such measurements, photosynthesis is excited by monochromatic light, and the production of oxygen per incident quantum of light is measured as a function of wavelength. The observed spectral variations in the yield of photosynthesis can be related to the proportion of light absorbed at each wavelength by the different pigments in the cells. Measurements of this kind have led to the conclusion that quanta absorbed by most carotenoids are 50–80% as effective as those absorbed by chlorophyll *a* in contributing energy to photosynthesis. An exception is fucoxanthol, the carotenoid that accounts for the color of brown algae (Phaeophyta) and that of the diatoms; it supplies light energy to photosynthesis about as effectively as chlorophyll *a*. The red and blue pigments of the Rhodophyta and cyanobacteria are also highly effective. They can be as effective as chlorophyll or somewhat less, depending, among other things, on the physiological status of the organism and the color of the light to which they have become adapted. The primary function of all these pigments is to harvest the light energy and transfer it to reaction-center chlorophyll molecules. However, in addition, several xanthophylls (violaxanthin, antheraxanthin, and zeaxanthin) and lutein are involved in photoprotecting photosynthetic organisms against excess light. In many cases, excess light energy is lost as "heat" via deexcitation of chlorophyll directly or via transfer to zeaxanthin.

Energy transfer between pigment molecules in antenna system. Chlorophyll *a* in vivo is weakly fluorescent, that is, some of the light quanta absorbed by it (up to 6%) are reemitted as light (**Fig. 7**, white curve). Observations of the action spectrum of chlorophyll *a* fluorescence in different oxygenic organisms closely parallel the action spectrum of photosynthesis. In other words, fluorescence of chlorophyll *a* is excited also by light absorbed by the accessory pigments. Excitation of chlorophyll *a* fluorescence by light quanta absorbed by phycoerythrin requires transfer of the excitation energy from the excited phycoerythrin molecule to a nearby chlorophyll molecule (somewhat as in acoustic resonance, where striking one bell causes a nearby bell to ring). Therefore, light quanta absorbed by accessory pigments, such as carotenoids and phycobilins, contribute to photosynthesis by being transferred to chlorophyll *a*. By this mechanism, red algae, growing relatively deep under the sea where only green light penetrates, can supply the energy of this light to chlorophyll *a*, which has very weak absorption in the green region of the spectrum.

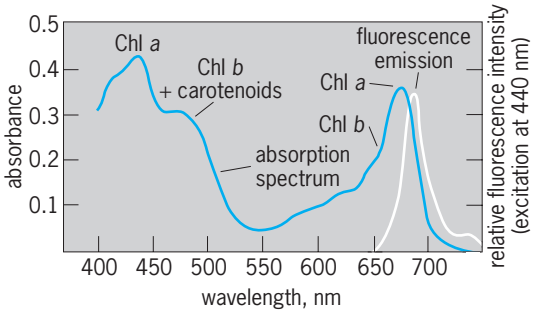

Fig. 7. Absorption spectrum of a maize (*Zea mays*) chloroplast suspension. Pigments responsible for specific bands are shown. Also shown is the fluorescence emission of chloroplasts from a maize chloroplast.

Excitation energy is transferred efficiently in the chloroplasts from accessory pigments to chlorophyll *a*. A similiar transfer (often referred to as energy migration) occurs also between different chlorophyll *a* molecules themselves. Excitation-energy transfer among chlorophyll *a* molecules or among phycobilin molecules, and excitation-energy transfer from accessory pigments (donor molecules) to chlorophyll *a* (acceptor molecules) or from various short-wavelength forms of chlorophyll *a* to the long-wavelength forms of chlorophyll *a*, has been demonstrated. The most widely accepted hypothesis, Förster's hypothesis, is that energy transfer is preceded by thermal relaxation in the donor molecules. The efficiency of energy transfer depends upon three basic factors: orientation of acceptor molecules with respect to the donor molecule; overlap of the fluorescence spectrum of the donor molecule with the absorption spectrum of the acceptor molecule; and the distance between the two molecules. The function of most of the pigments (including most of the chlorophyll *a* molecules) is to act as an antenna, harvest the energy, and transfer to very few (1 in 300) reaction-center Chl molecules, depending upon the pigment system. Energy is thus trapped and used for photochemistry. *See* CHLOROPHYLL; PLANT PIGMENT.

[Contributions of Rajni Govindjee to this article are acknowledged.] Robert E. Blankenship; Govindjee

Carbon Dioxide Fixation

The light-dependent conversion of radiant energy into chemical energy as adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) serves as a prelude to the utilization of these compounds for the reductive fixation of CO_2 into organic molecules. Such molecules, broadly designated as photosynthates, are usually but not invariably in the form of carbohydrates such as glucose polymers or sucrose, and form the base for the nutrition of all living things, as well as serving as the starting material for fuel, fiber, animal feed, oil, and other compounds used by people. Collectively, the biochemical processes by which CO_2 is assimilated into organic molecules are known as the photosynthetic dark reactions, not because they must occur in darkness but because, in contrast to the photosynthetic light reactions, light is not required. (We do recognize, however, that several enzymes need to be light-activated before they can function; see "Regulation of C_3 cycle enzymes" below.) CO_2 fixation by photosynthetic organisms is an important mechanism by which this "greenhouse" gaseous molecule is removed from the atmosphere during carbon cycling on Earth. Approximately 100 pentagrams of carbon (1 pentagram equals 10^9 metric tons) as CO_2 is assimilated annually into organic molecules by photosynthesis (about

