PHOTOSYNTHESIS

2.1 INTRODUCTION

Green plants obtain all their energy by photosynthesis, the process in which light is converted to chemical energy. The light reactions are the 'photo' part of photosynthesis, while the so-called 'dark' reactions constitute the 'synthesis' part of the process, which is also called carbon fixation. The light and dark reactions are intimately linked and are summarized by the left-to-right reaction in Equation 2.1, the overall equation for photosynthesis in plants, algae and cyanobacteria.

Note that $[CH_2O]$ denotes carbohydrate.

$$CO_2 + H_2O \underset{\text{RESPIRATION (in light and dark)}}{\overset{\text{PHOTOSYNTHESIS (in light)}}{\rightleftharpoons}} [CH_2O] + O_2 \qquad (2.1)$$

The right-to-left reaction in Equation 2.1 emphasizes that, like all other aerobic organisms, plants carry out respiration which, in summary, is the reverse of photosynthesis. This simple fact has profound implications when measuring rates of photosynthesis, depending on the level at which the process is being studied. Photosynthesis (like respiration) can be studied at the molecular level, but there are two other levels at which the process is studied and explained (see Table 2.1).

Table 2.1 Levels at which photosynthesis is studied and explained in different biological material using different experimental systems and time-scales.

Level of explanation	Subject area	Material studied	Common types of measurement	Measurement time-scale
level 1: molecules, organelles and cells	biochemistry	isolated chloroplasts, single -celled green algae or purified enzymes	O_2 release, CO_2 uptake, enzyme activity, chlorophyll fluorescence*	seconds (or below) to minutes
level 2: whole plants or leaves	plant physiology	single plants or leaves in controlled conditions	O_2 release, CO_2 uptake	minutes to hours
level 3: communities or crops	ecology or crop physiology (agronomy)	(i) groups of plants growing outside in the field.	gain in dry weight	weeks to months or years
		(ii) phytoplankton in open water	O_2 release, CO_2 uptake	minutes to hours

* Fluorescence is discussed in Box 2.1.

❍ Bearing in mind Equation 2.1, how might respiration affect measurements of the rate of photosynthesis at level 2 (whole plants or leaves)?

● Respiration reduces the apparent (measured) rate of photosynthesis because some of the CO_2 uptake (or O_2 release) is balanced by CO_2 release (or O_2 uptake) in respiration.

At level 2, what is measured is called **net photosynthesis (*NP*)**, which is total or **gross photosynthesis (*GP*)** minus respiration (*R*). In symbols:

$$NP = GP - R \qquad (2.2)$$

❍ Does respiration affect measurement of photosynthesis at levels 1 and 3, i.e. do these measurements relate to gross or net photosynthesis?

● For level 1, there is no effect of respiration: measurements with isolated chloroplasts inevitably relate to gross photosynthesis because respiratory organelles (mitochondria) are absent and, even with whole algal or plant cells, the very short time-scales of measurement mean that respiration can be ignored. For level 3, yes, respiration does have an effect: *net* gain in dry weight is measured and corresponds to net photosynthesis (carbon fixed) plus assimilation of all the other elements (nitrogen, phosphorus, etc.) that make up organic matter. Over long periods of time, a substantial loss of fixed carbon occurs through respiration.

The above case is a simple example of how the level of measurement or study affects interpretation of experimental results. Whenever you see data about the rate of photosynthesis, think about whether gross or net photosynthesis is implied.

This chapter is concerned mainly with levels 1 and 2 and tries to do three things:

(i) Consolidate (for the light reactions of photosynthesis) and introduce (for carbon fixation) the basic biochemical mechanisms that are common to all green plants.

(ii) Illustrate how these molecular processes are controlled and how environmental factors such as temperature and light intensity influence photosynthesis at both the molecular level and the whole-plant level.

(iii) Show how plants have adapted to different environments by modifications at both levels. For example, some plants can grow in deep shade below other plants and the mechanisms that enable them to do this include changes in leaf structure (level 2) coupled with molecular changes in the light-trapping systems and their control (level 1, discussed in Section 2.2). Variation in photosynthetic systems is one factor (among many) that contributes to plant diversity.

We begin (Section 2.2) with the light reactions, including their control, coordination and protection. Absorbing high intensity light, for example, is potentially dangerous because it can generate free radicals and cause oxidative damage, so elaborate protective mechanisms have evolved (Section 2.2.3).

Section 2.3 then describes the basic dark reactions of carbon fixation, the *C3* or *Calvin cycle* (Section 2.3.1). In green plants (and many autotrophic bacteria) this pathway alone gives a *net* fixation of CO_2. The first step in the pathway, in which CO_2 reacts, in a *carboxylase* reaction, is catalysed by a huge enzyme complex, *Rubisco* (whose name is explained in Section 2.3.2), which plays a key regulatory role. The active site for CO_2 on Rubisco also binds O_2 and catalyses an *oxidase* reaction. So oxygen acts as a competitive inhibitor of carbon fixation in the C3 cycle, and the product of the oxidase reaction is the substrate of an 'anti'-photosynthetic pathway known as the *C2 cycle* or *photorespiration* (Section 2.4). The overall reaction of the C2 cycle is the same as for respiration, i.e. O_2 uptake and CO_2 release, but unlike true respiration, no ATP is generated. Although it might seem paradoxical, the C2 cycle is now regarded as an integral and probably essential part of photosynthetic carbon metabolism.

In Sections 2.5 and 2.6 we focus more on whole plants and the ways in which environmental factors affect the rate of photosynthesis. Certain environmental conditions, notably high light intensity, high temperature and low availability of CO_2, strongly favour the oxidase reaction of Rubisco over the carboxylase reaction, resulting in a reduction of net photosynthesis and plant growth. However, some plants have evolved mechanisms that circumvent this problem by effectively concentrating CO_2 at the site of Rubisco action, so that CO_2 out-competes O_2. The mechanisms, involving both additional biochemical pathways and structural changes in green tissues, are found in so-called C4 and CAM plants (Sections 2.5.2 and 2.5.3) and are an important aspect of the diversification of plants and their adaptation to a wide range of environments.

The control of photosynthesis and what determines its overall rate are prominent themes in this chapter and are of more than academic interest, because photosynthesis in crop plants equates broadly with plant growth and food production. There is much controversy, for example, as to whether rising levels of atmospheric CO_2 might have the beneficial effect of increasing crop growth and this question is one we ask you to think about again after reading the chapter. Another question we consider is whether photosynthetic performance might be improved by introducing new or modified genes using the techniques of genetic engineering.

2.2 PHOTOSYNTHETIC LIGHT REACTIONS: CONTROL AND PROTECTION

2.2.1 CHLOROPLASTS AND THE STUDY OF THE LIGHT REACTIONS

You will already be familiar with the biochemistry of the light reactions. In this chapter, we consider the reactions more from the plants' perspective but, since you need to be completely familiar with the basic light reactions and with chloroplast structure, Figures 2.1 and 2.2 are included for easy reference.

❍ (i) *Before* looking at the legend to Figure 2.1, give the full names of all the components whose names are abbreviated (PSII, etc.). (ii) Using Figure 2.2b as a guide (if necessary), name the structures or regions labelled A–D in Figure 2.2a.

