

Photosynthesis

OM 4.1 PERCEPTION OF LIGHT BY PLANTS

Plants use light in two distinct ways: as the source of energy for photosynthesis (discussed in this chapter) and as a signal that enables many plant functions to be adjusted to changes in the light environment. These light-responsive functions – for example, chloroplast development, stem elongation, germination, and directional growth – are considered in Chapters 10 and 12.

Light is part of the spectrum of **electromagnetic radiation** (Figure OM4.1-1). The part of the spectrum visible to the human eye largely overlaps the part used by plants for photosynthesis and as a signal. The human eye perceives radiation with wavelengths between about 380 nm (violet) and 720 nm (red). The light that provides the energy for photosynthesis also falls in this range. Electromagnetic radiation perceived as signals by plants includes wavelengths on either side of this visible range, in the near-ultraviolet and far-red range.

Light is perceived by plants via absorption by pigments. Photosynthetically active light is absorbed by chlorophyll *a* and chlorophyll *b*. Both pigments have two peaks of absorption in the visible spectrum, between about 400 and 480 nm (the blue end) and between 550 and 700 nm (the red end). Neither chlorophyll absorbs light in the middle part of the visible range – the green part of the spectrum (Figure OM4.1-2A). This is why leaves appear green: the red and blue components of the visible spectrum are absorbed by chlorophyll, but the green wavelengths are reflected or pass through the leaf.

Several types of pigments are responsible for the perception of light as a signal. The best known of these light sensors – known as **photoreceptors** – is **phytochrome**, which absorbs light in the red and far-red parts of the spectrum. Phytochrome exists in two forms, with different biological activities and different absorption spectra (Figure OM4.1-2B). The two forms are interconvertible: when one form absorbs light, it is converted to the other form. Because the two forms have different signaling functions, the plant can respond to differences in the ratio of red to far-red light. This ratio varies with environmental conditions: for example, full sunlight is rich in red light and poor in **far-red light**, whereas the shade under a leaf canopy is enriched in far-red relative to red light, because the red component of sunlight is absorbed by chlorophyll in the canopy. The importance of the phytochrome system in plant responses to light is discussed in Section 12.1.

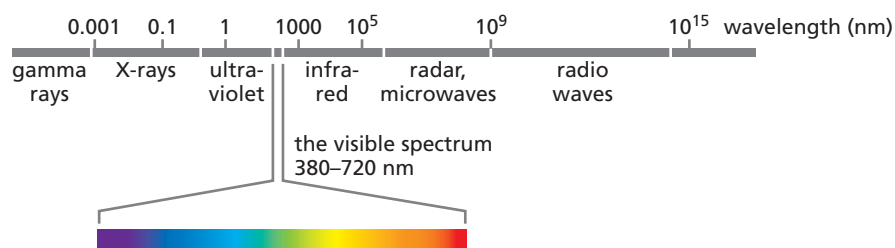


Figure OM4.1-1 The spectrum of electromagnetic radiation. Energy from the sun that reaches the Earth's surface has wavelengths in the range of 350 nm (in the ultraviolet) to 1500 nm (in the infrared, perceived as heat). The visible part of this energy – white light – is in the range 380–720 nm. White light consists of a spectrum of colors, seen when sunlight passes through a prism or through raindrops (in the form of a rainbow).

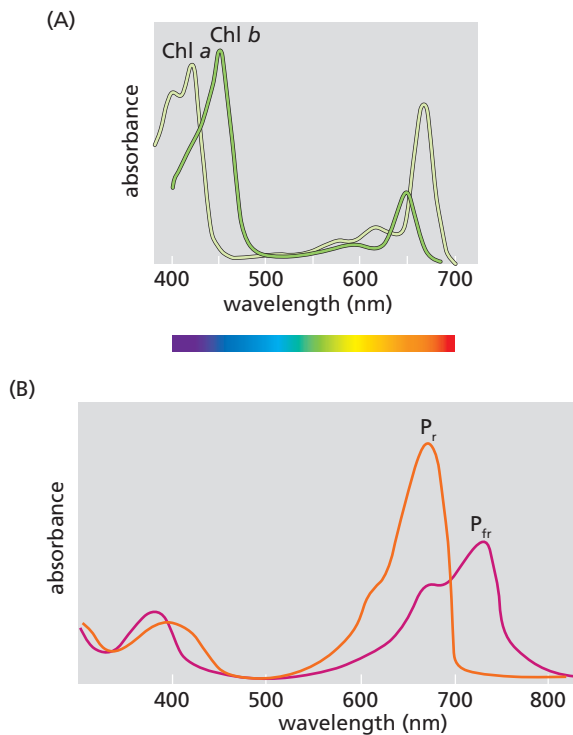


Figure OM4.1-2 Light absorption by chlorophylls and phytochromes. (A) Light absorption by chlorophyll *a* and chlorophyll *b*, plotted as a function of wavelength. This type of plot is known as an “absorption spectrum.” Note that both pigments absorb blue and red light, but not green light. (B) The absorption spectra of the two forms of phytochromes, P_r and P_{fr} .

In considering how much light is needed for a particular plant process, it is convenient to consider light in terms of particles, known as “photons,” rather than as a waveform. The primary unit for measuring light intensity related to photosynthesis is photosynthetic photon flux density, expressed in $\mu\text{mol m}^{-2} \text{s}^{-1}$. For most plants, the minimum amount of light required for net photosynthesis is in the range $10\text{--}20 \mu\text{mol m}^{-2} \text{s}^{-1}$. Below this value (called the **light compensation point**), there is a net loss of carbon dioxide from the plant through respiration (Figure OM4.1-3). Plants adapted to growth in low light conditions (e.g. plants of woodland floors) often have low light compensation points, in the range $1\text{--}5 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereas plants adapted to full sunlight have higher light compensation points. For the vast majority of plants (C_3 plants; see Section 4.5), photosynthesis is saturated (i.e. reaches a maximal value) with respect to light at values of about $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure OM4.1-3), well below the value for full sunlight, which is about $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$. For one group of plants, the C_4 plants, which have a higher energy requirement for photosynthesis (see Section 4.5), photosynthesis is not saturated even in full sunlight.

The amount of light required to trigger light-responsive functions in plants varies widely from one type of photoreceptor to another (see Section 12.1). In some cases, the amount required is tiny in comparison to the amount required for photosynthesis. For example, *Arabidopsis* seeds, which require light for maximum rates of **germination**, can be induced to germinate by light intensities as low as $1 \text{ nmol m}^{-2} \text{s}^{-1}$.