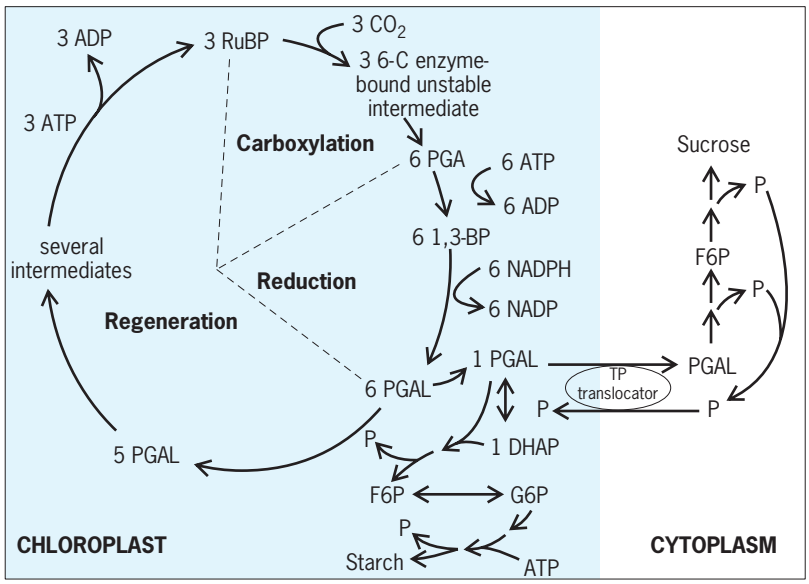

Fig. 8. Schematic outline of the C_3 (Calvin-Benson-Bassham) CO_2 assimilation cycle (the three phases are noted), showing the partitioning of assimilated carbon into starch within the chloroplast [via the phosphorylated 6-C intermediates, fructose-6-phosphate (F6P) and glucose-6-phosphate (G6P)], and the assimilate efflux (through the three-carbon triose phosphate [TP] transporter) across the chloroplast inner envelope to the cytoplasm (in exchange for inorganic phosphate) leading to sucrose synthesis. For the reactions of the C_3 cycle, shown in the chloroplast, the relative numbers of molecules involved in each specific step are shown to the left of the substrate and/or product.

half of this amount is assimilated by photosynthetic marine algae).

The route by which CO_2 is assimilated had been studied for over a century when it was discovered that photosynthesis leads to the accumulation of sugars and starch. Details of the biochemical pathway leading to CO_2 assimilation were worked out in the 1950s, when the availability of paper chromatographic techniques and $^{14}CO_2$ allowed Melvin Calvin, Andrew A. Benson, and James A. Bassham to develop the outline of the reductive pentose phosphate cycle (Calvin was awarded a Nobel Prize in Chemistry in 1961), now usually called the C_3 cycle. The name C_3 cycle refers to the first stable product generated from CO_2, which is a three-carbon organic molecule. The C_3 cycle forms the primary, or basic (with other, feeder pathways occurring in some plant types), route for the formation of photosynthate from CO_2.

C_3 photosynthesis. The essential details of C_3 photosynthesis can be seen in **Fig. 8**. The entire cycle can be separated into three phases—carboxylation, reduction, and regeneration. Since the smallest intermediate in the cycle consists of three carbons, we will start with three molecules of CO_2. During the initial carboxylation phase, these three molecules of CO_2 are combined with three molecules of the five-carbon compound ribulose 1,5-bisphosphate (RuBP) in a reaction catalyzed by the enzyme RuBP carboxylase/oxygenase (rubisco) to form three molecules of an intermediate, unstable enzyme-bound six-carbon compound. These unstable molecules are hydrolyzed by the enzyme into six molecules of the three-carbon compound phosphoglyceric acid (PGA). These products of the carboxylation phase, the six (three-carbon) PGA molecules, are phosphorylated by six molecules of ATP (releasing ADP to be used for photophosphorylation via the light reactions) to form six 1,3-bisphosphoglycerate (1,3-BP) molecules. The resulting compounds are reduced (that is, in the reduction phase of the C_3 cycle) by the NADPH formed in photosynthetic light reactions to form six molecules of the three-carbon compound phosphoglyceraldehyde (PGAL). PGAL is isomerized to form another three-carbon compound, dihydroxyacetone phosphate (DHAP). PGAL, the aldehyde, and DHAP, the ketone, are energetically equivalent, reduced compounds and can be considered the products of the reductive phase of the C_3 photosynthetic cycle. PGAL and DHAP together form the triose phosphate (TP) pool of the chloroplast. The chloroplast TP pool is primarily composed of PGAL; the isomerase responsible for PGAL:DHAP interconversion favors PGAL formation.