● (i) See the legend to Figure 2.1. (ii) A, double outer membrane or envelope (the outer and inner membranes are labelled separately in Figure 2.2b); B, stroma (the aqueous matrix); C, stroma lamellae; D, granum composed of a stack of membranous vesicles called thylakoids.

Some additional information about the antenna systems (antennae) is relevant to this chapter. First, they contain two other types of pigment in addition to chlorophyll *a*: chlorophyll *b* and carotenoids (the pigments that give carrots their orange colour and contribute to autumn leaf colours). These **accessory pigments** absorb light of shorter (i.e. higher energy) wavelengths than does chlorophyll *a*, so they increase the width of the spectrum available for photosynthesis. Energy transfer occurs in the sequence: carotenoids → chlorophyll *b* → chlorophyll *a* →

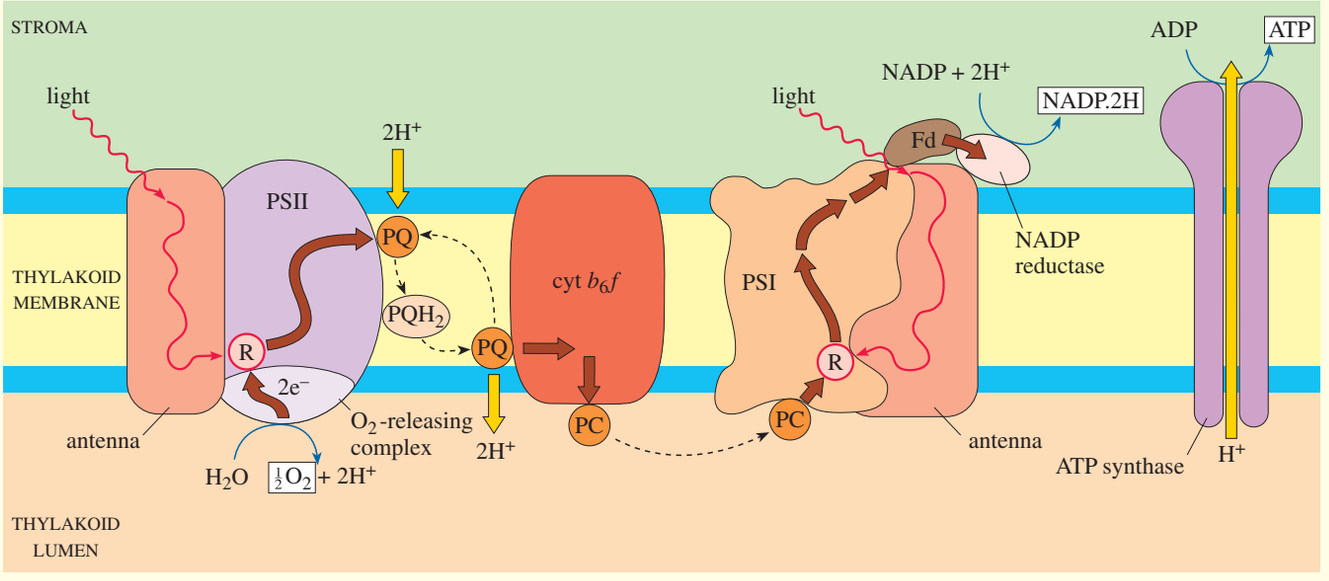

Figure 2.1 The components and pathway of non-cyclic photosynthetic electron transport and photophosphorylation in green plants. Light absorbed by groups of pigment molecules (**antennae**) is funnelled to the reaction centre (R), initially of **photosystem II (PSII)**. For each photon absorbed, an excited or energized electron is transferred to an electron acceptor in PSII and is replaced by electron donation from water via the oxygen-releasing complex. Two electrons are transferred per molecule of water oxidized and four electrons per molecule of oxygen released. Electrons from PSII pass via the mobile carrier plastoquinone (PQ) to the complex of cytochrome b_6 and cytochrome *f* (cyt b_6f) and thence to the mobile carrier plastocyanin (PC). PC donates electrons to photosystem I (PSI) after it has absorbed light and emitted excited electrons, which pass via the carrier ferredoxin (Fd) to NADP reductase. This last enzyme reduces NADP, generating reducing power, as NADP.2H. The proton gradient generated during electron transport (with protons accumulating in the interior or lumen of thylakoids) is discharged via the ATP synthase and used to power ATP synthesis. The brown arrows denote electron transfer.

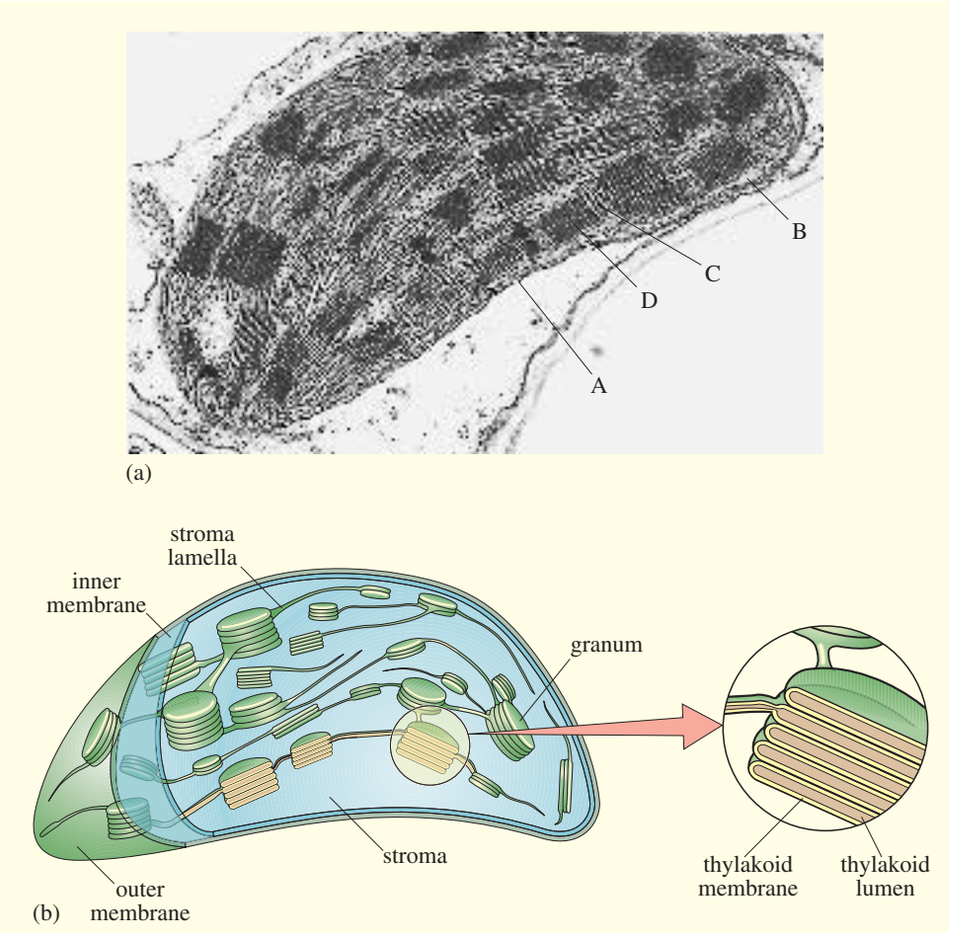

Figure 2.2 (a) Electron micrograph of a chloroplast. For labels A–D, see the answer to the text question on the previous page. (b) Schematic diagram of chloroplast structure.

reaction centre, with the whole antenna acting like a funnel, channelling the energy of absorbed quanta to the reaction centre. Different accessory pigments occur in other photosynthetic organisms; for example, red algae and cyanobacteria contain chlorophyll *d*, carotenoids and phycobiliproteins (which absorb green wavelengths).

