶ γ-glutamyl phosphate + ADP

(2) γ-Glutamyl phosphate + NH_4^+ ⟶ glutamine + P_i + H^+

Sum: Glutamate + NH_4^+ + ATP ⟶ glutamine + ADP + Pi + H^+ (22–1)

- Glutamine synthetase is found in all organisms.
- In addition to its importance for NH4 assimilation in bacteria, it has a central role in amino acid metabolism in mammals, converting toxic free NH4 to glutamine for transport in the blood.
- In bacteria and plants, glutamate is produced from glutamine in a reaction catalyzed by **glutamate synthase.**

 α-Ketoglutarate, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor:

- α-Ketoglutarate, an intermediate of the citric acid cycle, undergoes reductive amination with glutamine as nitrogen donor:

$$\alpha\text{-Ketoglutarate} + \text{glutamine} + \text{NADPH} + \text{H}^+ \longrightarrow 2\ \text{glutamate} + \text{NADP}^+$$

The net reaction of glutamine synthetase and glutamate synthase is:

$$\alpha\text{-Ketoglutarate} + \text{NH}_4^+ + \text{NADPH} + \text{ATP} \longrightarrow \text{L-glutamate} + \text{NADP}^+ + \text{ADP} + \text{P}_i$$

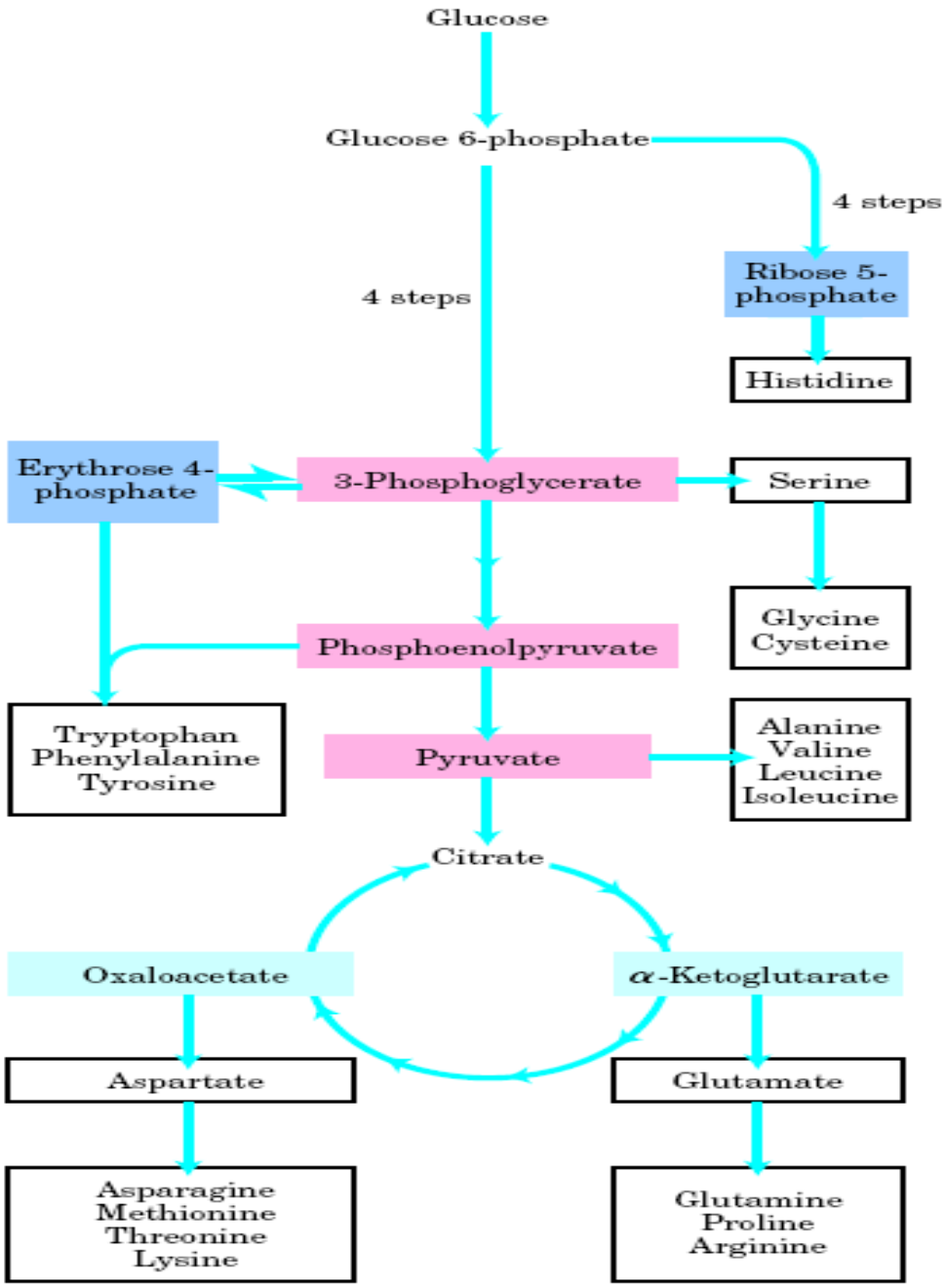
- Glutamate synthase is not present in animals, which, instead, maintain high levels of glutamate by processes such as the transamination of α -ketoglutarate during amino acid catabolism.

- Glutamate can also be formed in yet another pathway:
- the reaction of α-ketoglutarate and NH_4 to form glutamate in one step.
- This is catalyzed by L-glutamate dehydrogenase, an enzyme present in all organisms.
- Reducing power is furnished by NADPH:

$$\alpha\text{-Ketoglutarate} + NH_4^+ + NADPH \longrightarrow$$
$$\text{L-glutamate} + NADP^+ + H_2O$$

Biosynthesis of Amino Acids

- All amino acids are derived from intermediates in glycolysis, the citric acid cycle, or the pentose phosphate pathway .
- Nitrogen enters these pathways by way of glutamate and glutamine.
- Some pathways are simple, others are not. Ten of the amino acids are just one or several steps removed from the common metabolite from which they are derived.
- The biosynthetic pathways for others, such as the aromatic amino acids, are more complex.


Glucose
Glucose 6-phosphate
4 steps
Ribose 5-phosphate
Histidine
4 steps
Erythrose 4-phosphate
3-Phosphoglycerate
Serine
Glycine
Cysteine
Phosphoenolpyruvate
Tryptophan
Phenylalanine
Tyrosine
Pyruvate
Alanine
Valine
Leucine
Isoleucine
Citrate
Oxaloacetate
α-Ketoglutarate
Aspartate
Glutamate
Asparagine
Methionine
Threonine
Lysine
Glutamine
Proline
Arginine

- Organisms vary greatly in their ability to synthesize the 20 common amino acids.
- Whereas most bacteria and plants can synthesize all 20, mammals can synthesize only about half of them—generally those with simple pathways. These are the **nonessential amino acids,** not needed in the diet
- The remainder, the **essential amino acids,** must be obtained from food.

TABLE 18–1 Nonessential and Essential Amino Acids for Humans and the Albino Rat

Nonessential	*Conditionally essential**	*Essential*
Alanine	Arginine	Histidine
Asparagine	Cysteine	Isoleucine
Aspartate	Glutamine	Leucine
Glutamate	Glycine	Lysine
Serine	Proline	Methionine
	Tyrosine	Phenylalanine
		Threonine
		Tryptophan
		Valine