The rest of the C_3 photosynthetic cycle (the regeneration phase) involves enzymatic steps that allow regeneration of RuBP, the initial carboxylation substrate. One molecule of PGAL is made available for combination with DHAP isomerized from a second PGAL (requiring a second "turn" of the Calvin-Benson-Bassham cycle wheel) to form a six-carbon sugar. The other five PGAL molecules, through a complex series of enzymatic reactions, are rearranged into three molecules of RuBP, which can again be carboxylated with CO_2 to continue the cycle.

It should be noted that the enzyme that incorporates CO_2 into an organic compound, RuBP carboxylase/oxygenase (rubisco), also allows oxygen (O_2) to react with RuBP, hence the "oxygenase" in the name. This reaction initiates the process called photorespiration, which results in the release of one previously incorporated molecule of CO_2 for every two molecules of O_2 that are allowed to react. *See* PHOTORESPIRATION.

Due to its low catalytic efficiency, rubisco (**Fig. 9**) can be up to half of the soluble protein in C_3 chloroplasts, and most likely it is the most abundant protein found in nature. Rubisco is a large and

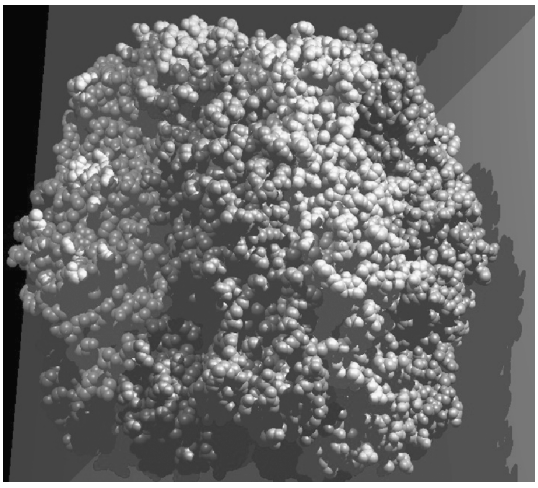

Fig. 9. Structural model of Rubisco.

complex enzyme, comprising eight large polypeptide subunits and eight small subunits. Interestingly, the small subunit polypeptide is produced (as a larger precursor form) in the cytoplasm from mRNA which is encoded in the nucleus. The precursor polypeptide is then transported across the chloroplast membrane (the mature form of this polypeptide cannot be transported in this manner); processed into the shorter, mature polypeptide; and then combined with large subunits (encoded in the chloroplast DNA and produced in the stroma) to form the mature enzyme.

The net product of two "turns" of the cycle, a six-carbon sugar (G6P or F6P), is formed either within the chloroplast in a pathway leading to starch (a polymer of many glucose molecules) or externally in the cytoplasm in a pathway leading to sucrose (condensed from two six-carbon sugars, glucose and fructose). This partitioning of newly formed photosynthate leads to two distinct pools; starch is stored in the photosynthesizing "source" leaf cells, and sucrose is available either for immediate metabolic requirements within the cell or for export to "sinks" such as developing reproductive structures, roots, or other leaves. Factors within the photosynthesizing cell, such as energy requirements in different compartments (mitochondria, cytoplasm, and chloroplasts), along with energy needs of the plant (such as increased sink requirements during different developmental stages) and external, environmental factors (such as light intensity and duration) ultimately regulate the partitioning of newly formed photosynthetic product (PGAL) into starch or sucrose. *See* PLANT METABOLISM.

This profound control of photosynthate partitioning is accomplished through regulation of PGAL export from the chloroplast to the cytoplasm, as well as by regulation of the enzymes that convert PGAL to sucrose in the cytoplasm and starch in the chloroplast. Under conditions where sink demand is low (and sucrose is not transported through the phloem away from source leaf cells), metabolic effectors accumulate in the cytoplasm that lower the activities of the sucrose-forming enzymes and increase the activities of the starch-forming enzymes. This results in a condition that reduces PGAL export from the chloroplast, and hence more PGAL is retained in the chloroplast for starch formation. Also, under conditions which cause low chloroplast PGAL levels (such as low light), PGAL transport out of the chloroplast is restricted, resulting in decreased substrate for sucrose formation, increasing the relative amount of starch production. The energy status of the cell affects sucrose formation (and therefore photosynthate partitioning) because cytoplasmic uridine triphosphate (used in the formation of sucrose) level is dependent on ATP generation, and also because PGAL export to the cytoplasm is coupled obligatorily to inorganic phosphate (formed when ATP is metabolized in the cytoplasm) import into the chloroplast.

In addition to providing carbon skeletons for starch and sucrose synthesis, PGAL is fed back into the C_3 cycle to allow for the regenerative phase of the reactions to synthesize more RuBP, the carboxylation substrate. We have known for quite some time that the maximal measurable activities of fructose 1,6-bisphophatase (FBPase) and sedoheptulose 1,7-bisphosphatase (SBPase), two enzymes involved in RuBP regeneration, are not much greater than the rate of photosynthetic carbon assimilation and concomitant carbon flow through the C_3 cycle. Thus, carbon flow through these enzymes might contribute to rate limitation of photosynthetic carbon assimilation. Studies with transgenic plants overexpressing these enzymes support this contention; increasing the amount of either enzyme led to a higher level of the carboxylation substrate RuBP as well as higher photosynthetic rates.