❍ Three pigments from plant chloroplasts absorb maximally light of wavelengths (i) 540 nm, (ii) 670 nm and (iii) 650 nm; identify these pigments as either chlorophyll *a*, chlorophyll *b* or carotenoid.

● (i) is carotenoid (because it absorbs maximally the shortest wavelength), (ii) is chlorophyll *a* and (iii) is chlorophyll *b*.

In addition to pigments, antennae also contain structural proteins, which are different for photosystems I and II and play an important protective role (Section 2.2.2): the terms *light-harvesting complexes I and II* (LHCI and LHCII) are often used to describe the pigment–protein complexes associated with PSI and PSII respectively.

Photosynthetic electron transport and photophosphorylation occur on the membranes or lamellae (singular lamella) in chloroplasts, but the molecular components (Figure 2.1) are not arranged randomly, as you can see in Figure 2.3. This non-random arrangement is significant for control and coordination of the light reactions, so it is worth examining closely.

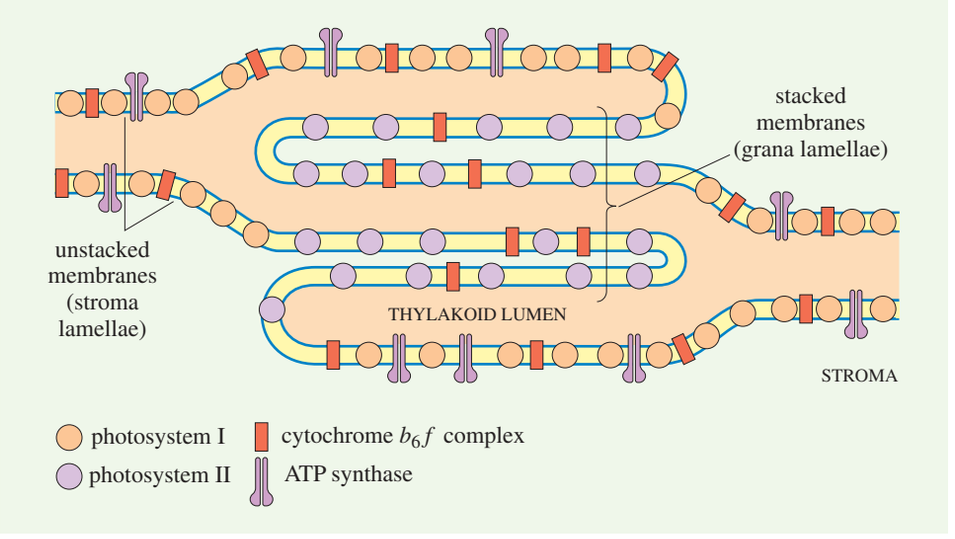

Figure 2.3 The location of molecular complexes on grana and stroma lamellae.

Notice first that one molecular complex is restricted mainly to the inner membranes (lamellae) of grana where they lie close together and are described as appressed membranes.

❍ Which complex is localized in this way?

● PSII.

Two other complexes, PSI and ATP synthase, are restricted to the unstacked stroma lamellae (Figure 2.2), which link grana across the chloroplast stroma, and to the outermost lamellae of grana; and the cytochrome b_6f complex occurs on all lamellae. So most of PSII is relatively far from PSI. Bearing in mind the pathway of non-cyclic electron transport (PSII → cytochrome b_6f → PSI, Figure 2.1), consider the functional implications of this structural arrangement. It must involve some remarkable long-distance shuttling by the mobile electron carriers plastoquinone (PQ) and plastocyanin (PC), so why should there be such a wide separation of PSI and PSII?

One possibility is that spatial separation of the photosystems is necessary in order to regulate and balance light input so that each photosystem receives the same number of quanta during non-cyclic electron flow; the molecular machinery can be damaged if light input is unbalanced (see Section 2.2.2). Another possibility is that physical separation facilitates *cyclic electron flow*, which is purely as an ATP-producing system and generates no reducing power (NADP.2H).

❍ What are the components and pathway of cyclic electron flow in chloroplasts?

- Cyclic electron flow involves absorption of light by PSI and transfer of excited electrons via ferredoxin to plastoquinone, cytochrome b_6f and then, via the mobile carrier plastocyanin, back to PSI. No NADP.2H is produced, but the coupled proton pumping can be used for ATP synthesis.

This cyclic flow operates mainly in the stroma lamellae and provides a flexible way of generating ATP in situations where there is a need for ATP but relatively less demand for reducing power. At present, however, there is no *completely* satisfactory explanation for the wide separation of the two photosystems in chloroplast lamellae. The arrangement is strikingly different from that in mitochondria, where electron carriers are all packed closely together.

You might wonder how information was obtained about the location of molecular complexes on chloroplast membranes. Box 2.1 describes three approaches and now would be a suitable time to study it. The rest of this section is concerned with how plants can grow over a huge range of light intensities.

BOX 2.1 STUDYING CHLOROPLAST MEMBRANES

MEMBRANE FRACTIONATION

In order to locate molecular complexes, chloroplast membranes must first be separated into stroma and grana lamellae. A rough separation can be obtained by exposing isolated chloroplasts to high-frequency sound waves (sonication) and/or high pressure, followed by differential centrifugation to separate the dense grana membranes from the less dense stroma membranes. A more refined method is illustrated in Figure 2.4. Membranes are fragmented under very high pressure and the membrane fragments then re-seal to form vesicles. Vesicles from stroma lamellae and the outer thylakoids of grana re-seal so that the originally outside surface remains outside (normal or right-side-out vesicles). Vesicles from the inner, appressed grana lamellae re-seal so that the original outer surface is now inside (inverted or inside-out vesicles). The two types of vesicle, which differ in surface charge, can be separated in solutions of polymers of different molecular mass, as shown here.