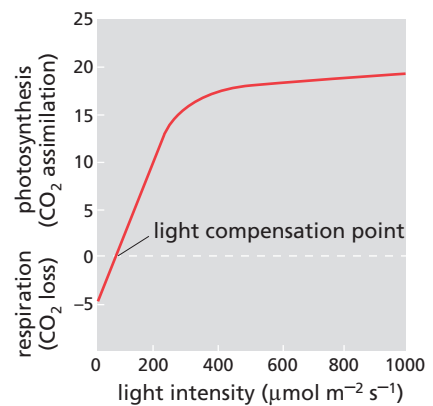


Figure OM4.1-3 A typical plot of the effect of light intensity on plant CO₂ exchange. At light intensities below the light compensation point, the rate of CO₂ assimilation by photosynthesis is less than the rate of CO₂ loss by respiration. Light intensities of about $500 \mu\text{mol m}^{-2} \text{s}^{-1}$ are sufficient to give maximal rates of photosynthesis in most plants. The values on the y-axis are a commonly used way of expressing the rate of CO₂ loss or assimilation – as $\mu\text{mol of CO}_2 \text{ m}^{-2}$ (of leaf surface) s^{-1} .

OM4.2 CYCLIC ELECTRON TRANSPORT

Cyclic electron transport provides a means by which light energy can be used to produce ATP without concomitant NADPH production. Rather than a linear flow of electrons from PSII to NADP⁺ via PSI, in cyclic electron transport electrons are passed from PSI to ferredoxin but are not then used to reduce NADP⁺. Instead, electrons from reduced ferredoxin are passed - via plastoquinone - to the cytochrome *b₆f* complex in the thylakoid membrane and then back to PSI via the plastocyanin pool (Figure OM4.2-1). This cyclical electron transport chain brings about the movement of protons from the stroma to the thylakoid lumen (at the cytochrome *b₆f* step). Cyclic electron transport thus provides a proton motive force to drive ATP synthesis in the absence of NADPH production.

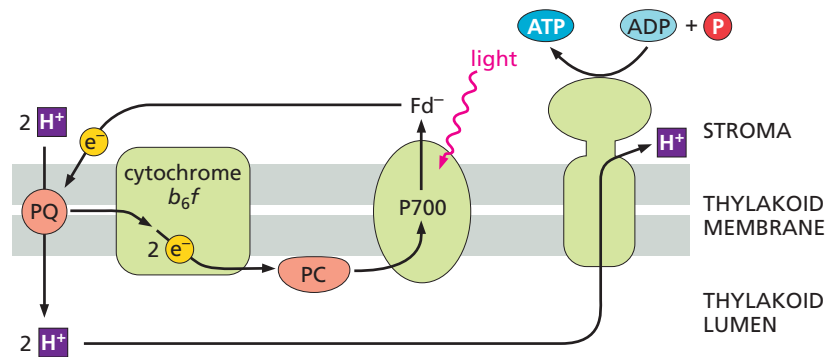


Figure OM4.2-1 ATP synthesis via cyclic electron transport.

OM4.3 REGULATION OF SUCROSE PHOSPHATE SYNTHASE

Two mechanisms render sucrose phosphate synthase highly sensitive to the ratio of glucose 6-phosphate to Pi in the cytosol (**Figure OM4.3-1**). First, these two metabolites directly modulate enzyme activity: a rise in the ratio of glucose 6-phosphate to Pi levels activates the enzyme. Second, sucrose phosphate synthase is phosphorylated and dephosphorylated by enzymes that are themselves modulated by the ratio of glucose 6-phosphate to Pi in the cytosol. Phosphorylation of a specific serine (Ser) residue in the sucrose phosphate synthase protein by a protein kinase – a sucrose phosphate synthase kinase called **SnRK1** (see Section 8.1) – reduces the activity of the enzyme, and removal of the phosphate by a protein phosphatase activates it. The kinase is inhibited by glucose 6-phosphate, and the phosphatase is inhibited by Pi. A rise in the ratio of glucose 6-phosphate to Pi thus decreases the level of phosphorylation of sucrose phosphate synthase, activating the enzyme and reinforcing the direct stimulation of activity brought about by this change in metabolite levels.

The importance of feed-forward regulation of sucrose phosphate synthase by the ratio of glucose 6-phosphate to Pi is illustrated when the rate of photosynthesis rises. An increased ratio of triose phosphate to Pi in the cytosol activates fructose 1,6-bisphosphatase, resulting in increased fructose 6-phosphate and hence glucose 6-phosphate levels. The consequent elevation of the ratio of glucose 6-phosphate to Pi in the cytosol activates sucrose phosphate synthase, so that its activity rises in concert with the rise in fructose 1,6-bisphosphatase activity and allows an increase in flux to sucrose. Feedback regulation of sucrose phosphate synthase is not fully understood; changes in sugar levels in the leaf are reflected in changes in the degree of activation of sucrose phosphate synthase but the mechanism by which this occurs remains uncertain.

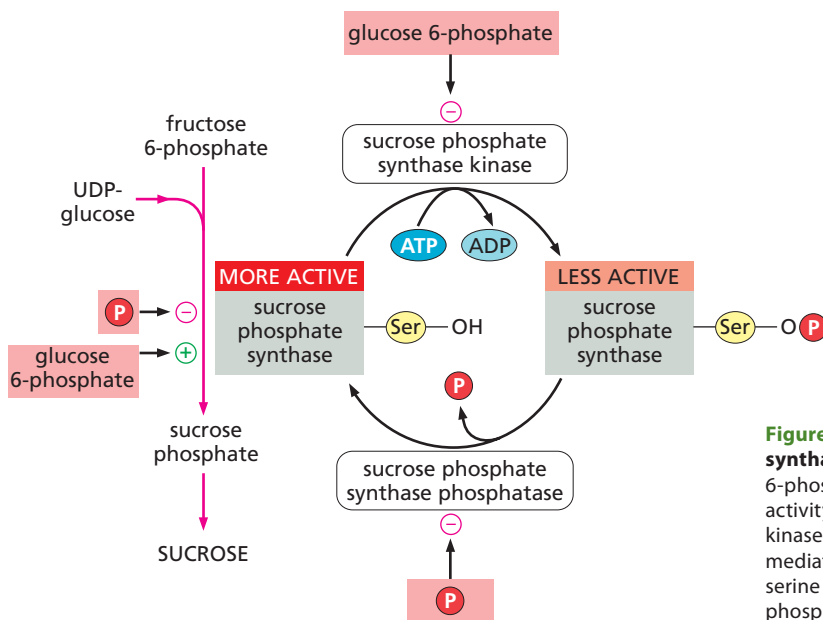


Figure OM4.3-1 Regulation of sucrose phosphate synthase by phosphorylation. Levels of glucose 6-phosphate and inorganic phosphate modulate enzyme activity both directly and through their effects on a protein kinase and a protein phosphatase. These two enzymes mediate the phosphorylation and dephosphorylation of a serine residue of the sucrose phosphate synthase protein; phosphorylation inactivates the enzyme.