The autocatalytic nature of the cycle (that is, more substrate for initial carboxylation can be generated as carbon flows through the steps of the pathway) can be best understood by considering that the net product of one "turn" of the cycle (representing three carboxylations), that is, a PGAL molecule, can be fed back into the cycle. Thus the rate of carboxylation during an initial lag phase (as chloroplasts are initially illuminated) is dependent on the level of newly formed RuBP. If all newly fixed carbon were fed back into the cycle, the level of RuBP would double after five carboxylations. Since the rate of photosynthetic carbon fixation is initially dependent on the level of intermediates such as the substrate (RuBP) for the carboxylation reaction, the next five carboxylations would occur in a shorter amount of time, resulting in an exponential increase in the rate of photosynthesis until factors other than intermediate levels become limiting.

Regulation of C_3 cycle enzymes. The photosynthetic carbon assimilation cycle is regulated at a number of enzymatic steps. The initial carboxylation catalyst, rubisco, as well as some of the enzymes involved in the regeneration phase, including glyceraldehyde-3-phosphate dehydrogenase, phosphoribulose kinase, SBPase, and FBPase, require activation. These enzymes are inactivated in the dark and activated in the light. Several conditions are required for activation, including high concentrations of Mg^{2+}, high pH, and a reductant (supplied in the chloroplast by the enzyme thioredoxin). Thioredoxin is reduced by NADPH generated in the light. Thioredoxin acts as a protein disulfide oxidoreductase, converting disulfide (S-S) bonds of the target proteins (all of the above enzymes except rubisco) to a reduced (−SH) form. Rubisco activity is also modulated by a specific mechanism involving another enzyme, called rubisco activase. All of the aforementioned activating conditions within the chloroplast stroma are facilitated by light-dependent processes but are reversed in darkness. This regulatory mechanism conveniently allows for the synthesis pathway to be "shut off," preventing a futile cycle during the night, when starch reserves are mobilized to meet cell energy requirements via intermediates which, if C_3 cycle enzymes were activated, would be reconverted to starch.

C_4 photosynthesis. Initially, the C_3 cycle was thought to be the only route for CO_2 assimilation,

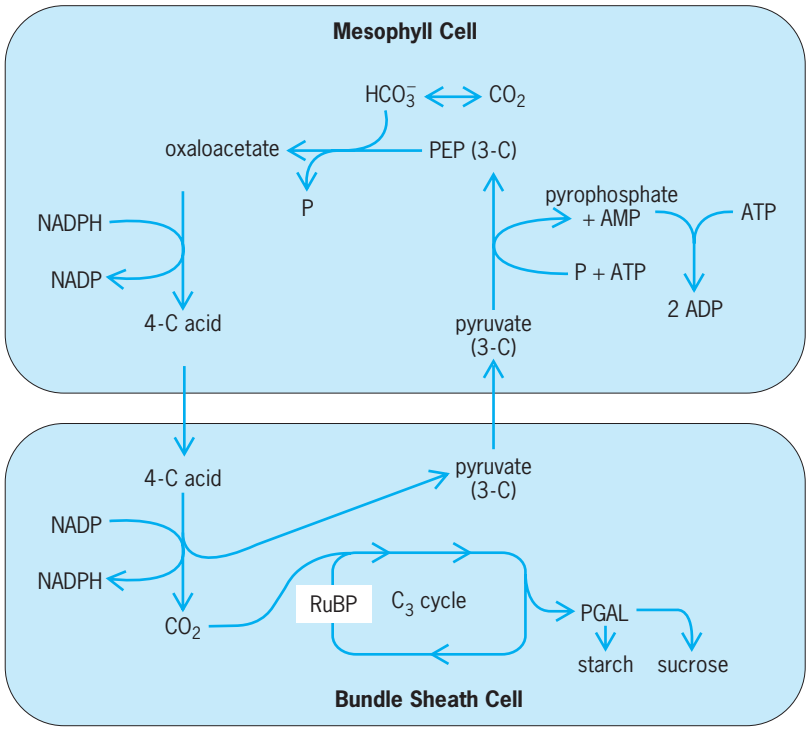

Fig. 10. Schematic outline of the C_4 carbon dioxide assimilation process in the two cell types of a NADP-ME-type plant.

although it was recognized by plant anatomists that some rapidly growing plants (such as maize, sugarcane, and sorghum) possessed an unusual organization of the photosynthetic tissues in their leaves (Kranz morphology). Work by Hugo Kortschak, Constance Hartt, and colleagues in Hawaii as well as that of M. D. (Hal) Hatch and Roger Slack in Australia demonstrated that plants having the Kranz anatomy utilized an additional CO_2 assimilation route now known as the C_4-dicarboxylic acid pathway (**Fig 10**). Carbon dioxide enters a mesophyll cell where it is combined (in the form of bicarbonate) with the three-carbon compound phosphoenolpyruvate (PEP) via the enzyme PEP carboxylase to form a four-carbon acid, oxaloacetate, which is reduced to malic acid or transaminated to aspartic acid. The four-carbon acid moves into bundle sheath cells where the acid is decarboxylated and the CO_2 reassimilated via the C_3 cycle. The resulting three-carbon compound, pyruvic acid, moves back into the mesophyll cell and is transformed into PEP (at the cost of 2 ATP molecules) via the enzyme pyruvate phosphate dikinase located in the mesophyll chloroplasts to complete the cycle. The net effect of this cycle is to increase the CO_2 concentration around rubisco, thereby reducing photorespiration via the competing oxygenase activity of this enzyme.

