CHAPTER 2

Oxygenic Photosynthesis

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2.1 INTRODUCTION

2.1.1 Importance of Photosynthesis: Why Study Photosynthesis?

In a general sense the term *photosynthesis* is synthesis of chemical compounds by the use of light. In the more restricted sense, as we shall use it here, it stands for the process by which plants, algae, cyanobacteria, and phototrophic bacteria convert light energy to chemical forms of energy. Most photosynthesis is coupled to assimilation of carbon in the form of carbon dioxide or bicarbonate ions, but there exists also assimilation of CO_2 that is not coupled to photosynthesis, as well as photosynthesis that is not coupled to assimilation of carbon.

All life on Earth, with some exceptions, is completely dependent on photosynthesis. Most organisms that do not live directly by photosynthesis depend on the organic compounds formed by photosynthesis and, in many cases, also on the molecular oxygen formed by the most important type of photosynthesis, oxygenic photosynthesis. Even much of the energy fueling the ecosystems at deep-water hydrothermal vents depends on photosynthesis, since it is made available to organisms using molecular oxygen of photosynthetic origin. In addition, photosynthesis is biologically important in a number of more indirect ways. The stratospheric ozone layer protecting the biosphere from dangerous ultraviolet radiation from the sun is formed from photosynthesis-derived oxygen by a photochemical process. The photosynthetic assimilation of CO_2, and associated processes such as formation of carbonate shells by aquatic organisms, has (so far) helped to maintain the climate of our planet in a life-sustainable state. For basic descriptions of photosynthesis, see Rabinowitch [1] and Blankenship [2], and for reviews on all aspects of *Advances in Photosynthesis and Respiration Including Bioenergy and Other Processes*, see many volumes at the following web site: `http://www.springer.com/series/5599`.

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Rosing et al. [3] speculate that photosynthesis has also caused the formation of granite and the emergence of continents. Granite is common, among bodies in the solar system, only on Earth. After oceans were first formed there were no continents, the surface of the Earth was completely aquatic. Granite with its lower density is, in contrast to the heavier basalt, able to "float high" on the Earth's liquid interior. Thus photosynthesis is very important for life on Earth, and worth a thorough study just for its biological and geological importance. In recent years it has also attracted much interest in connection with the search for a sustainable energy source that can replace nuclear power plants and systems that release greenhouse gases to the atmosphere. There is much interest in solar fuels today: see `http://blogs.rsc.org/cs/2012/09/25/a-centenary-for-solar-fuels/` for a special collection of articles and opinions to mark the centenary of Ciamician's paper "The Photochemistry of the Future" [4].

2.1.2 Oxygenic Versus Anoxygenic Photosynthesis

The form of photosynthesis that first comes to mind when the term is mentioned is that carried out by the plants we see around us. It is called *oxygenic photosynthesis* because one of its products is molecular oxygen, resulting from the oxidation of water [5]. This form of photosynthesis is also carried out by algae and by cyanobacteria (formerly called blue-green algae) (for a perspective on cyanobacteria, see Govindjee and Shevela [6]). Photosynthesis by bacteria other than cyanobacteria, on the other hand, does not involve evolution of O_2. Instead of water (H_2O), other electron donors, for example, hydrogen sulfide (H_2S), are oxidized. This latter type of photosynthesis is called *anoxygenic photosynthesis* [7,8]. In addition to these processes, some members of the "third domain of life," the Archaea, as well as some other organisms, carry out conversion of light into electric energy by carrying out light-dependent ion transport. Although this biological process, which strictly speaking is not photosynthesis, could also be a useful guide to technological applications, we shall not deal with it in this chapter (see, however, Oesterhelt et al. [9]).

The reactions of oxygenic photosynthesis in algae and plants take place within a special cell organelle, the *chloroplast* (see Fig. 2.1). The chloroplast has two outer membranes, which enclose the *stroma*. Inside the stroma is a closed membrane vesicle, the *thylakoid*, which contains the *lumen*. The stroma is the site where the CO_2 fixation reactions occur (the *dark reactions* of photosynthesis; described in Section 2.5); the thylakoid membrane is the site for the conversion of light energy into energy of the chemical bonds (the *light reactions*; discussed in Sections 2.2 and 2.3). In cyanobacteria, however, the thylakoid membrane is within the cytoplasm.