OM4.4 ORIGINS AND FUNCTION OF PHOTORESPIRATION

The evolutionary origins of the oxygenase function of Rubisco and of the photorespiratory cycle have been the subject of much debate among plant physiologists. There is general agreement from comparisons of Rubisco sequence and structure in bacteria and plants that a Rubisco-like protein was responsible for CO₂ assimilation in the very earliest organisms capable of using inorganic carbon for growth, at least 3.5 billion (3.5 × 10⁹) years ago. These organisms existed in an essentially anoxic (oxygen-free) environment (see Chapter 1). Over about 1.5 billion years, the oxygen concentration in the Earth's atmosphere started to increase due to production of oxygen by microorganisms using water as an electron donor in photosynthesis. As the oxygen concentration increased, so did the oxygenase reaction of Rubisco.

The deleterious impact of the oxygenase reaction on photosynthetic CO₂ assimilation generated selection pressure for mechanisms that either converted the oxygenase product phosphoglycolate to a photosynthetic metabolite (as in the photorespiratory cycle) or prevented the oxygenation reaction. The ratio of oxygenase to carboxylase activity of Rubisco probably fell over evolutionary time due to selection for modifications to the active site of the enzyme that favored CO₂ rather than oxygen binding. Consistent with this idea, the Rubisco of modern photosynthetic bacteria that occupy anoxic environments has a higher ratio of oxygenase to carboxylase activity than the Rubisco of most plants. Some plants (C₄ plants, described in Section 4.5) have a mechanism that concentrates CO₂ to high levels in the chloroplasts that contain Rubisco, thereby eliminating oxygenation: the Rubiscos of these plants also have somewhat higher ratios of oxygenase to carboxylase activity than the Rubiscos of other plants (C₃ plants). This evolutionary change in properties of Rubisco is relatively small, however, and the enzyme of all modern plants retains a very substantial oxygenase activity.

Why has the apparently deleterious oxygenase reaction of Rubisco been retained? First, it may be impossible to reduce the oxygenase activity of Rubisco without adversely affecting the carboxylase activity. The Rubisco of modern plants may represent the most favorable ratio of oxygenase to carboxylase activity achievable by natural selection from the ancestral Rubisco – in other words, the oxygenase reaction is an inevitable consequence of the structural requirements for the carboxylase reaction. Support for this idea comes from extensive attempts to engineer an “improved” Rubisco, carried out from the 1970s onward. Researchers tried to change the amino acid sequence around the active site (the region of an enzyme protein that catalyzes conversion of the enzyme's substrates to its products) to improve the binding of CO₂ relative to oxygen. Although this work resulted in much valuable information about the reaction mechanism (the way in which substrate is converted to product in the active site of the enzyme), improvements in Rubisco properties have so far been limited.

Another possible explanation for the retention of the oxygenase reaction is that, under some conditions, it can help to prevent photoinhibition. As described in the main text (see Figure 4.33), when the stomatal pores of illuminated leaves shut to reduce water loss from the leaf to the atmosphere, the concentration of CO₂ inside the leaf falls as it is used up in photosynthesis. This low CO₂ condition could potentially lead to photoinhibition: the electron transport chain could become highly reduced, with consequent damage to the photosynthetic apparatus, because there is little CO₂ assimilation in the Calvin-Benson cycle and thus little consumption of NADPH and regeneration of NADP⁺ to accept electrons from PSI. Under these conditions, photorespiration helps to prevent the occurrence of photoinhibition. The consumption of ATP and NADPH in the photorespiratory cycle when CO₂ assimilation is low makes a major contribution toward dissipating excitation energy from the photosystems. The importance of photorespiration in preventing photoinhibition when CO₂ levels in the leaf are low is illustrated by experiments in which oxygen levels as well as CO₂ levels are strongly reduced in leaves in the light. When oxygen levels are normal and CO₂ levels are very low, no photoinhibition is observed. However, strong photoinhibition is observed when oxygen is also reduced to low levels, because there is no consumption of NADPH or ATP in the absence of photorespiration.

OM4.5 MODELING AND MEASUREMENT OF PHOTOSYNTHESIS

Our current understanding of the process of CO₂ assimilation in photosynthesis is founded to a large extent on a major conceptual breakthrough made over 40 years ago, combined with exciting technical innovations in our capacity to measure photosynthetic parameters. Here the first conceptual breakthrough – in the form of a model of the whole photosynthetic process – is outlined, followed by brief descriptions of four major technical innovations that underpin and complement the modeling approach: infrared gas analysis, leaf fluorescence measurement, measurement of carbon isotope ratios, and free-air CO₂-enrichment (FACE) experiments. This list of experimental approaches is by no means exhaustive. It is intended to provide a brief introduction to commonly used methods in photosynthesis research.

The FvCB model

The original model on which much of today's research in photosynthesis is based was first put forward in the early 1980s by Graham Farquhar and Susanne von Caemmerer at the Australian National University in Canberra, and Joe Berry at the Carnegie Institute, Stanford, California (hence it is known as the **FvCB model**). It has undergone many further elaborations and refinements, but the original concept remains of central importance. In essence, the model links experimentally determined aspects of photosynthetic biochemistry with measurements of leaf gas exchange. It employs a mathematical description of photosynthesis that integrates light-driven electron transport with assimilation of CO₂ via Rubisco and the Calvin–Benson cycle and its release in photorespiration. A major innovation of the model was the recognition that the rate of CO₂ assimilation (referred to as *A*) is a function of both light-dependent reactions and the reactions of the Calvin–Benson cycle, so it can be represented as the minimum of the potential values of these two processes under a given set of conditions. To illustrate this point, **Figure OM4.5-1A** shows the modeled changes in *A* with increasing CO₂ concentration in the chloroplast at three different light levels (solid lines), compared with the potential changes if *A* were limited by Calvin–Benson cycle reactions (largely Rubisco: blue lines) or light-dependent reactions alone (red line). The graph shows that at low CO₂ concentrations *A* is limited primarily by Calvin–Benson cycle reactions whereas at high CO₂ concentrations it is primarily limited by light.