As depicted in Fig. 10, extensive transport of metabolites must occur between the two cell types that are found in most C_4 plants. The diffusion of metabolites between two cell types is facilitated by the presence of plasmodesmata connecting the cells to form a cytoplasmic continuum. However, in some cases the two cell types, mesophyll and bundle sheath, are not necessarily adjacent (sedges are an example). Exotic plants have now been found that perform C_4 photosynthesis in single cells in which the chloroplasts that initially combine carbon dioxide with PEP are spatially separated from the chloroplasts where the carbon dioxide is reassimilated via the C_3 cycle.

C_4 metabolism is classified into three types, depending on the primary decarboxylation reaction used with the four-carbon acid in the bundle sheath cells. The majority of C_4 species (exemplified by sugarcane, maize, crabgrass, and sorghum) are of type 1 (see below), and employ NADP-malic enzyme (NADP-ME) for decarboxylation. NAD-malic enzyme (NAD-ME) C_4 plants (type 2) include amaranthus, atriplex, millet, pigweed, and purslane. Type 3 C_4 types use phosphoenol pyruvate carboxykinase (PCK) for decarboxylation and include Panicum grasses. The decarboxylases are also located in different intracellular compartments as indicated:

1. NADP-ME type,

$$\text{NADP}^+ + \text{malic acid} \xrightarrow{\text{NADP-malic enzyme (chloroplasts)}} \text{pyruvic acid} + CO_2 + \text{NADPH} \quad (6)$$

2. NAD-ME type,

$$\text{NAD}^+ + \text{malic acid} \xrightarrow{\text{NAD-malic enzyme (mitochondria)}} \text{pyruvic acid} + CO_2 + \text{NADH} \quad (7)$$

3. PCK type,

$$\text{Oxaloacetic acid} + \text{ATP} \xrightarrow{\text{phosphoenolpyruvate carboxykinase (cytosol)}} \text{PEP} + CO_2 + \text{ADP} \quad (8)$$

In addition to differing decarboxylation reactions, the particulars of the CO_2 fixation pathway in NAD-ME and PCK plant types differ from those depicted in Fig. 10 with respect to the three-carbon compound transported from bundle sheath to mesophyll cells. With NAD-ME types, the three-carbon compound can be either pyruvic acid or alanine, and in PCK types this compound is PEP. Therefore, the three variations in the C_4 pathway necessarily predicate different energy (ATP and NADPH) usage in the two cell types. The generation of ATP from ADP, and NADPH from NADP via noncyclic electron flow through photosystem I (PSI) and photosystem II (PSII), is tightly coupled: neither compound can be produced without sufficient substrate for both. Therefore, the different usage of ATP and NADPH in the mesophyll and bundle sheath chloroplasts of the three C_4 plant types (due both to variations in the pathway of carbon flow in the photosynthetic cycle and to variations in partitioning of portions of the pathway between cell types) is supported by variations in the photochemical apparatus which allow for differing ability to produce ATP without concomitant NADPH production. These alternative pathways of ATP production (which result in different ratios of ATP:NADPH produced) are cyclic and pseudocyclic

photophosphorylation, with the cyclic pathway considered the major pathway of uncoupled ATP production in chloroplasts, and the pseudocyclic pathway possibly acting as a "fine-tuning" modulator.

Variations in the photochemical apparatus that indicate enhanced cyclic photophosphorylation capacity (utilizing only PSI) are a high chlorophyll *a/b* ratio, low Chl/P700 ratio, and a low PSII reaction. These characteristics are found in bundle sheath chloroplasts of NADP-ME-type plants, indicating that the primary function of the photochemical apparatus in these chloroplasts is the generation of ATP. NADPH is supplied via the decarboxylation of malic acid to support the C_3 cycle activity (PGA conversion to PGAL) in these chloroplasts. Assays of chlorophyll *a/b* ratio, Chl/P700 ratio, and PS II activity indicate that NAD-ME mesophyll chloroplasts also have a primary role of cyclic photophosphorylation, while NAD-ME bundle sheath chloroplasts have a primary role of noncyclic electron flow. In PCK-type plants, mesophyll chloroplasts appear to have a photochemical apparatus similar to C_3 chloroplasts, while bundle sheath chloroplasts appear to have a low PSII activity. The enhanced ability of PCK bundle sheath chloroplasts to produce ATP via cyclic photophosphorylation supplies the extra ATP needed to convert pyruvic acid to PEP. These variations in the C_4 pathway and photochemical apparatus among the C_4 plant types demonstrate the close relationship that has evolved between light reactions and the biochemical processes of carbon dioxide assimilation, and show the highly integrated cooperation between the cell types involved.