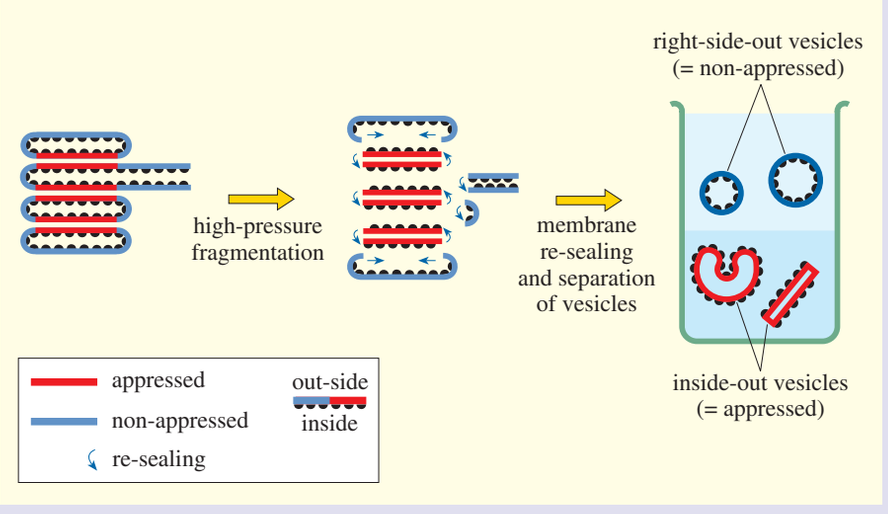

Figure 2.4 Diagram to illustrate how normal (right-side-out) and inverted (inside-out) vesicles are formed from different chloroplast membranes and then separated.

IDENTIFYING MOLECULAR COMPLEXES

The location of molecular complexes on membrane fractions can be demonstrated using three main methods:

1 *Immunohistochemistry* The molecular complexes can be visualized (and hence located) by raising antibodies to them, labelling the antibodies with a suitable marker (e.g. a fluorescent tag), and exposing chloroplast membranes to a labelled antibody.

2 *Freeze-etch/freeze fracture electron microscopy of photosynthetic mutants* If photosynthetic membranes are rapidly frozen and surface water evaporated under vacuum, molecular complexes which protrude from the membrane surface become visible in electron micrographs as characteristic knobs. Parts of complexes that are embedded in the membrane can be made visible by cleaving the lipid bilayer (freeze fracture). Using these techniques, the distribution of knobs on inside-out vesicles derived from grana was compared between normal plants and mutants known to be deficient in PSII or PSI. The results indicated that PSI mutants are deficient in knobs characteristic of stroma vesicles whereas PSII mutants are deficient in knobs characteristic of grana vesicles.

3 *Low-temperature fluorescence emission spectroscopy* A chlorophyll molecule excited by absorption of a photon of light may get rid of this energy either by transferring it to another molecule (the route to photosynthetic electron transport) or by releasing the energy partly as heat and partly by emission of a photon of light of lower energy (and hence longer wavelength) than that of the absorbed photon. Such emission is called **fluorescence**. The two photosystems fluoresce at different wavelengths when illuminated at very low temperature (77 K or −196 °C): the fluorescence emission maximum for PSII is 685 nm and that for PSI is 735 nm. Hence by comparing fluorescence emission spectra of different chloroplast membrane fractions, the locations of the photosystems can be deduced.

❍ Figure 2.5 shows the low-temperature fluorescence emission spectra of two chloroplast membrane fractions, one derived from internal grana membranes (inside-out vesicles) and the other from stroma membranes (right-side out vesicles). Knowing the distribution of PSI and PSII, identify the source (grana or stroma) of membrane fractions 1 and 2 in Figure 2.5.

● Membrane fraction 1 derives from internal grana membranes because the maximum fluorescence emission occurs at 685 nm, characteristic of PSII, which is located mainly on these membranes (Figure 2.3). Membrane fraction 2 derives from stroma membranes, which contain only PSI.

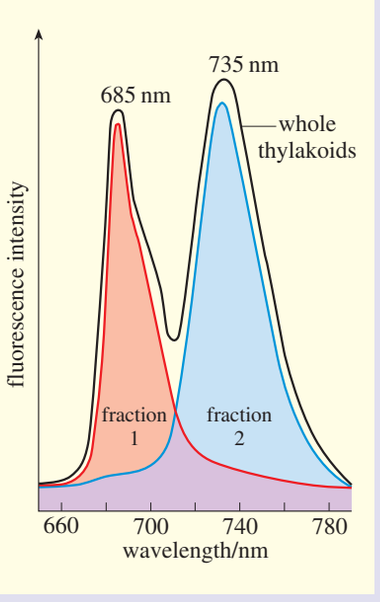

Figure 2.5 Low-temperature fluorescence emission spectra of isolated chloroplast membranes derived from internal grana or stroma.

2.2.2 ADAPTATIONS TO DIFFERENT LIGHT ENVIRONMENTS

The difference in light intensity, or 'strength', between a deeply shaded forest floor and midday tropical sun in the open is about 160-fold, i.e. from 15–2400 μmol m^{-2} s^{-1} of light quanta of wavelengths 400–700 nm (the **photosynthetic photon flux density** or **PPFD**), yet plants flourish over this whole range of light conditions. Some species are genetically adapted to permanent shade, others to full sun and yet others tolerate or adapt physiologically to a wide range of light

intensities. Such physiological adaptation is properly described as **acclimation**. We examine first some of the structural and biochemical mechanisms that allow plants to survive and grow in shady habitats.

In addition to low average light intensity, shady habitats — which nearly always occur beneath a plant canopy — have two other characteristics that affect plants:

(i) The quality of light, or spectral composition (i.e. the relative numbers of quanta of different wavelengths) is changed because of selective absorption by the canopy.

❍ From Figure 2.6, describe how the light beneath a canopy differs from that above it.

● Compared to full sun, shade light contains proportionately fewer quanta in the range 400–680 nm, but proportionately more quanta above 680 nm, especially in the far-red and infrared.

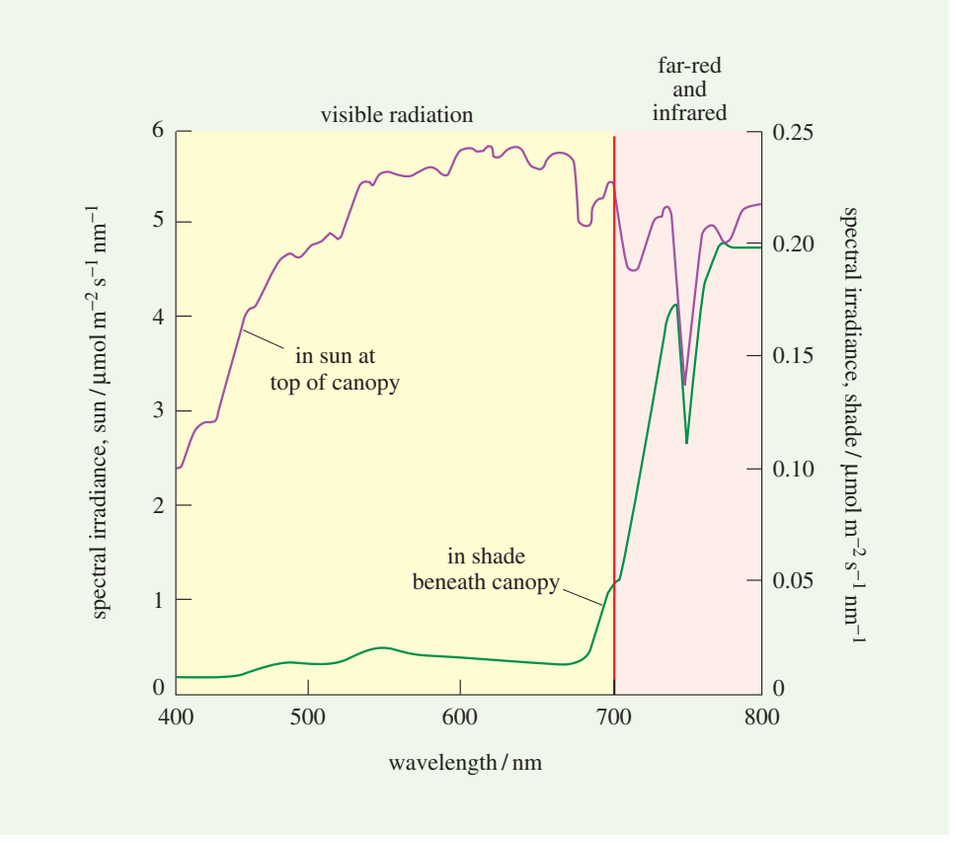

Figure 2.6 The spectral distribution of sunlight at the top of a plant canopy and under the canopy. The units of photosynthetic photon flux density are μmol m^{-2} s^{-1} nm^{-1} (i.e. μmoles of light quanta per square metre of leaf surface per second per unit wavelength).