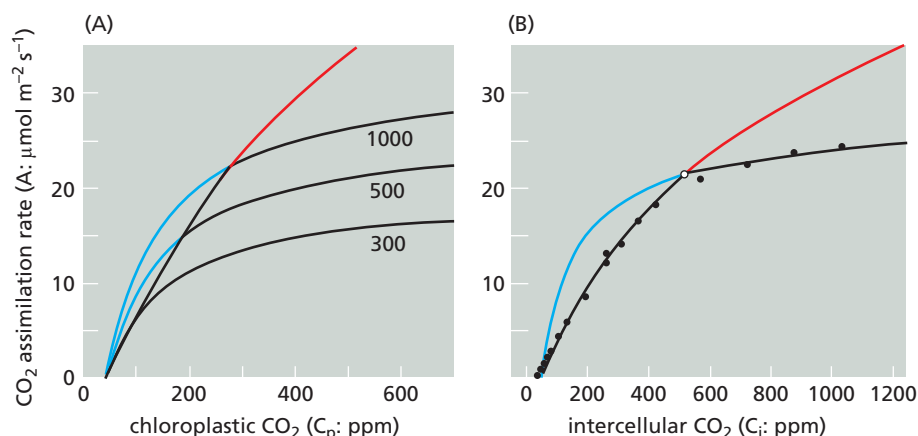


Figure OM4.5-1 Modeled and experimentally generated assimilation rate versus CO₂ concentration curves. (A) FvCB-modeled CO₂ assimilation rates (solid lines) as a function of chloroplastic CO₂ concentration ($p\text{CO}_2$) (an A/C_p curve) at irradiances of 300, 500, and 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The red and blue lines are the rates if limitation were by Rubisco alone (red) or electron transport alone (blue). (B) The FvCB model applied to an experimentally generated A/C_i curve. The black dots are experimental data from IRGA measurements; the black line is generated with the FvCB model. The experimental material was needles of balsam spruce at 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Blue and red lines are as in (A). (A, from Von Caemmerer S *et al.*, Biochemical model of C₃ photosynthesis. In Laisk A *et al.* (1990) *Photosynthesis in silico. Understanding Complexity from Molecules to Ecosystem*. Springer, Dordrecht, the Netherlands, pp. 209–230. With permission from Springer Nature. B, from Coursolle C *et al.* (2019) *Front Plant Sci* 10:1276. Distributed under CC BY license.)

The explanatory and predictive power of this model (and its many subsequent adjustments and refinements) lies at the heart of attempts to solve the interconnected global challenges of providing adequate food (food security) and adapting to climate change caused by the continuing rapid rise in atmospheric CO₂ concentration. In broad terms, FvCB-based modeling underpins research into global carbon cycling (see Figure 1.19) through its capacity to predict the impact of rising CO₂ and associated changes in temperature and rainfall on the rate at which the Earth's vegetation can assimilate and lock up (as wood and organic deposits) atmospheric carbon and thus ameliorate the impact of CO₂ produced by burning of fossil fuels.

Related research on crop photosynthesis uses FvCB-based modeling to predict how climate changes will affect crop productivity, and to propose ways in which productivity could be increased. The unprecedented development of plant biotechnology in recent decades offers potential ways of increasing photosynthesis and hence crop productivity through genetic manipulation and genome editing. Approaches being tested include introduction of CO₂ capture and concentration mechanisms into C₃ crops, changing the capacity of specific Calvin-Benson cycle reactions, manipulation of the light-driven reactions through, for example, changes in the composition of photosynthetic pigments in the leaf, and modulation of the rate at which leaf electron transport adjusts to changing light levels (also discussed in Online Material 4.4 and 4.6 and the Further Reading literature cited below).

Infrared gas analysis

The FvCB model was originally introduced to provide a rigorous framework for the evaluation of leaf gas exchange measurements made by infrared gas analysis. This technique of estimating CO₂ uptake by leaves makes use of the fact that CO₂ absorbs energy from infrared wavelengths (maximum at 4.25 μm; for comparison, chlorophyll *a* has an absorbance maximum at 0.68 μm). Comparison of absorbance at 4.25 μm of gas before and after exposure to a photosynthesizing leaf enables calculation of the amount of CO₂ taken up by the leaf. In its simplest form, an infrared gas analyzer (IRGA) measures CO₂ concentration of a gas stream before it enters and after it exits a chamber in which is clamped all or part of a leaf. From knowledge of the volume and flow rate of gas in the chamber, the area of the leaf and the drop in CO₂ concentration caused by passage of the gas through the chamber, the rate of assimilation, *A*, can be calculated as the amount of CO₂ taken up per unit time and per unit leaf area.

Chambers may contain a temperature controller, an adjustable light source and measuring device (a quantum sensor), and a fluorescence detector (see later). The gas used in the chamber is usually an atmosphere-like mix of precisely known composition, in which the CO₂ concentration entering the chamber can be adjusted. IRGAs usually also measure water vapor released into the gas passing over the leaf, using the fact that water vapor, like CO₂, absorbs wavelengths in the infrared. The amount of water vapor released by the leaf from its vapor-saturated intercellular spaces – its transpiration rate – can be used to calculate the resistance to gas flow imposed by the stomata. From these data, it is possible to estimate the concentration of CO₂ inside the leaf, and thus to construct experimentally an *A* versus CO₂ concentration curve (known as an *A/C_i* curve, where *C_i* is the concentration of CO₂ in the intercellular spaces: **Figure OM4.5-1B**). These curves, and *A*/irradiance curves, allow analysis of many aspects of leaf photosynthetic performance.

Early IRGAs were large pieces of equipment that were slow and cumbersome to use and unsuitable for field measurements. The development of hand-held, lightweight IRGAs with sophisticated software and environmental controls in the chamber has enabled much more rapid and comprehensive photosynthesis measurements in a wide range of field conditions (**Figure OM4.5-2**). Nonetheless, difficulties remain in extrapolating from measurements on single leaves in defined conditions to photosynthesis in the whole canopy of a crop or natural stand of vegetation in which individual leaves experience widely different ranges of light level and quality, and local variation in CO₂, temperature, and water deficit.



Figure OM4.5-2 A portable IRGA in use in a wheat field. The researcher is supporting a box containing a computer, gas supplies, and batteries. The silver box on the hand-held device is the leaf chamber, clamped onto a wheat leaf. (From "Measuring photosynthetic efficiency using an LCpro-SD," by Steveaducuk, Wikimedia.)

Chlorophyll fluorescence measurement

Measurement of chlorophyll fluorescence is the most widely used method for rapid assessment of photosynthetic performance. Small, hand-held fluorimeters that employ a technique called **pulse-amplitude modulation (PAM)** can be used to make many, rapid measurements of fluorescence emission on attached leaves in controlled or field conditions, potentially yielding a wealth of information about the efficiency of photosynthesis. A brief description of the principles underlying this technique is as follows.

As discussed in Section 4.2, excitation energy in PSII can be dissipated (quenched) in two main ways. First, it can be dissipated through the electron transport chain in the thylakoid membranes, ultimately resulting in the synthesis of ATP and NADPH (photochemical quenching). Second, if excitation builds up in PSII because of limited capacity for electron transport, two main mechanisms of non-photochemical quenching (NPQ) come into play. They are the dissipation of excitation energy as heat (for example through the xanthophyll cycle), and its dissipation as fluorescence. In a leaf with a high rate of CO₂ assimilation, fluorescence is very low because excitation in PSII is photochemically quenched. PSII is said to be in an "open" state, able to receive electrons from its antennae. If the rate of ATP and NADPH consumption is low and excitation energy builds up in PSII, fluorescence emission is high. PSII is said to be in a closed state – it cannot accept any more electrons from antennal chlorophylls.