2.1.3 What Can We Learn from Natural Photosynthesis to Achieve Artificial Photosynthesis?

Natural photosynthesis is characterized by a number of features, which are useful to keep in mind when trying to construct useful and economically viable artificial systems [10]:

1. Use of antenna systems that concentrate the energy.
2. Regulation of antenna systems by light.

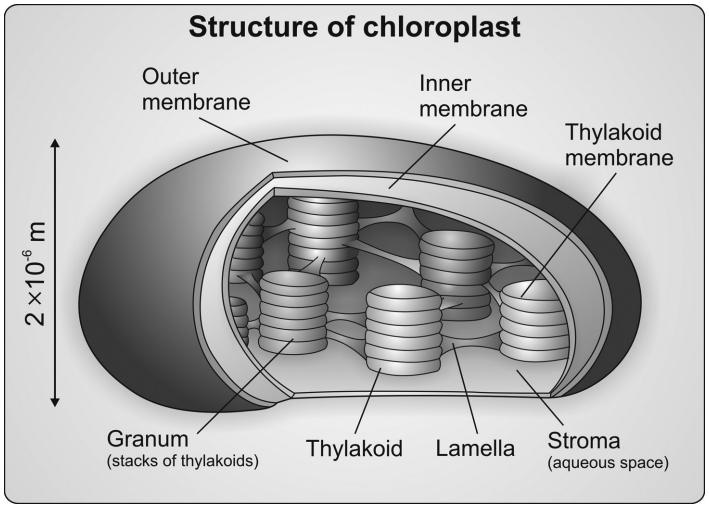

FIGURE 2.1 Three-dimensional diagrammatic view of a chloroplast.

3. Use of quantum coherence to increase efficiency.
4. Connection, in series, of two photochemical systems to boost electrochemical potential difference.
5. Protective systems and safety valves to prevent overload and breakdown.
6. Self-repair of damaged components.

We must consider constructing artificial systems from common and cheap materials that may be available everywhere. One should also bear in mind that perhaps plants do not optimize the process toward the same goal as we wish them to do. Maximizing energy conversion is not always the best strategy for an organism; they have evolved for survivability.

Attempts are being made on many fronts including mimicking the manganese–calcium cluster of PSII for energy storage (for more details see Chapters 3 and 4, and Refs. [11–18]).

2.1.4 Atomic Level Structures of Photosynthetic Systems

By means of X-ray diffraction studies of protein crystals and other methods, the detailed atomic structure of some photosynthetic systems are now available (for recent reviews on the structures of photosynthetic complexes, see Refs. [19–22]). They have revealed great similarity between the "cores" of the two photosynthetic systems (PSI and PSII) present in oxygenic organisms, and the "cores" of photosystems present in various groups of bacteria carrying out anoxygenic photosynthesis, indicating a common evolutionary origin of all photosynthesis (e.g., see Refs. [23–25]). Among many other structures of photosynthetic systems, we mention, at

the very outset, that we now have available atomic level structure of the PSII at 1.9 Å resolution [26].

2.1.5 Scope of the Chapter

This chapter is intended as a background on natural photosynthesis for those interested in artificial photosynthesis. We start with a description of how light is used for creating positive and negative charges, and continue with how these charges are transferred through the molecular assemblies in the membranes. Next, we describe how the charge transport leads to creation of a pH difference across the photosynthetic membrane, and how charge and pH differences lead to the production of high-energy phosphate that can be used in chemical synthesis. Finally, we deal with the time dimension, how the type of photosynthesis present today has evolved over billions of years, and what can we expect of the future that we are ourselves able to influence. In addition, in the end, we consider some interesting photosynthesis-related questions relevant to whole land and aquatic plants.