(ii) Light conditions may change abruptly during the day. For example, discontinuities in the canopy mean that sunbeams occasionally pass right down to ground level, producing **sunflecks**. Quite suddenly and lasting for only a few minutes, deeply shaded leaves may receive a tenfold increase in light flux from sunflecks.

Shade plants have a range of adaptations to their environment, which may be determined genetically or result from acclimation. For example, they have very low rates of respiration (discussed further in Section 2.6.1). Here, however, we consider the adaptations that allow them to harvest light very efficiently when it is available at low average intensity, is relatively enriched in longer wavelengths compared with sunlight and may show brief periods of high intensity during sunflecks.

The first type of adaptation is at the level of whole leaves and is illustrated in Figure 2.7. Compared with a leaf that developed in the open (sun leaf), a leaf that developed in shade is much thinner overall and, in particular, has a very shallow layer of palisade mesophyll (Chapter 1) and patchy spongy mesophyll with more air spaces. It takes energy and resources to construct and maintain thick leaves, so this minimal structure of shade leaves is an efficient way in which to harvest the meagre supply of light normally available.

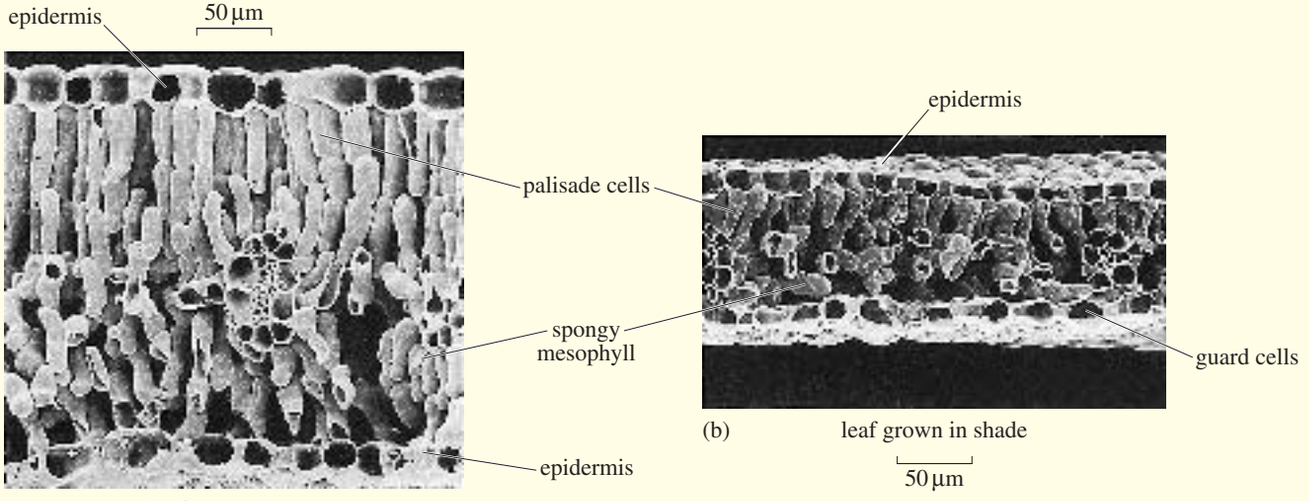

Figure 2.7 Scanning electron micrograph of vertical sections of *Impatiens parviflora* leaves that developed in (a) strong light (a sun leaf) and (b) low light (a shade leaf).

Other types of adaptation occur at a biochemical level within chloroplasts. For example, shade leaves have more chlorophyll molecules in the antenna systems that collect and feed light energy to each reaction centre. In addition, there is much greater proportion of PSII relative to PSI, which is often reflected in the presence of wide grana containing large numbers of stacked thylakoids, giving a ratio of appressed to non-appressed lamellae up to five times greater than in sun leaves.

- ❍ Why should this arrangement increase the ratio of PSII to PSI?
- ● PSII is located mainly on the appressed lamellae of grana and PSI on the non-appressed lamellae on the outside of grana or in the stroma (Figure 2.3).

The increase in PSII in shade plants relates to the far-red enrichment of shade light. The reaction centre of PSII shows maximum light absorption at a slightly shorter wavelength (680 nm) compared with the reaction centre of PSI (700 nm) — hence the names of the reaction centres, P680 and P700 respectively, where P stands for 'pigment'. This difference in absorption properties means that in far-red-enriched light, PSI is relatively more excited (emitting energized electrons at a faster rate) than PSII. But the smooth operation of non-cyclic electron transport requires that the two photosystems are excited *equally*, hence the requirement in shady habitats for increased absorption by PSII, by either increasing the number of reaction centres or the size of the pigment funnels channelling energy to each PSII reaction centre.

Finally, there is the question of sunflecks which, for leaves in deep shade, may provide nearly half the daily light income, but which are potentially damaging for shade leaves. Damage may arise because sunflecks are not far-red-enriched and hence over-excitation of PSII can occur. Such over-excitation — literally, funnelling more energy to PSII reaction centres than they can deal with or, alternatively, having too small a pool of electron acceptors to cope with the flow of excited electrons from reaction centres — leads to a form of reversible inhibition of photosynthesis called **photoinhibition**. This phenomenon is widespread in plants and is not restricted to shade plants exposed to sunflecks, so in Section 2.2.3 we discuss the general nature of photoinhibition and plant defences against it. Other adaptations that are necessary for the efficient use of precious sunfleck light do not involve the light reactions *per se* but rather:

- an ability to increase rapidly the rate of carbon fixation, i.e. make use of the increased supply of NADP.2H and ATP (discussed in Section 2.3.3);
- an ability to tolerate abrupt increases in leaf temperature (as much as 20 °C during prolonged, bright sunflecks), which increase water loss and may cause wilting; there is evidence that plants growing in moderately shaded habitats where sunflecks are relatively prolonged or abundant have larger root systems, which facilitate water uptake compared with plants adapted to more deeply shaded sites.

So adaptation of individual leaves or whole plants to variable light conditions (i.e. sunflecks) involves many aspects of their physiology. We explore these physiological adaptations further in later chapters.