Chlorophyll fluorescence analyzers first measure fluorescence emission when PSII is in a closed state. This is achieved by applying a 1- or 2-second pulse of saturating light. Comparison of this value, F_m , with values obtained under various other conditions can provide insight into many aspects of photosynthetic performance, including the extent of NPQ, the efficiency of PSII photochemistry, the rate of electron transport, and the occurrence of photoinhibition. Fluorescence measurements are used in field conditions to assess general plant health: deviations of fluorescence values from those of a healthy plant can indicate disease, poor mineral status, and stress from heat, cold, or high light for example. With appropriate equipment, fluorescence can be imaged across the surface of whole plants or – using drones or elevated cameras – on canopies of crops and natural vegetation to reveal the spatial distribution of fluorescence variation. **Figure OM4.5-3** shows how data from fluorescence imaging can be used to detect the spread of infection by the hemibiotrophic fungus *Colletotrichum orbiculare* across a leaf before any symptoms become visible to the naked eye. A further example of the use of fluorescence imaging – to reveal damage from cold – is shown in Figure OM13.2-1.

Although fluorescence measurements provide a rapid and convenient means of assessing photosynthetic performance, and provide types of information not obtainable from infrared gas analysis, they do not give a direct measurement of CO₂ assimilation. It is now possible to combine PAM, IRGA, and sophisticated software into a single hand-held device, providing an unprecedented wealth of information about photosynthetic performance in controlled and field conditions.

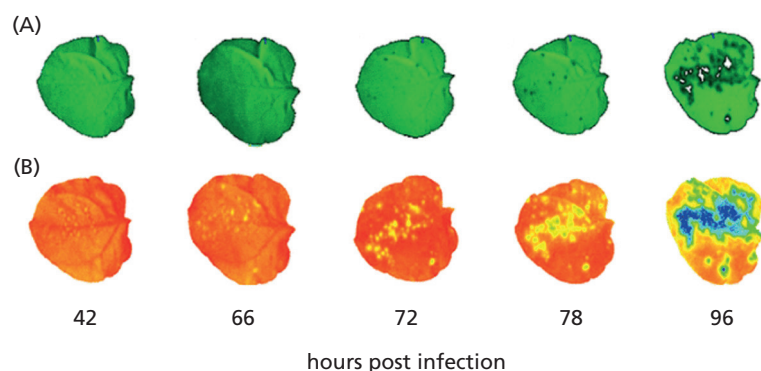


Figure OM4.5-3 Fluorescence emission by leaves infected with a hemibiotrophic fungus. The figure shows progressive stages in fungal infection from 48 to 96 hours post infection. (A) Leaves are imaged in visible light. (B) Fluorescence emission is imaged and presented as a heat map of Fv/Fm values. Fv is variable fluorescence: in essence it is the difference between Fm (PSII closed) and F0, the fluorescence emission when PSII is fully open. Fv/Fm values are used as a measure of PSII efficiency and, by extrapolation, photosynthetic performance. Damage to PSII, for example by photoinhibition, results in lower Fv/Fm values than those of a leaf with high photosynthetic performance. Orange areas have the highest Fv/Fm values; blue areas have the lowest. Damage to PSII is revealed as yellow areas about 30 hours before symptoms of infection are visible to the eye. (From Tung J *et al.* (2013) *Eur J Plant Pathol* 136:301–315. With permission from Springer Nature.)

Carbon isotope discrimination

Information about several important photosynthetic processes can be gained by measuring the carbon isotope ratio of plant material. The basis of these measurements is as follows. The carbon isotope ratio (known as a $\delta^{13}\text{C}$ value, pronounced delta thirteen C) of a carbon-containing material is defined as the abundance of the scarce carbon isotope ^{13}C (1.1% of carbon atoms in atmospheric CO_2) relative to that of the abundant isotope ^{12}C (98.9% of carbon atoms in atmospheric CO_2), expressed with reference to the ratio in an accepted standard (the Late Cretaceous Peedee limestone of North and South Carolina, USA) that is set at zero. The atmosphere has a $\delta^{13}\text{C}$ value of -8‰ (minus 8 parts per thousand). C_3 plants have more negative values than the atmosphere from which their carbon is derived; typically in the range -20 to -37‰ , meaning that they have a lower ratio of ^{13}C to ^{12}C than the atmosphere. In general terms, this difference between plants and the atmosphere arises because $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ molecules differ in the speed at which they diffuse into the plant and are processed during carbon assimilation. Precise understanding of how discrimination against ^{13}C happens inside the plant has allowed researchers to use $\delta^{13}\text{C}$ values to study processes as diverse as the extent of water deficit experienced during growth of different species or ecotypes, the abundance and geographic distribution of C_4 and CAM species in the world's flora, and the contribution of photosynthesis in the biosphere to global carbon cycling.

Discrimination against ^{13}C during photosynthesis occurs by two main routes. Most of the discrimination arises at the level of Rubisco. The active site of Rubisco is competed for by $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$. The slower speed of reaction of $^{13}\text{CO}_2$ results in products of assimilation that are enriched in ^{12}C relative to the atmosphere: in other words their $\delta^{13}\text{C}$ value is more negative than that of atmospheric CO_2 . The second cause of discrimination against ^{13}C relates to the pathway of diffusion of CO_2 from the atmosphere into the intercellular spaces of the leaf via the stomata. As described in Section 4.4, this route presents significant resistance to CO_2 diffusion. Because ^{13}C diffuses more slowly than $^{12}\text{CO}_2$, CO_2 available for assimilation in the chloroplast is enriched in ^{12}C relative to C_4 . The degree of enrichment depends on stomatal conductance: the lower the conductance (in other words the greater the degree of resistance to diffusion), the greater the discrimination.

These causes of discrimination against ^{13}C by plants provide many opportunities to gain information about photosynthesis from measurements of the $\delta^{13}\text{C}$ value of plant material. Two (out of many) important uses are outlined briefly.

First, $\delta^{13}\text{C}$ values can be used to determine whether a given plant employs C_3 or C_4 photosynthesis. As described in Section 4.5, primary assimilation of carbon from the atmosphere in C_4 plants occurs via PEP carboxylase in the outer, mesophyll cells. PEP carboxylase uses bicarbonate (not CO_2) to generate a four-carbon compound that moves into the inner bundle sheath cells where it is decarboxylated to provide a high concentration of CO_2 for assimilation via Rubisco. There is no discrimination against ^{13}C during the primary assimilation step. In theory, if the bundle-sheath cells were gas tight, Rubisco would also exercise no discrimination because it would consume all of, and only, the CO_2 pool provided by decarboxylation of the PEP carboxylase products. In practice the bundle sheath is somewhat leaky to CO_2 , so Rubisco does discriminate to a limited extent against ^{13}C in C_4 plants. Overall, however, the $\delta^{13}\text{C}$ of photosynthetic products of

C₄ plants is considerably less negative than that of C₃ plants (-12 to -16‰, compared with C₃ values of around -20 to -37‰). Measurements of δ¹³C values of plant material can therefore be used to identify whether a particular species is C₃ or C₄. The method can be used on small pieces of dried material, enabling knowledge of the occurrence of C₄ plants to be extended by measurements on herbarium collections as well as living plant collections.