2.2 PATH OF ENERGY: FROM PHOTONS TO CHARGE SEPARATION

2.2.1 Overview: Harvesting Sunlight for Redox Chemistry

The initial event in photosynthesis is the light absorption by pigments: chlorophylls (Chls), carotenoids (Cars), and phycobilins (in cyanobacteria and in some algae), contained in antenna protein complexes (for overviews of light-harvesting antenna, see Green and Parson [27], for Chls, see Scheer [28] for Cars, see Govindjee [29], and for phycobilins, see O'hEocha [30]). The absorbed energy is transferred from one antenna pigment molecule to another in the form of *excitation energy* until it reaches *reaction centers* (RCs), located in two large membrane-bound pigment–protein complexes named photosystem I (PSI) and photosystem II (PSII) (see Figs. 2.2 and 2.3). Due to the primary photochemistry, which takes place after trapping of the excitation energy by special photoactive Chl molecules in the RCs of these two photosystems, light energy is converted into chemical energy. This energy becomes available for driving the redox chemistry of the stepwise "extraction" of electrons from water and their transfer to $NADP^+$ (oxidized form of nicotinamide adenine dinucleotide phosphate) (for further details, see Section 2.3). In this section we briefly describe how photosynthetic organisms capture light energy and how this energy migrates toward the RC Chl molecules, where the primary photochemical reactions occur.

2.2.2 Light Absorption and Light-Harvesting Antennas

The function of all light-harvesting antennas in photosynthetic organisms is common to all, that is, capture of light energy through absorption of photons of different

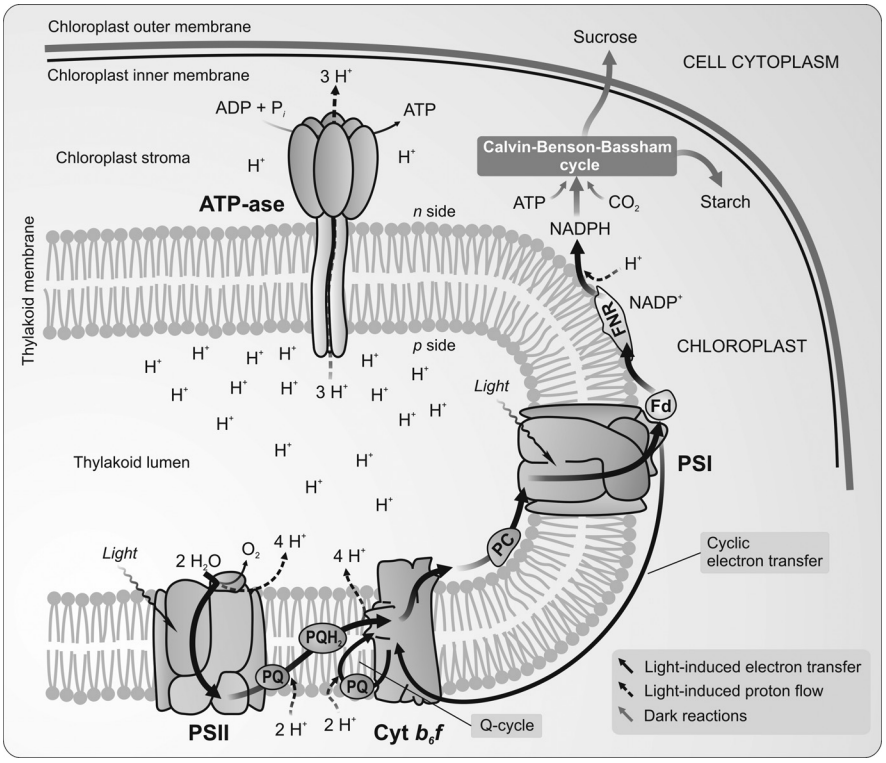

FIGURE 2.2 A schematic view of the photosynthetic thylakoid membrane and the protein complexes involved in the light-induced electron transfer (black solid arrows) and proton transfer (black dashed arrows) reactions in the thylakoid membrane of chloroplast in plants and algae. The end result of these light reactions is the production of NADPH and ATP. NADPH and ATP drive the "dark reactions" (grey arrows) of CO_2-fixation in stroma of the chloroplast via a cyclic metabolic pathway, the so-called the Calvin–Benson–Bassham cycle (also called by some as Calvin cycle, or Calvin-Benson cycle). This results in the reduction of CO_2 to energy-rich carbohydrates (e.g., sucrose and starch). See text for abbreviations and further details. Adapted from Messinger and Shevela [247].

wavelengths, and its transfer to RC complexes where photochemistry (the primary charge separation) takes place (see Fig. 2.3).