Not all sunflecks are intense enough to cause photoinhibition, however, and in shade leaves, redistribution of light energy from PSII to PSI must occur so that both photosystems are again equally excited. **Energy redistribution** is thought to occur frequently in shade leaves, as the spectral composition of incident light shifts at different times of day or with different atmospheric conditions. It is a short-term, fine tuning of the photosynthetic light reactions, which allows light to be used with maximum efficiency. Figure 2.8 illustrates the mechanism of energy redistribution which was unravelled by research in the 1980s and 90s. PSII is now thought to exist as a huge dimeric complex with two reaction centres plus associated proteins and two pigment–protein funnels or light-harvesting complexes (LHCII).

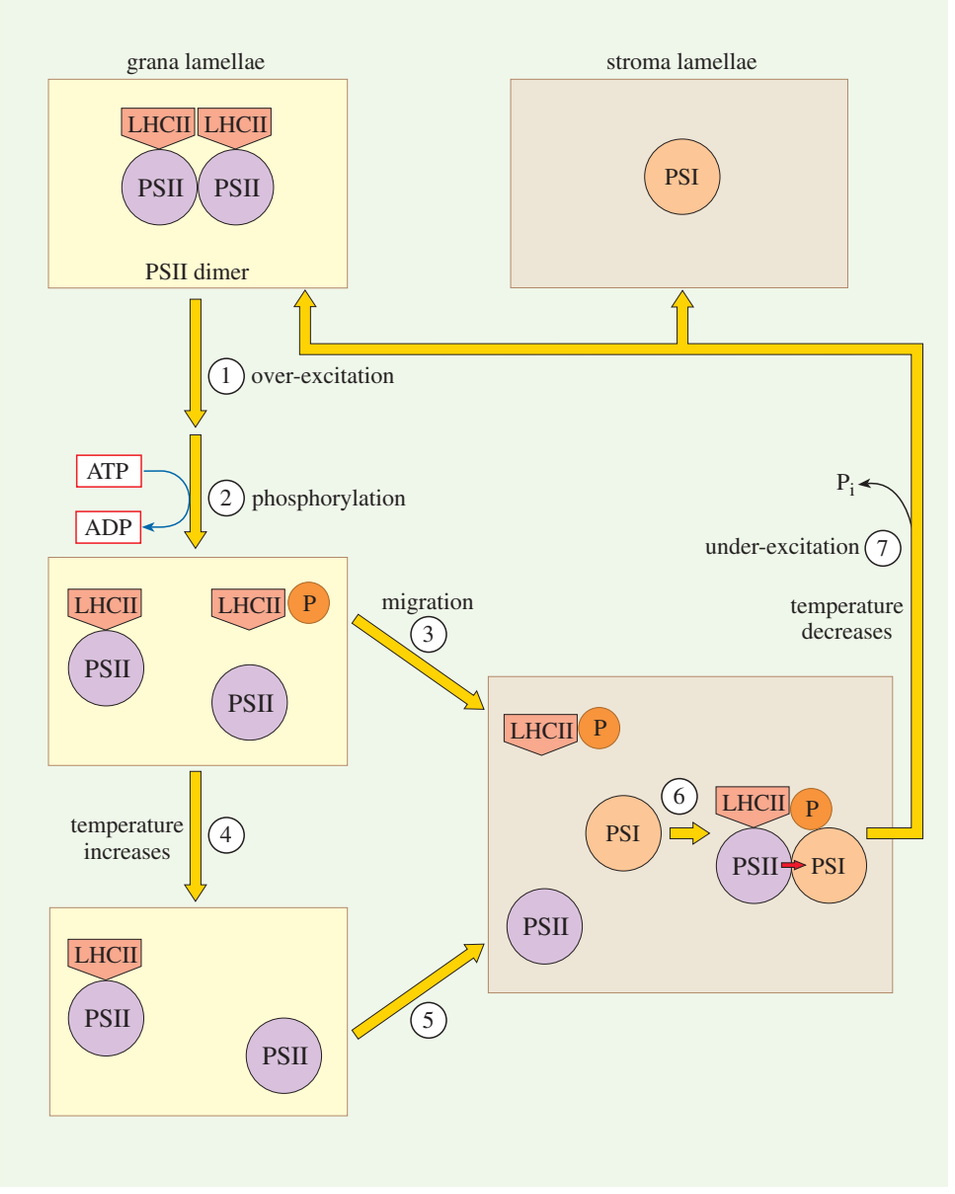

Figure 2.8 Energy redistribution after over-excitation of photosystem II, and its reversal. Numbered steps are described in the text. The small red arrow indicates energy transfer to PSI during step 6.

Over-excitation of PSII (step 1) has two consequences:

(a) A kinase enzyme is activated and this enzyme then phosphorylates a protein in one of the two LHCII complexes in the dimer (step 2). This change alters the electrical charge of the LHCII, reducing the adhesion between stacked lamellae and causing the dimer to split and then the phosphorylated LHCII to separate from its PSII and migrate into the non-appressed stroma region (step 3).

(b) Temperature increases (step 4) because some of the excess light energy entering PSII is dissipated as heat, which causes isolated PSII complexes to migrate into the stroma lamellae (step 5).

Reassembly of phosphorylated LHCII, PSII and PSI in the stroma lamellae (step 6) effectively channels more energy into PSI. The whole process can proceed in reverse (step 7) if PSII becomes under-excited (dephosphorylation of LHCII, fall in temperature and reverse migrations of the PSII and LHCII components).

This process is a good example illustrating how covalent modification of a protein (phosphorylation of the LHCII complex) can alter its properties. Energy redistribution is one of the mechanisms that helps plants to survive in low light conditions when light quality and quantity vary erratically.

2.2.3 PROTECTION AGAINST TOO MUCH LIGHT: PHOTOINHIBITION AND PHOTO-OXIDATION

As explained above, excess light can be harmful and plants have evolved a wide variety of mechanisms that prevent or minimize this harm, with the minimum 'cost' in terms of energy and resources. A first line of defence operates at the level of leaf behaviour and structure and involves decreased light absorption. Some plants adjust the orientation of leaf blades so that they lie parallel to the Sun's rays and thus minimize light interception. The shade plant *Oxalis oregana* (redwood sorrel), for example, grows in the redwood forests of North America and is often exposed to sunflecks lasting 20 minutes or more. Within a few minutes of sunfleck exposure, this species folds down its leaves. Table 2.2 shows the consequences of this behaviour for photosynthesis compared with those when leaves were artificially prevented from folding.

Table 2.2 The effects on photosynthesis in *Oxalis oregana* of leaf folding during exposure to sunflecks. The efficiency of the light reactions was estimated by measuring fluorescence characteristics which are proportional to quantum efficiency (the number of light quanta absorbed per molecule of O_2 evolved). Data from Powles and Björkman (1981).

Conditions during sunfleck exposure	% reduction in efficiency of light reactions during sunfleck	% reduction in rate of CO_2 uptake in low light following sunfleck
leaves allowed to fold down	9	0
leaves prevented from folding down	47	30

❍ From Table 2.2, suggest two reasons why leaf folding during sunflecks appears advantageous to *O. oregana*.

● Leaf folding (compared with not folding) results in: (1) greater efficiency of the light reactions, i.e. less photoinhibition, during sunflecks; and (2) no inhibition of photosynthesis after the sunfleck. Post-sunfleck inhibition in non-folding leaves results from damage, which takes time (and energy) to repair.