Second, because δ¹³C values are related to stomatal conductance, they can be used in various ways to assess plant-water relationships. For example, the degree of drought stress experienced by plants during growth is reflected in the δ¹³C values of their tissues. Plants that have experienced water deficit will have more negative δ¹³C values than well-watered plants. δ¹³C values have been evaluated for use in breeding drought-resistant crops, as a means to select lines likely to have high water-use efficiency (amount of carbon gained per amount of water consumed) and hence a lower water requirement for a given yield. Lines are selected that have both high yields and low (very negative) δ¹³C values. However, because numerous environmental and genetic factors can influence δ¹³C values of a crop over its growing season, the association between these values and water-use efficiency is often not sufficiently strong for use as a robust tool in plant breeding.

Free-air CO₂ enrichment

It is abundantly clear that the current and continuing increase in atmospheric CO₂ levels will have a major impact on the Earth's vegetation. Early predictions were that there would be a "CO₂ fertilization" effect, in which rising CO₂ would lead to substantial increases in the rate of plant growth because of increased rates of photosynthesis. However, the knock-on effects on plants and plant communities of elevated CO₂ were largely unknown. The speed and magnitude of the increase in atmospheric CO₂ (from <360 to 430 ppm in 30 years) has precluded the possibility that plants could adapt to their new conditions by natural selection.

Initial experiments with elevated CO₂ were done on plants in controlled environments that did not replicate conditions in natural vegetation or crop fields. Over the last 30 years, equipment has been devised to elevate CO₂ concentrations in otherwise natural, outdoor conditions, and a wide range of crops, natural grasslands, and forests have been studied over long periods. Free-air CO₂ enrichment equipment consists of a large (up to about 300 m²) plot of vegetation surrounded by a ring of pipes with valves that release CO₂ or CO₂-enriched air into the plot. CO₂ monitors at the center of the plot are linked to computer control of the valves so that the desired elevation of CO₂ is maintained across the plot (**Figure OM4.5-4**). The short- and long-term responses of plants in the plot (using IRGA and PAM analysis for photosynthesis and evaluating growth, yield, composition, etc.) are compared with those in a control plot without CO₂ enrichment.



Figure OM4.5-4 Free-air CO₂ enrichment (FACE) experiments on forested land in Wisconsin.

The vertical pipes surrounding areas of shrubs and trees release CO₂ at different levels in the canopy so that an even spatial distribution of supplied CO₂ is achieved throughout the plot. (Courtesy of David F. Karnosky.)

FACE experiments have revealed complex, highly variable responses of plants to elevated CO₂. Part of the variation is due to the use of different equipment, plant species, and environments, and different degrees of elevation of CO₂ relative to atmospheric levels at the time the experiments were set up. Some common conclusions can be drawn however. The following are a few examples: the reader is referred to the references for more information. First, it is clear that yields of most species are not increased to the extent predicted from consideration of photosynthesis in isolation. Second, plants grown under high CO₂ are often enriched in carbohydrates and relatively depleted in other elements and minerals relative to controls. Third, some environmental conditions including water and nitrogen availability and temperature strongly affect the extent to which elevated yields of biomass can be achieved. Fourth, microbial infections and predation by pests may be increased in high CO₂. Fifth, plants grown at high CO₂ have different influences from control plants on the composition and microbiome of their soils.

The conclusions from FACE experiments have profound implications for food security and quality, for sustainable farming, and for the capacity of the Earth's vegetation to buffer the CO₂ concentration in the atmosphere. The detailed results also allow researchers to suggest how genetic manipulation of specific components of the photosynthetic apparatus can boost photosynthetic efficiency at high CO₂. Modeling and experiments to test these suggestions are in progress.

Further reading

Ainsworth EA, Long SP (2021) 30 years of free-air carbon dioxide enrichment (FACE):

What have we learned about future crop productivity and its potential for adaptation? *Glob Chang Biol* 27:27–49.

Simkin AJ, López-Calcagno PE, Raines CA (2019) Feeding the world: improving photosynthetic efficiency for sustainable crop production. *J Exp Bot* 70: 1119–1140.

OM4.6 C₄ PHOTOSYNTHESIS HAS ARISEN MANY TIMES DURING THE EVOLUTION OF ANGIOSPERMS

When the C₄ pathway was discovered in the late 1960s by Hal Hatch and Roger Slack in Australia, its potential advantages over C₃ photosynthesis in environments with high light and temperature and low water availability were soon apparent. Hatch and Slack studied the highly productive tropical crop sugar cane, and analyses of the distribution of other C₄ species showed that they dominated several hot, dry regions of the Earth. For example, 60–80% of the grass species in three natural habitats on the USA–Mexico border were found to be C₄, whereas 0% and 12% of the grass species were C₄ in two natural habitats in central Canada. It was initially supposed that C₄ photosynthesis had arisen as an adaptation to hot, dry climates. However, this idea proved to be simplistic. Subsequent work showed that many C₃ species are also confined to hot, arid habitats, and that although C₄ species are abundant in hot, dry areas of North America, in some other parts of the world there are very few C₄ species in habitats with the same climate. This means that C₄ metabolism is only one of many adaptations that allow survival in these climates, and it does not represent an overwhelming selective advantage under these conditions. (Note that a related type of photosynthetic metabolism, crassulacean acid metabolism (CAM), has obvious selective advantages in hot, dry conditions: CAM is discussed in the main text.)

Increasing understanding of when C₄ plants first appeared and of changes in the CO₂ concentration in Earth's atmosphere gave rise to more sophisticated ideas about the selective pressures that gave rise to C₄ photosynthesis. C₄ plants are of recent evolutionary origin: the first traces in the fossil record are from about 30 million years ago. This point saw a fall in the CO₂ concentration in the atmosphere, from levels higher than today to values below 300 ppm (see Section 1.3). The coincidence of these events indicates that the original appearance of C₄ photosynthesis was favored by the fall in CO₂ concentration in the atmosphere, which would have strongly reduced growth rates of plants living at that time. The major expansion of C₄ species occurred around 10 million years ago, at a time when dry, open landscapes became more dominant in tropical and subtropical regions.

Remarkably, C₄ has arisen independently many times since its first appearance 30 million years ago (i.e. it is **polyphyletic**) – an example of **convergent evolution** (acquisition of similar functional characteristics in plant species after their divergence from a common ancestor: another striking example is the acquisition of carnivory in diverse species of nutrient-poor habitats, described in Section 13.5). About 20 families and more than 60 genera of angiosperms contain C₄ species, including both monocots and eudicots. All of the families with C₄ species also contain C₃ species (**Figure OM4.6-1**). The complexity of the C₄ mechanism – involving a host of changes to both the anatomy and the biochemistry of the leaf – raises interesting questions about how it has evolved so many times in such diverse genetic backgrounds.