Benefits of C_4 cycle. The concentration of CO_2 in air is presently about 0.037% by volume (and increasing with time due to burning of fossil fuels), a concentration that does not fully saturate the C_3 cycle when it is operating at capacity due to the low affinity of rubisco for CO_2. It would be necessary to have about 0.1% CO_2 to saturate photosynthesis in C_3 plants, which can be achieved only under controlled conditions (CO_2-enriched greenhouses or growth chambers). Leaf photosynthesis in C_4 plants, however, is fully saturated at air CO_2 concentrations. Thus, C_4 photosynthesis may be considered to be an evolutionary adaptation to current-day CO_2 levels in air. During the C_4 cycle, CO_2 is rapidly captured via biochemical reactions in mesophyll chloroplasts and released near rubisco in bundle sheath chloroplasts. This serves to increase the CO_2 concentration around the enzyme, increasing its catalytic efficiency and decreasing its reaction with O_2 and thus photorespiration; thus the ambient concentration of CO_2 in air is not rate-limiting. The spatial compartmentalization of portions of CO_2 assimilation into the two cell types not only allows C_4 plants to assimilate air CO_2 rapidly with minimal photorespiration, but also partly explains other physiological characteristics and responses to the external environment of C_4 plants. C_4 plants have a higher efficiency of water use. Water vapor exits from leaves through the same stomatal pores through which CO_2 enters the leaf. Since the C_4 plant is more efficient at fixing CO_2 than C_3 plants, more CO_2 is incorporated per unit water lost. C_4 plants have a greater efficiency of nitrogen usage. Since rubisco is produced only in bundle sheath cells in C_4 plants, only 10–35% of the leaf nitrogen is tied up in this enzyme, as opposed to 40–60% in C_3 plants. Since C_4 plants have to "expend" less carbon on producing the protein rubisco, they have higher rates of sugar formation, which can facilitate the rapid growth rates seen in such C_4 plants as maize, sugarcane, sorghurn, and crabgrass. Other differences in response to the environment between C_3 and C_4 plants are as follows: C_4 plants exhibit a nonsaturating response curve of leaf photosynthesis to light levels found in nature. In addition, C_4 plants tolerate more salinity and higher temperatures than do C_3 plants. The higher energy requirements of C_4 plants (2 ATPs per CO_2 assimilated) are also reflected by the fact that quantum yields of photosynthesis for C_3 plants are higher than for those possessing the auxiliary C_4 system. At 2% oxygen partial pressure and 30°C, maximum quantum yield for C_3 plants is about 0.073 mole CO_2 assimilated per absorbed Einstein (an Einstein is a mole of photons) of light, while for C_4 plants the maximum quantum yield is 0.054. However, at normal O_2 partial pressures (21% O_2), quantum yields are almost identical. This is due to the presence of high photorespiration in C_3 plants, and thus represents a net quantum yield rather than a true photosynthetic yield. *See* PHOTORESPIRATION.

Crassulacean acid metabolism photosynthesis. Under arid and desert conditions, where soil water is in short supply, transpiration during the day when temperatures are high and humidity is low may rapidly deplete the plant of water, leading to desiccation and death. By keeping stomata closed during the day, water can be conserved, but the uptake of CO_2, which occurs entirely through the stomata, is prevented. Desert plants in the Crassulaceae, Cactaceae, Euphorbiaceae, and 15 other families have evolved, apparently independently of C_4 plants, a similar strategy of concentrating and assimilating CO_2 by which the CO_2 is taken in at night when the stomata open; water loss is low because of the reduced temperatures and correspondingly higher humidities. Although these succulent plants with thick, fleshy leaves were known since the nineteenth century as being unusual, the biochemical understanding of the process did not occur until the 1960s and 1970s when the details of C_4 photosynthesis were being worked out. It was first studied in plants of the Crassulaceae; thus, the process has been called crassulacean acid metabolism (CAM).

In contrast to C_4, where two cell types usually cooperate, the entire CAM process occurs within an individual cell; the separation of C_4 and C_3 is thus temporal rather than spatial. At night, CO_2 combines with PEP through the action of PEP carboxylase, resulting in the formation of oxaloacetic acid and its conversion into malic acid. The PEP is formed from starch or sugar via the glycolytic route