Some plants from open habitats show similar leaf movements, but it is not a common response to excess light. More common in high-light environments are longer-term adaptations such as the development of a thick waxy layer on the leaf surface. Such layers may perform several functions, including protection from insect or fungal attack and reduction of water loss, but they also reflect light very effectively. Experimental removal of surface wax from the succulent plant *Cotyledon orbiculata*, for example, increased light absorption by 50% and greatly increased photoinhibition in strong light.

The other lines of defence against excess light all involve molecular mechanisms that are universal among plants but are developed to different degrees in different habitats. Before describing these mechanisms, we need to examine more closely *why* light may cause damage. The basic cause is the formation of highly dangerous forms of oxygen, known as **reactive oxygen species** (**ROS**), which include the superoxide anion radical, $O_2\bullet^-$ (essentially an oxygen molecule with an extra electron). ROS are all free radicals and are especially harmful to membranes. During the photosynthetic light reactions, ROS may form either by energy transfer from excited chlorophyll molecules to O_2 (when reaction centres are too few to accept all the absorbed energy), or by transfer of electrons to O_2 from carriers such as ferredoxin (when there are too few suitable electron acceptors).

MOLECULAR PROTECTIVE MECHANISMS

Molecular protective mechanisms against light-induced ROS operate at three levels:

1 Prevention of ROS formation by dissipating as heat the excess light energy absorbed by pigments. Heat dissipation is mediated by a pigment called zeaxanthin, a type of carotenoid pigment (Section 2.2.1) belonging to a group called the xanthophylls. As light levels increase, zeaxanthin is synthesized enzymically from another, precursor xanthophyll and accumulates in chloroplasts. The reverse occurs as light levels fall, i.e. conversion of zeaxanthin to its precursor, so that a **xanthophyll cycle**, finely tuned to prevailing light conditions, operates to protect leaves from light through thermal energy dissipation.

2 Rapid destruction of any ROS that form. The enzyme superoxide dismutase (SOD), for example, converts $O_2\bullet^-$ very efficiently to hydrogen peroxide, H_2O_2, which is then disposed of by other enzymes because its bleaching action is also potentially damaging.

3 If ROS start to build up, damage occurs first to PSII, culminating in the destruction of a particular protein, named D1, which acts as a weak link within the PSII complex. D1 has a very rapid turnover rate, with a half-life of about 2 h, so it can be replaced within hours or days. So, by breaking the electron transport chain at one easily repaired link, damage to the rest of the photosynthetic machinery is minimized.

❍ Suppose that you wish to carry out experiments to test the hypothesis that repair of photoinhibitory damage at level 3 involves the synthesis of new D1 protein. The first need is to establish that protein synthesis is required in order to recover from level 3 photoinhibition. For this investigation (Experiment 1), you are provided with: leafy plants that had experienced strong photoinhibition at level 3; an inhibitor of protein synthesis which is taken up by leaves; a suitable incubation medium; and apparatus to measure the rate of photosynthesis by O_2 evolution.

(a) Describe (i) how you would carry out Experiment 1 with an appropriate control; and (ii) the result that would support the hypothesis.

Assuming that Experiment 1 supported the hypothesis, a second experiment is needed to establish that more D1 protein is synthesized in leaves recovering from photoinhibition than in controls. To carry out Experiment 2 you are provided with: leaves from photoinhibited plants and from similar plants that had not experienced photoinhibition; radiolabelled amino acids; incubation medium; equipment to isolate chloroplasts and to extract chloroplast proteins and separate them by electrophoresis; information about the location of D1 after electrophoresis; and equipment to measure the amount of labelled D1 protein present.

(b) Describe (i) how you would carry out Experiment 2 with an appropriate control; (ii) the result that would support the hypothesis.

● (a) (i) For Experiment 1, incubate photoinhibited leaves in the incubation medium under low light conditions (which would allow recovery from photoinhibition) and with and without (controls) the protein synthesis inhibitor. At the same time, monitor rates of photosynthesis (O_2 release). (ii) An increase in the rate of photosynthesis in controls but not in leaves incubated with the inhibitor would support the hypothesis, i.e. protein synthesis is necessary for recovery from level 3 photoinhibition.

(b) (i) For Experiment 2, incubate photoinhibited and non-inhibited (control) leaves in the presence of radiolabelled amino acids (which are incorporated into newly synthesized proteins). Isolate chloroplasts and then extract chloroplast proteins from these leaves and separate the proteins by electrophoresis. Measure the amount of radioactivity associated with separated D1 protein. (ii) If D1 from previously photoinhibited leaves was much more heavily labelled with radioactivity than that from control leaves, this result would suggest that more D1 was synthesized during recovery from photoinhibition, which is consistent with and supports the hypothesis.

If, despite all the protective measures, ROS continue to accumulate, then irreversible damage occurs, a process described as **photo-oxidation**. Bleached leaves are a common sign of photo-oxidative damage preceding leaf death. Figure 2.9a illustrates the different stages as a leaf passes from relatively low light through increasing light fluxes when different protective mechanisms come into play up to the stage of photo-oxidation. The green area represents an environment

where light limits the rate of photosynthesis and not only do the light reactions cope fully with all the harvested light but, equally important, the carbon fixation reactions utilize all of the ATP and NADP.2H as fast as these products are made, hence the label 'utilization' for this stage.

Figure 2.9b shows, for three different kinds of leaf, the relative magnitude of the utilization, protection and damage stages in a light gradient. Study this Figure carefully and look back at the descriptions of sun and shade leaves in Section 2.2.1.

❍ (a) Why do shade leaves (Figure 2.9b, top) have such a narrow window of light utilization and enter the photoprotection stages at such a low light flux? (b) Why do they enter the damage stage at such a low light flux compared with sun leaves?

● (a) Shade leaves are adapted to low light conditions and they have a high ratio of light-harvesting pigments to reaction centres. Reaction centres are rapidly over-excited as light levels rise and, in addition, carbon fixation cannot keep pace with the light reactions. Hence photoprotection becomes necessary at low light fluxes. (b) The only explanation is that protection at levels 1 and 2 (p. 66) is weak.