The process of photosynthesis starts in femtosecond time scale ($\sim 10^{-15}$ s) by light absorption in pigments, located in the light-harvesting antenna. Within less than a second, thylakoid membranes release O_2 and produce reducing power (reduced form of nicotinamide adenine dinucleotide phosphate or NADPH) and adenosine triphosphate (ATP). Kamen [31] used a pts (negative log of time) scale, analogous to the pH scale, to describe this process that spans pts of $+15$ to -1. The process of light absorption in any pigment molecule in the antenna, say, a Chl *a* molecule, implies that when a photon has the right energy ($E = hc/\lambda$, where h is Planck's constant, c is velocity of light, and λ is the wavelength of light), the molecule, which

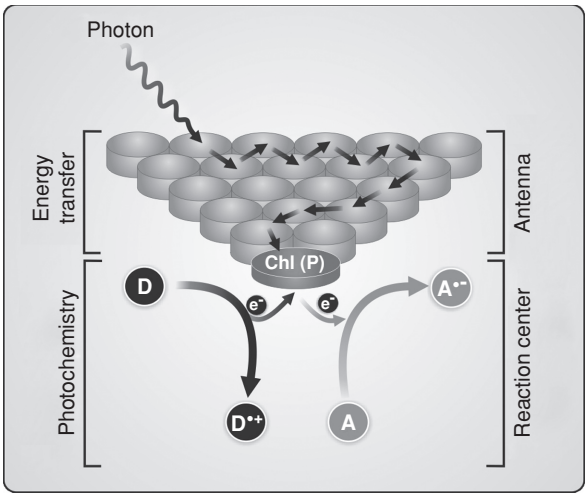

FIGURE 2.3 Excitation energy transfer in light-harvesting antenna that leads to primary photochemistry (charge separation) at the reaction center of photosynthetic organisms. Light energy is transferred through photosynthetic pigments of the light-harvesting antenna until it reaches reaction centers, where primary charge separation takes place. Abbreviations: P, reaction center Chl *a* molecule; e^-, electron; A, an electron acceptor; D, an electron donor. For further description, see text. Adapted from Messinger and Shevela [247].

is in the ground state, will go to its excited state (Chl*): one of the two outermost electrons, spinning in the opposite directions, is transferred to the higher excited states. An excited singlet state is produced (^{1}Chl a^*). This process is very fast: it occurs within a femtosecond, as mentioned above. Figure 2.4 shows the relation between the absorption spectrum of a Chl *a* molecule and its energy level diagram, the Jabłonski–Perrin diagram [32]. It shows that blue light (440 nm) will take the molecule to the *n*th excited state, whereas the red light (672 nm; or 678 nm, depending on the Chl *a* species) will take the molecule to its first excited state. The higher excited state is very unstable and within a pts of $+14$ to $+13$, the electron falls down to the lowest excited state; the extra energy is lost as heat. No matter what color of light is absorbed, the photochemical processes begin from this lowest excited state.

Plants and green algae have major and minor antenna complexes in both the photosystems (I and II). In PSII, there is a major complex, the LHCII (light-harvesting complex II), with many subcomplexes, and minor complexes that include CP43 (Chl–protein complex of 43 kDa mass) and CP47 (Chl–protein complex of 47 kDa mass). LHCII contains both Chl *a* and Chl *b* (the latter has absorption maxima at 480 nm and 650 nm), whereas CP43 and CP47 contain only Chl *a*. Chl *b* transfers energy to Chl *a* with 100% efficiency as has been known for a very long time (e.g., see Duysens [33, 34]). In addition, there are Cars that also transfer excitation energy to Chl *a*, with different efficiencies (see Govindjee [29]); the Cars, in general, are of two types: carotenes and xanthophylls (the mechanism of their energy transfer to