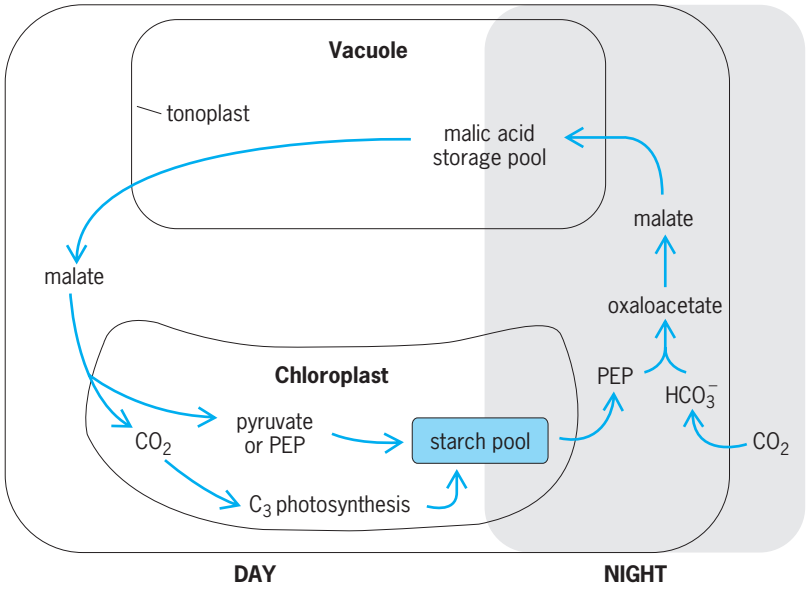

Fig. 11. Scheme for the flow of CO_2 within a single crassulacean acid metabolism (CAM) cell over a day, showing initial dark CO_2 fixation, malic acid storage in the vacuole at night, followed by decarboxylation and the C_3 cycle the next day.

of respiration. Thus, there is a daily reciprocal relationship between starch (a storage product of C_3 photosynthesis) and the accumulation of malic acid (the terminal product of nighttime CO_2 assimilation; **Fig. 11**).

As in C_4 plants, there may be variations in the decarboxylase that provides the CO_2 for assimilation via the C_3 cycle. In some CAM plants (such as pineapple) PCK is used, while in others (cactus) the decarboxylase is the NADP-malic enzyme (NADP-ME type). A few CAM species use NAD-ME for decarboxylation. Since the stomata are closed most of the day, decarboxylation of the stored malate (or oxaloacetate) results in an elevation of its concentration around rubisco. The **table** summarizes the major physiological differences between C_3, C_4, and CAM plants.

Other CO_2 assimilation mechanisms. Both the C_4 cycle and CAM involve the synthesis of oxaloacetic acid, which is also one of the intermediates in the tricarboxylic acid (TCA) cycle of respiration. In the late 1960s a light-driven reversal of the TCA cycle was discovered. This CO_2 fixation cycle, called the reductive carboxylic acid cycle, results in the net synthesis of pyruvic acid via the reversal of the three decarboxylation steps in the TCA cycle (pyruvic acid to acetyl coenzyme A, isocitric acid to α-ketoglutaric acid, and succinyl CoA to succinic acid). The pathway has been detected in some photosynthetic bacteria. *See* CITRIC ACID CYCLE.

In most photosynthetic bacteria, the C_3 cycle is functional despite some differences in detail. The green sulfur bacteria, however, carry out C_3 photosynthesis poorly or not at all. *Chlorobium thiosulfatophilum* (alternate name: *Chlorobium limicola*), lacking the key enzyme rubisco, utilizes a reductive carboxylic acid cycle in which reduced ferredoxin drives the TCA cycle in reverse, resulting in carboxylation reactions much like those of the reductive carboxylic acid cycle. Heterocysts of cyanobacteria do not have a functional C_3 cycle because, in contrast to the normal cells of these bacteria, the heterocyst cell (implicated in nitrogen fixation) lacks the key enzyme rubisco. Here, CO_2 fixation in heterocysts may occur through PEP carboxylase as in C_4 and CAM photosynthesis. Guard cells in

Some characteristics of the three major plant groups

Characteristics	C_3	C_4	CAM
Leaf anatomy in cross section	Diffuse distribution of organelles in mesophyll and palisade cells with less chloroplasts in bundle sheath cells if present	Layer of bundle sheath cells around vascular tissue with a high concentration of chloroplasts; layers of mesophyll cells around bundle sheath	Spongy, often lacking palisade cells; mesophyll cells have large vacuoles
Theoretical energy requirement for net CO_2 fixation (CO_2:ATP:NADPH)	1:3:2	1:5:2	1:6.5:2
Carboxylating enzyme	Rubisco	PEP carboxylase, then rubisco	Darkness: PEP carboxylase; light: mainly rubisco
CO_2 compensation concentration, ppm CO_2	30–70	0–10	0–5 in dark
Transpiration ratio, g H_2O/g dry weight increase	450–950	250–350	50–55
Maximum net photosynthetic rate, mg CO_2/(dm^2 leaf)(h)	15–40	40–80	1–4
Photosynthesis sensitive to high O_2	Yes	No	Yes
Photorespiration detectable	Yes	Only in bundle sheath	Difficult to detect
Leaf chlorophyll *a*/*b* ratio	2.8 ± 0.4	3.9 ± 0.6	2.5–3
Maximum growth rate, g dry wt/(dm^2 leaf)(day)	0.5–2	4–5	0.015–0.018
Optimum temperature for photosynthesis	15–25° C (59–77° F)	30–40° C (86–104° F)	About 35° C (95° F)

C_3 plants, which regulate the opening of stomatal pores for gas exchange in leaves, also lack rubisco and apparently use PEP carboxylase exclusively to fix CO_2.

Contributions of the late Martin Gibbs to this article are acknowledged.