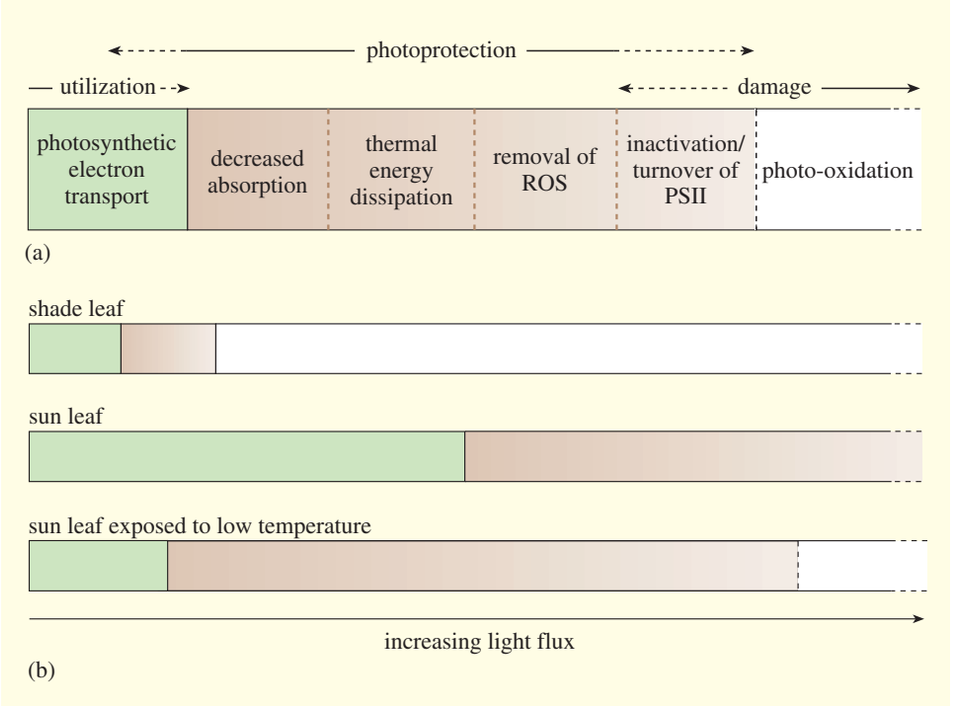

Figure 2.9 (a) The response of leaves to increasing fluxes of light from the utilization stage when all or most of the light absorbed is translated into photosynthetic electron transport (green), through the various, easily reversible photoprotection stages (brown) to the damage stages, which take much longer to reverse or are irreversible (photo-oxidation, white). (b) The relative magnitude of the stages shown in (a) for three kinds of leaves.

Shade leaves have only limited capacity to form zeaxanthin and contain low levels of protective enzymes such as SOD. The interesting point to notice about sun leaves in Figure 2.9b (middle) is that they never enter the damage stage at all! Sun leaves have very large pools of xanthophyll pigments and may dissipate harmlessly as heat over 50% of the light energy absorbed by these pigments. They also have high levels of photoprotective enzymes, so that even in the

strongest sunlight only slight photoinhibition occurs. If raised in strong light, some shade plants can adapt physiologically (i.e. acclimate) to these conditions, performing almost as effectively as true sun plants. Such acclimation is an example of **phenotypic plasticity** (the production of different phenotypes in different environments by organisms with the same genotype) and contrasts with the genetically determined adaptations of obligate shade plants, which cannot acclimate to high light and are confined to shaded habitats.

Finally there is the situation illustrated in Figure 2.9b (bottom): that of sun leaves exposed to low temperature. In laboratory experiments, these leaves often show strong photoinhibition at light fluxes equivalent to only half full sunlight and may show photo-oxidative damage at higher fluxes. This is why house-plants of tropical origin, exposed to strong light in a cool room (around 10 °C), commonly grow poorly and may be permanently damaged. To explain this phenomenon, you need to bear in mind that photochemical reactions are insensitive to temperature but that the rates of enzyme-catalysed reactions and electron transport are reduced at low temperatures.

❍ Given this information, suggest an explanation for the altered behaviour of sun leaves at low temperatures.

● Firstly, the utilization of absorbed light is greatly slowed down; rates of photosynthetic electron transport and carbon fixation do not keep pace with photochemical reactions as light levels increase. Secondly, protection by the xanthophyll cycle and SOD is less effective at low temperatures because both processes involve enzyme-catalysed reactions.

Strong light on cold days can have detrimental effects on both crops and wild plants. Crop plants such as maize (*Zea mays*), for example, showed photoinhibition of up to 45% when sunny conditions coincided with morning temperatures of 10 °C or less, with recovery requiring 2–3 days of warm dull weather. Crops such as winter rape (*Brassica napus*) and evergreen trees such as holly (*Ilex aquifolium*) and Scots pine (*Pinus sylvestris*) likewise show increased photoinhibition on sunny winter days. In fact, any stress that reduces the rate of carbon fixation — such as shortage of water, high or low temperatures or shortage of mineral nutrients — tends to exacerbate photoinhibition by reducing the magnitude of the utilization stage. There is a delicate and closely regulated balance between the light and dark reactions of photosynthesis. The next section looks in more detail at the latter, i.e. the process of carbon fixation.

SUMMARY OF SECTION 2.2

1 The molecular components of photosynthetic light reactions are arranged on chloroplast membranes such that PSII is largely confined to appressed lamellae inside grana and PSI to the non-appressed stroma lamellae.

2 Information about molecular arrangements on chloroplast membranes can be obtained using low-temperature fluorescence emission spectra, immunohistochemistry and freeze-etch/freeze fracture electron microscopy of particular membrane fractions.

3 Shade plants are genetically or physiologically adapted to conditions of low light, rich in far-red wavelengths, and to exposure to sunflecks. They harvest light efficiently under these conditions and minimize damage due to over-excitation of PSII.

4 Shade-plant characteristics include thin leaves, low rates of leaf respiration, large antenna (or light-harvesting) systems and a high ratio of PSII to PSI. Protection during sunflecks is achieved by physiological and molecular mechanisms (energy redistribution involving covalent modification and migration of PSII components).

5 Strong light (high PPFD) can damage leaves through the production of free radicals (ROS). General protection against ROS may be through leaf movements and a range of molecular mechanisms, which include the xanthophyll cycle (preventing formation of ROS), enzymatic destruction of ROS, and rapid destruction and repair of D1 protein in PSII.

6 Damage due to strong light which is reversible (by repair mechanisms) is called photoinhibition. Irreversible damage is called photo-oxidation. Shade leaves have only very limited capacity for damage repair and are easily photo-oxidized, whereas sun leaves have a high repair capacity (unless stressed, e.g. by low temperatures) and do not usually become photo-oxidized.

2.3 CARBON FIXATION: THE C3 CYCLE

Carbon fixation, the conversion of inorganic carbon (CO_2 or hydrogen carbonate, HCO_3^-) to organic compounds, is usually taken as the defining metabolic activity of autotrophs and, from cyanobacteria to eukaryotic algae to the plant kingdom, only one series of reactions achieves *net* carbon fixation: the **C3** or **Calvin cycle**. Other processes in both plants and animals may fix CO_2, but for every molecule of CO_2 fixed, a molecule is released later in the process, so there is no net carbon fixation. There are also other pathways that achieve net carbon fixation among the anaerobic photosynthetic bacteria and chemosynthetic archaeons but, on a global scale, the C3 cycle dominates. Here we begin by describing what the reactions of the C3 cycle are and some aspects of product synthesis (Section 2.3.1); then we examine Rubisco, the most important enzyme in the cycle (Section 2.3.2); and finally, we look at how the cycle is controlled and coordinated with the light reactions (Section 2.3.3).

2.3.1 REACTIONS OF THE C3 CYCLE

In the C3 cycle, CO_2 and water combine with a 5-carbon acceptor molecule and the products are converted to sugars (using reducing power (NADP.2H) and ATP from the light reactions) with regeneration of the acceptor. The cycle occurs in the chloroplast stroma (Section 2.2.1) and involves many small steps, but it can be divided into three main stages. They are illustrated in Figure 2.10a, with more detail provided in Figure 2.10b.