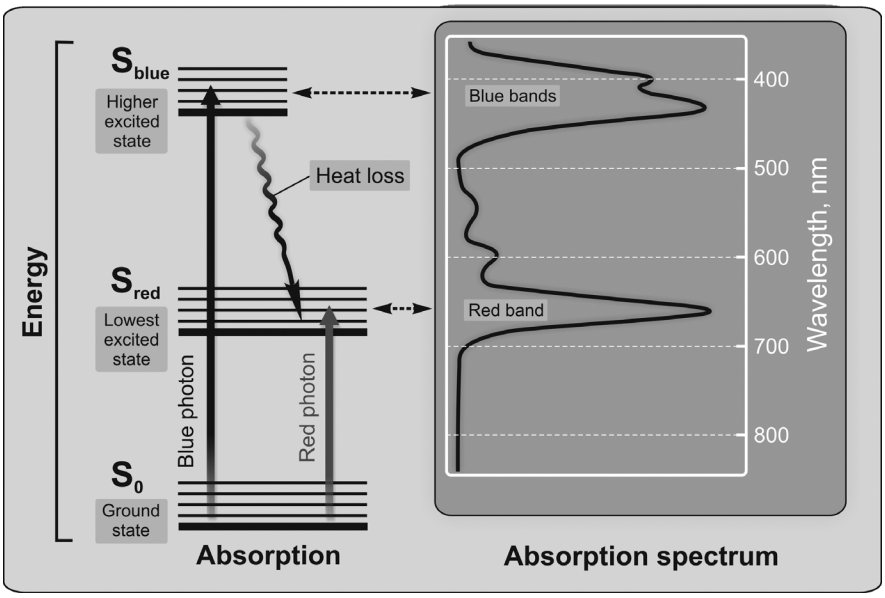

FIGURE 2.4 A Jabłonski–Perrin diagram of the energy levels in a Chl molecule with spectral transitions between them (vertical arrows) and absorption spectrum (turned 90° from the usual orientation) of Chl *a* corresponding to these levels. Diagram shows heat loss as radiationless energy dissipation (downward-pointing wiggly arrow): other radiationless energy dissipation processes such as fluorescence emission and intersystem crossing are not shown here. Note that the short- (blue) and long-wavelength (red) absorption bands of Chl absorption spectrum correspond to the absorption by this molecule of blue and red photons, respectively. Thus the red absorption band corresponds to the photon that has energy required for the transition from the ground state to the lowest excited state, while the blue absorption band reflects the transition to a higher excited state.

Chl *a* is, however, unique and different; e.g., see Zigmantas et al. [35] and Zuo et al. [36] for a discussion).

Brown algae, yellow-brown and golden-brown algae, and diatoms contain, in addition to Chl *a*, fucoxanthin as a xanthophyll, and various forms of Chl *c*, instead of Chl *b* [37]. Chls c_1 and c_2 have absorption maxima at ∼630 nm; and Chl c_3 at 586 nm. Fucoxanthin absorbs in the green (535 nm) and gives the organisms brown color; cryptomonads and dinoflagellates contain peridinin (absorption peak at 440–480 nm [38]) instead of fucoxanthin. On the other hand, red algae have water-soluble red and blue pigment-proteins, the phycobilins: phycoerythrins (absorption peak at 570 nm), phycocyanins (at 630 nm), and allophycocyanins (at 650 nm), absorbing green to orange light [39, 40].

The oldest oxygenic photosynthesizers are cyanobacteria (they were called blue-green algae before their prokaryotic nature was realized) (for a perspective, see Govindjee and Shevela [6]). Being prokaryotes they do not have chloroplasts. They

contain Chl *a* and phycobilins like the red algae, and phycoerythrin, the red pigment, is also present in some cyanobacteria; these cyanobacteria capture light that is not absorbed by green algae [41, 42], and thus they have different ecological niches in nature [43, 44]. The major LHCs of cyanobacteria are the *phycobilisomes* (PBS) that are made of the *phycobiliproteins* attached to the cytoplasmic surface of thylakoid membrane (for further details on the cyanobacterial PBS, see Mimuro et al. [45] and Sidler [46]). Interestingly, Chl *b*, that is, with some exceptions, not present in wild type cyanobacteria, can be introduced by genetic engineering into cyanobacteria [47]. For recent overviews on the LHCs of photosynthetic organisms, see Collines et al. [48] and Neilson and Durnford [49].