It is clear that the evolution of C₄ photosynthesis involves changes in the functions of proteins encoded by existing genes, rather than the appearance of new genes not found in C₃ species. The acquisition of new functions generally involves changes in spatial and temporal patterns of expression of particular genes, often affecting one copy of a gene duplicated by a previous genome duplication event (see Section 2.4). The advent of large-scale **transcriptome** sequencing (sequencing of all of the mRNAs present in a particular tissue or set of conditions) has been particularly helpful in shedding light on which genes are involved, and whether the mechanisms are the same in each instance of C₄ evolution. Patterns of gene expression found in C₄ species but absent from C₃ species have emerged from transcriptome comparisons of large numbers of C₃ and C₄ species from different families. It seems that changes in relatively few genes (a few hundred) underlie the C₄ mechanism. Many of these genes encode proteins with metabolic functions in photosynthesis, photorespiration, and related metabolic processes. Alterations in their expression or properties in C₄ plants have been brought about at several different levels depending on the species and enzyme. They include changes at epigenetic, transcriptional, and translational levels, post-transcriptional modifications, and protein turnover. For example, genes encoding some key enzymes of the C₄ cycle possess *cis* elements absent from the equivalent genes of C₃ plants. These elements direct spatial patterns of expression and responses to environmental factors such as light. Other genes important for the changes that allowed the evolution of C₄ species encode transcription factors. The emergence of Kranz anatomy is thought to have been brought about largely by changes in developmental transcription factors.



Figure OM4.6-1 Some closely related C_3 and C_4 species. (A) *Suaeda maritima*, a C_3 species found on seashores (left), and *Suaeda moquinii*, a C_4 species of deserts in the southwestern USA (right). *Suaeda* is in the family Chenopodiaceae, in which C_4 photosynthesis has arisen independently several times. (B) A C_3 and a C_4 species in the Cleomaceae (*Tarenaya hassleriana* and *Gynandropsis gynandra*) that are very similar in leaf and flower morphology (left) but radically different in leaf anatomy (right). *T. hassleriana* leaves have an upper palisade and a lower spongy mesophyll layer of cells typical of C_3 leaves; *G. gynandra* leaves have Kranz anatomy typical of C_4 leaves. The Cleomaceae is closely related to the Brassica family that includes *Arabidopsis*. (A left, courtesy of Bob Gibbons/Science Photo Library. A right, courtesy of Keir Morse, www.keiriosity.com. B, photos kindly provided by Tina Schreier and Julian Hibberd.)

Some clues about the process by which C_4 photosynthesis may have evolved come from the study of so-called “ C_3 - C_4 intermediate” plants. These were originally discovered because their CO_2 compensation points are lower than those of C_3 plants but not nearly as low as those of C_4 plants. Like C_4 plants, they have arisen multiple times during the evolution of higher plants. The genus *Moricandia* (Brassicaceae) contains C_3 and C_3 - C_4 members, and the genera *Panicum* (Poaceae) and *Flaveria* (Asteraceae, or Compositae) contain C_3 , C_4 , and C_3 - C_4 members. Like C_4 plants, C_3 - C_4 plants have a mechanism to reduce the release of photorespiratory CO_2 . However, most of these species do not have true Kranz anatomy, a C_4 cycle, or differentiation of chloroplast function. Instead, the reduction in compensation point is achieved by a differentiation of mitochondrial function such that photorespiratory CO_2 is released only from cells adjacent to the vascular bundle (Figure OM4.6-2). This spatial arrangement increases the efficiency with which photorespiratory CO_2 is recaptured by the Calvin-Benson cycle before it can escape from the leaf.

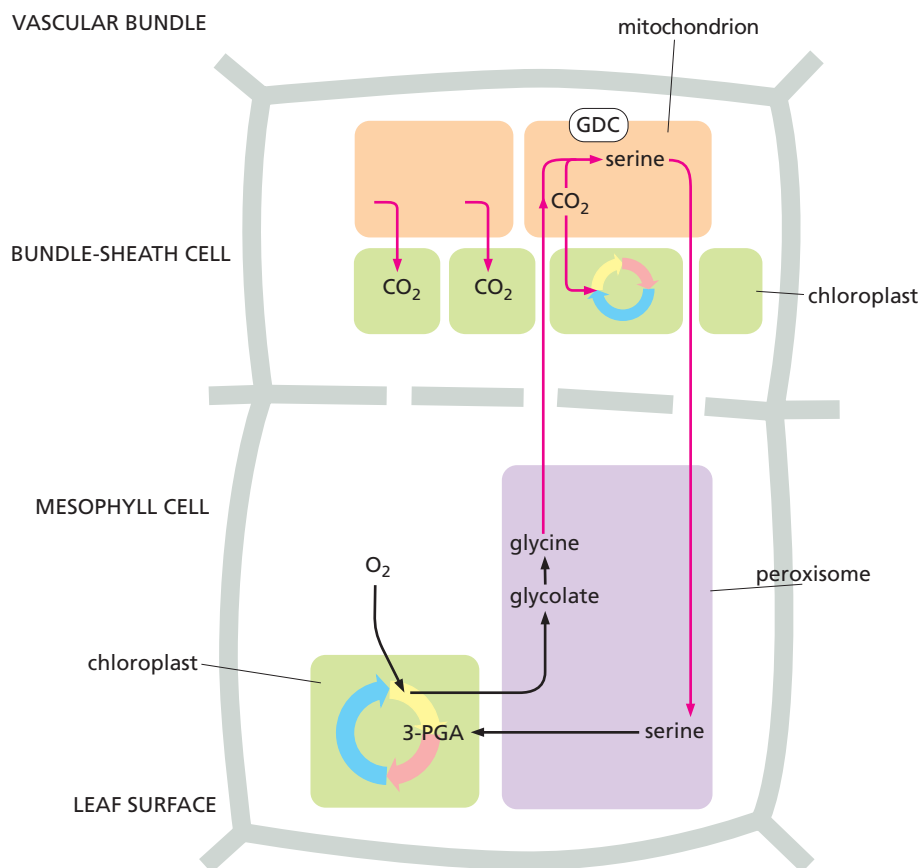


Figure OM4.6-2 Metabolism and leaf anatomy of C₃-C₄ intermediate species. Glycine produced in mesophyll cells during photorespiration diffuses to the bundle-sheath cells, the only leaf cells that contain glycine decarboxylase (GDC). In the bundle sheath, the CO₂ released via GDC must pass through a layer of chloroplasts, increasing the chance that it will be reassimilated before it can escape from the leaf. 3-PGA, 3-phosphoglycerate.

Can CO₂-concentrating mechanisms be engineered into C₃ crops?

Concerns about global food security in the face of population increase and urbanization have prompted large-scale international research projects to understand better existing CO₂-concentrating mechanisms such as C₄ photosynthesis, and to introduce these mechanisms into C₃ crops such as wheat and rice. It is likely that these mechanisms would increase rates of net CO₂ assimilation, and thus could potentially increase crop yields. These projects are discussed in outline here. In parallel, new research on Rubisco seeks to explore potentially useful variation in Rubisco and Rubisco activase properties across photosynthetic organisms. Although direct attempts to engineer Rubisco have had limited success, it is possible that the introduction into crop plants of Rubisco or Rubisco activase proteins from other species will improve photosynthetic efficiency. Other researchers are exploring the possibility of introducing alternative methods of recapture of phosphoglycolate into the leaves of C₃ crops. Several schemes that use bacterial enzymes to convert 2-phosphoglycolate to photosynthetic metabolites with greater efficiency than the photorespiratory cycle have been proposed, and some of these new pathways have been shown to increase net CO₂ assimilation when introduced into transgenic plants (Figure OM4.6-3).