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Bacterial Photosynthesis

Certain bacteria have the ability to perform photosynthesis. This was first noticed by Sergey Vinogradsky in 1889 and was later extensively investigated by Cornelis B. Van Niel, who gave a general equation for bacterial photosynthesis. This is shown in reaction (9).

$$2H_2A + CO_2 + \text{light} \xrightarrow[\text{enzymes}]{\text{bacteriochlorophyll}} \{CH_2O\} + 2A + H_2O \quad (9)$$

where A represents any one of a number of reductants, most commonly S (sulfur).

Photosynthetic bacteria cannot use water as the hydrogen donor and are incapable of evolving oxygen. They are therefore called anoxygenic photosynthetic bacteria. The prokaryotic cyanobacteria (formerly called blue-green algae) are excluded in this discussion of bacterial photosynthesis, since their photosynthetic system closely resembles that found in eukaryotic algae and higher plants discussed above. Anoxygenic photosynthetic bacteria can be classified in four major groups:

1. *Proteobacteria*. Two groups with somewhat different properties are known.

(A) Nonsulfur purple bacteria (Rhodospirillaceae). In these bacteria, H_2A is usually an organic H_2 donor, such as succinate or malate; however, these bacteria can be adapted to use hydrogen gas as the reductant. They require vitamins for their growth and usually grow anaerobically in light, but they can also grow aerobically in the dark by using respiration to utilize organic compounds from the environment. They are thus facultative photoheterotrophs. Examples of this group are *Rhodospirillum rubrum* and *Rhodobacter sphaeroides*.

(B) Sulfur purple bacteria (Chromatiaceae). These cannot grow aerobically, and H_2A is an inorganic sulfur compound, such as hydrogen sulfide, H_2S; the carbon source can be CO_2. These bacteria are called obligate photoautotrophic anaerobes. An example is *Chromatium vinosum* (alternate name: *Allochromatium vinosum*).

2. *Green sulfur bacteria* (Chlorobiaceae). These bacteria are capable of using the same chemicals as Chromatiaceae but, in addition, use other organic H_2 donors. They may then be called photoautotrophic and photoheterotrophic obligate anaerobes. An example of the green sulfur bacteria is *Chlorobium tepidum*.

3. *Green gliding bacteria* (Chloroflexaceae) [also known as filamentous anoxygenic phototrophs, FAP]. These are primarily photoorganotrophic bacteria which can grow under anaerobic conditions in light by photosynthesis or in aerobic conditions in the dark by using respiration to utilize organic compounds from the environment. They are thermophilic bacteria found in hot springs around the world. They also distinguish themselves among the photosynthetic bacteria by possessing mobility. An example is *Chloroflexus aurantiacus*.

4. *Heliobacteria* (Heliobacteriaceae). These are strictly anaerobic bacteria that contain bacteriochlorophyll *g*. They grow primarily using organic substrates and have not been shown to carry out autotrophic growth using only light and inorganic substrates. An example is *Heliobacterium chlorum*.

Like plants, algae, and cyanobacteria, anoxygenic photosynthetic bacteria are capable of photophosphorylation, which is the production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (P_i) using light as the primary energy source. Several investigators have suggested that the sole function of the light reaction in bacteria is to make ATP from ADP and Pi. The hydrolysis energy of ATP (or the proton-motive force that precedes ATP formation) can then be used to drive the reduction of CO_2 to carbohydrate by H_2A in reaction (9).

Photochemical apparatus. Photosynthetic bacteria do not have specialized organelles such as the chloroplasts of green plants. Electron micrographs of certain photosynthetic bacteria show tiny spherical sacs, with double-layered walls, as a result of invaginations which form stacks of membranes (**Fig. 12***a*). Other photosynthetic bacteria have invaginations which form thylakoids (Fig. 12*b*). These intracytoplasmic membranes, often called chromatophores, contain the photosynthetic apparatus and can be isolated easily by mechanical disruption of bacteria followed by differential centrifugation. Isolated chromatophores are often used for biochemical and biophysical studies of bacterial photosynthesis.

Reaction centers. The pigment bacteriochlorophyll (BChl) is a necessary component for bacterial

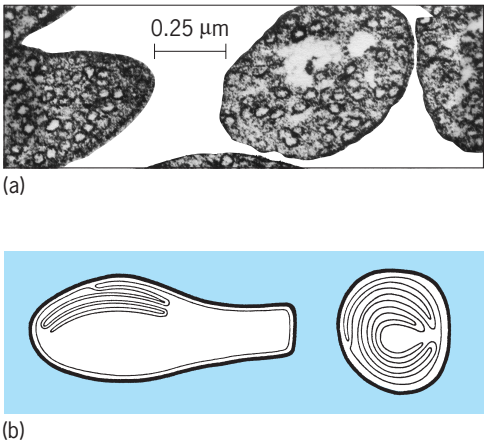

Fig. 12. Photosynthetic bacteria. (*a*) Electron micrograph of *Rhodobacter sphaeroides* with vesicle-like invaginations (*from T. W. Goodwin, ed., Biochemistry of Chloroplasts, vol. 1, Academic Press, 1966*). (*b*) Pictorial representation of a stacked invagination in a photosynthetic bacterium; at left is a longitudinal section and at right is a transverse section (*after R. Whittenbury and A. G. McLee, Archiv. für Mikrobiologie, 59:324–334, 1967*).