2.2.3 Excitation Energy Transfer: Coherent Versus Incoherent or Wavelike Versus Hopping

2.2.3.1 A Bit of History In 1936, Gaffron and Wohl [50] were the first to discuss excitation energy transfer (or migration) among hundreds of Chl molecules, in what we now call "antennas" before it reaches what we now call "RCs", and what Emerson and Arnold in 1932 [51] had called a "unit" (that could be interpreted as a "photoenzyme"). The concept of the "photosynthetic unit" serving a photoenzyme (RC in today's language) was born in the experiments of Emerson and Arnold [51,52], who found that a maximum of only one oxygen molecule evolved per thousands of Chl molecules present (for a review, see Clegg et al. [53]). In 1943, Dutton et al. [54] and in 1946 Wassink and Kersten [55] were among the first to demonstrate efficient excitation energy transfer from fucoxanthin to Chl *a* in a diatom (*Nitzschia* sp.) using the method of sensitized fluorescence: excitation of fucoxanthin led to as much Chl *a* fluorescence as excitation of Chl *a* did (see Govindjee [29]). Using the same sensitized fluorescence method, Duysens (in 1952) [33] showed 100% excitation energy transfer from Chl *b* to Chl *a* in the green alga *Chlorella*, and about 80% transfer from phycocyanin to Chl *a* in the cyanobacterium *Oscillatoria*. In 1952, both French and Young [39] and Duysens [33] showed efficient excitation energy transfer, in red algae, from phycoerythrin to phycocyanin and from phycocyanin to Chl *a* (however, later, it was realized that a distinct kind of phycobiliprotein, allophycocyanin, carries energy from phycocyanin to Chl *a*). Such excitation energy transfers from one type of pigment to another may be dubbed "heterogeneous" excitation energy transfer. On the other hand, Arnold and Meek [56] showed for the first time that when Chl *a* molecules in *Chlorella* cells were excited with polarized light, an extensive depolarization of fluorescence was observed; this was evidence of excitation energy migration among Chl *a* molecules. Such energy migration can be dubbed as "homogeneous" energy transfer since it is between the same type of pigment molecules. One of the first measurements of the time of excitation energy transfer was performed by Brody in 1958 [57] when he observed a delay of about 500 ps for energy transfer from phycoerythrin to Chl *a,* using a home-built instrument for measuring lifetime of fluorescence. Furthermore, excitation energy transfer from various pigments to Chl *a*, and one spectral form of Chl *a* to another, was found to be

temperature dependent down to 4 K (see Refs. [58–60]). For further historical details, see Govindjee [61].

2.2.3.2 Mechanism of Excitation Energy Transfer

There are two extreme cases [1]: (1) When there is a very strong coupling between the neighboring pigment molecules, the net result is that the exciton (excitation energy) formed from the absorbed photon belongs to all the pigment molecules, but not to one. There is thus quantum *coherence* in the system, and the motion of the exciton has a wavelike character. The exciton is *delocalized* [53, 62, 63]. (2) When there is a very weak (or even weak) coupling between the neighboring pigment molecules, the net result is that the excited pigment molecule formed from the absorbed photon belongs to that specific molecule only. There is thus quantum *incoherence* in the system, and the motion of the excitation energy has a *hopping* character. At one specific time, the excitation energy is said to be *localized* on a specific molecule [64–66]. Excitation energy transfer in this case is by the *Förster resonance energy transfer* (FRET), the magnitude of which depends (i) inversely on R^6, where R is the distance between the donor and the acceptor molecules; (ii) on the overlap integral of the absorption spectrum of the acceptor molecule and the emission spectrum of the donor molecule; and (iii) the so-called orientation factor, κ^2 [67, 68]. In photosynthetic systems, both the coherent (delocalized, wavelike) and incoherent (localized, hopping) mechanisms exist (for basics on fluorescence spectroscopy, see Colbow and Danyluk [69] and Lakowicz [70]).

Other sophisticated theories, besides the Förster theory, have evolved, which incorporate additional details and concepts and are applicable to several photosynthetic systems. They are the Redfield theory, the modified Redfield theory, and the generalized Förster theory [71–75].