Much of the recent progress in understanding C₄ photosynthesis has come from scientific consortia with the ultimate goal of introducing the C₄ mechanism into the C₃ crop rice. This goal is hugely ambitious, but it has already driven important discoveries about photosynthesis and leaf anatomy. In parallel, researchers are trying to introduce into plants **CO₂-concentrating mechanisms (CCMs)** that are found in single-celled algae and in cyanobacteria. These CCMs involve fewer genes than C₄ photosynthesis, but there is uncertainty about whether they can operate successfully in a multicellular leaf. The algal and cyanobacterial cells live in water, in which CO₂ availability is very low but bicarbonate is plentiful. In essence, the cells import bicarbonate via specific transporters in their outer membranes, elevating the concentration in the cell. The Rubisco proteins in the cells are tightly clustered together: in **carboxysomes** in cyanobacteria and in **pyrenoids** in algae. The clusters are associated with the enzyme

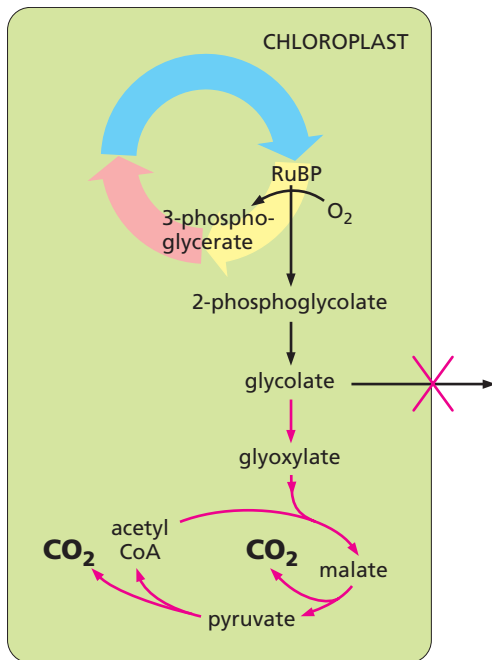


Figure OM4.6-3 Example of new reactions introduced into chloroplasts in transgenic plants to reduce photorespiratory CO_2 loss. Black arrows are endogenous reactions; magenta arrows show new steps catalyzed by introduced enzymes. In addition to the new steps, the expression of the transporter that exports glycolate from the chloroplast was down-regulated. This scheme does not prevent production of CO_2 from 2-phosphoglycolate, but it ensures that CO_2 is released in the chloroplast and not the mitochondrion as in the photorespiratory cycle (see Figure 4.34). This greatly enhances recapture in the Calvin-Benson cycle of the released CO_2 . Testing in transgenic tobacco plants in the field showed that these modifications could enhance plant productivity.

carbonic anhydrase, which interconverts bicarbonate and CO_2 . This arrangement generates high concentrations of CO_2 adjacent to Rubisco, saturating the enzyme with CO_2 and suppressing photorespiration (Figure OM4.6-4). Some components of these mechanisms have been introduced into plant leaves, but assembly of a complete and effective CCM is likely to prove very challenging.

Although the introduction of CCMs is an important goal for improvement of C_3 crop productivity over the next few decades, the predicted doubling of CO_2 in the atmosphere by the end of this century will present different challenges for the maintenance of crop productivity. At atmospheric CO_2 concentrations over 700 ppm, photorespiration will be strongly suppressed in C_3 plants and some advantages of C_4 over C_3 photosynthesis at the present time may well be lost.

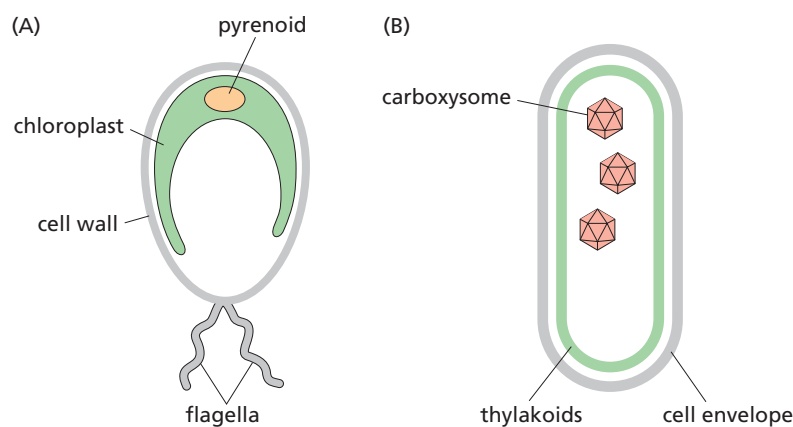


Figure OM4.6-4 Carbon-concentrating mechanisms in single-celled photosynthetic organisms. (A) The freshwater alga *Chlamydomonas*. The cell has a single, large, cup-shaped chloroplast in which Rubisco and a Rubisco-binding protein aggregate and undergo a **phase separation** that concentrates Rubisco, Rubisco activase, and carbonic anhydrase in a structure called a pyrenoid. Bicarbonate is transported into the cell and then into the pyrenoid. Release of CO_2 from bicarbonate inside the pyrenoid saturates Rubisco even when external CO_2 concentrations are low. (B) In prokaryotic cyanobacterial cells, Rubisco and carbonic anhydrase occur together in regular arrays inside a polyhedral protein shell called a carboxysome. Bicarbonate is pumped into the cell and converted to CO_2 by carbonic anhydrase inside the carboxysome, saturating Rubisco with CO_2 .

OM4.7 EVOLUTION OF CAM PHOTOSYNTHESIS

As for the evolution of C_4 from C_3 photosynthesis, the evolution of CAM and the switch in facultative CAM plants between C_3 and CAM involves altered spatial and temporal patterns of expression of a limited set of genes, under the control of a handful of transcription factors. Changes in the way in which enzyme activities are controlled are a major feature. For example, the control of PEP carboxylase activity changes as a facultative CAM plant transitions from C_3 to CAM in response to water stress. The CAM PEP carboxylase enzyme is activated specifically at night by phosphorylation by a protein kinase, the gene for which is expressed at night under the control of the circadian clock. These mechanisms for control of PEP carboxylase activity disappear if the plant is rewatered and reverts to C_3 photosynthesis.

The changes required to convert C_3 to CAM can be regarded as less complex than those required for the evolution of C_4 from C_3 plants. CAM occurs within a single cell, and there is no requirement for a separate cell type as there is in C_4 . The lower complexity of CAM may help to account for its extremely wide distribution among plant species. Like C_4 photosynthesis it has arisen multiple times independently, but rather than being confined to some families of angiosperms it is also found in ferns, lycophytes, and gnetales (see Chapter 1).