When the pigment–pigment excitonic interaction coupling is weak, but the pigment–protein excitonic interaction–vibrational coupling is strong, and the excitation energy is localized, the classical FRET [66] mechanism applies (see Kleima et al. [76] as, for example, in the case of peridinin–Chl *a* system). However, when the pigment–pigment interaction coupling is strong, and the pigment–protein interaction coupling is weak, and the excitation is delocalized, a different mechanism called the *Redfield theory* applies (see Redfield [77] and Renger et al. [78]). On the other hand, when both the pigment–pigment and pigment–protein interaction couplings are strong, a *modified Redfield theory* applies [73]. For the case of energy transfer in LHCII, see Novoderezhkin et al. [79].

In PSII and PSI complexes, and in anoxygenic bacterial photosystems, we have pigment–protein domains that have strong coupling within them, but weak coupling between the domains. Thus interdomain excitation energy transfer would be by Förster theory, but the integrated mechanism would require extension of this theory to include coherence within individual domains (using Redfield or modified Redfield theory); the final description is called *generalized Förster theory* (see, e.g., a description of excitation energy transfer in PSII core complexes [80]).

In our opinion, the Förster theory must be applicable to excitation energy transfer within the phycobiliproteins, when excitation energy is transferred from phycocyanin

to allophycocyanin and then from allophycocyanin to Chl *a* [41]. For examples and discussion of Förster energy transfer in other photosynthetic systems, see Şener et al. [81] and Jang et al. [82] and, for a historical perspective, see Clegg et al. [53]. On the other hand, Ishizaki and Fleming [62] and Ishizaki et al. [83] discuss the ramifications of coherent energy transfer in photosynthetic systems, whereas Collini et al. [84] show coherent energy transfer in marine algae at room temperature. Quantum coherence is inferred when oscillations of exciton state populations, lasting up to a few hundred femtoseconds, are observed. This happens when pigment–pigment interaction coupling is very strong. The reality is that both coherent and incoherent mechanisms occur in natural systems. However, there are many open questions that remain to be answered!

2.2.4 Concluding Remarks and Future Perspectives for Artificial Photosynthesis

Sunlight is a dilute form of energy traveling at a speed that is beyond our comprehension. Photosynthetic organisms have learned over billions of years of trial and error how to catch it, concentrate it, and convert it in an efficient way to electrical energy for further use. We have attempted here to describe what is known about this process. An urgent task for humanity is to explore this further and adapt the process for solving our present technological energy crisis by what we may call "artificial photosynthesis."

2.3 ELECTRON TRANSFER PATHWAYS

2.3.1 Overview of the Primary Photochemistry and the Electron Transfer Chain

The photosynthetic electron transfer chain (ETC) from water to $NADP^+$ is energized by two membrane-bound photosystems. Thus the end result of the steps of light harvesting by the antenna complexes of PSI and PSII (or by phycobilisomes in cyanobacteria) is the capture of excitation energy by the ensemble of unique photoactive Chl molecules, denoted P, in the RC of photosystems (see Fig. 2.3). In PSII, this special photoactive RC is composed of several Chl *a* molecules, dubbed P680, and in PSI, the special RC is a "heterodimeric" complex of Chl *a* and Chl a', dubbed P700 (the numbers 680 and 700 are based on the wavelengths of the absorption maxima of these Chls in the red region). The singlet excited states of these RC Chls ($^1P^*$) is where the primary photochemistry begins. This is followed by fast charge separation between $^1P^*$ and the neighboring primary electron acceptor (symbolized as A in Fig. 2.3) and thus the formation of the radical pair $P^{\bullet+}$ $A^{\bullet-}$. The cation radical $P^{\bullet+}$ is reduced by the electron donor (denoted D in Fig. 2.3). An important point to realize here is that as soon as the cation radicals $P680^{\bullet+}$ (in PSII) and $P700^{\bullet+}$ (in PSI) are formed, the light energy has already been converted into chemical energy; this process has a very high quantum efficiency [85].

However, the steps involved in photosynthetic energy conversion are catalyzed not only by PSII and PSI, but also by two other protein complexes that are also located in the thylakoid membrane. These complexes are the cytochrome (Cyt) b_6f and the ATP synthase (ATP-ase) (see Fig. 2.2). PSII, PSI, and Cyt b_6f complexes contain almost all the redox active cofactors that allow light-induced transfer of electrons from H_2O to $NADP^+$ through the thylakoid membrane in the following sequence: $H_2O \rightarrow$ PSII $\rightarrow$ Cyt $b_6f \rightarrow$ PSI $\rightarrow$ $NADP^+$. PSII is linked with the Cyt b_6f complex via the mobile lipophilic hydrogen atom carrier plastoquinone (PQ), in the membrane, while the Cyt b_6f is linked with PSI via a mobile water-soluble redox carrier plastocyanin (PC) in the thylakoid lumen (Fig. 2.2). This copper-containing protein PC can, in some cases, be substituted by the iron-containing carrier Cyt c_6 (also sometimes called Cyt c_{533}) [86, 87]. In addition to these mobile electron transfer carriers, there is a soluble [2Fe-2S]-containing protein ferredoxin (Fd), a one-electron carrier connected with PSI. Upon receiving an electron from PSI, Fd reduces $NADP^+$ to NADPH. This reduction is catalyzed by the membrane-associated flavoprotein called ferredoxin-$NADP^+$ reductase (FNR) [88, 89]. The scheme that depicts the ETC from water to $NADP^+$ via redox-active cofactors is called the *zig-zag* or the *Z-scheme* (Fig. 2.5). Its origin and development have been described recently by Govindjee and Björn [90].

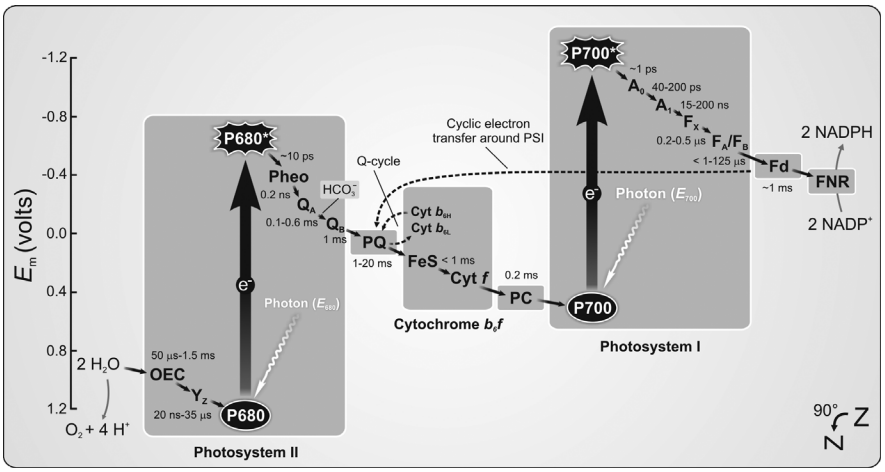

FIGURE 2.5 The zig-zag or Z-scheme of oxygenic photosynthesis representing the energetics of linear electron transfer from H_2O to $NADP^+$ plotted on redox midpoint potential (E_m, at pH 7) scale. The diagram also shows a cyclic electron transfer around PSI, Q-cycle, and half-times of several linear electron transfer steps. The two black vertical arrows symbolize the excitation of RC Chl *a* molecules (P680 and P700 in PSII and PSI, respectively); these lead to electrons in the ground state to be raised into a higher (singlet) excited state in response to the absorption of excitation energy from the light-harvesting antenna or by direct absorption of photons (wiggly white arrows). For further details and abbreviations of the components involved in the electron transfer, see text. Adapted from Govindjee et al. [115] and Shevela [248].

The light-driven flow of electrons in the photosynthetic ETC also involves the flow of protons (see Fig. 2.2). There are two steps in the ETC from water to $NADP^+$, where protons are released into the lumen of the thylakoids: (1) during water oxidation (oxidation of two water molecules results in release of four protons), and (2) during oxidation of plastoquinol (PQH_2) (protons are released into the lumen that were initially taken up from the stromal side during the formation of PQH_2) (Fig. 2.2). The accumulation of these protons in the lumen of thylakoid generates the *proton motive force* (*pmf*) across the membrane. As a consequence of dissipation of the pmf (which includes membrane potential and proton gradient) through the ATP synthase, ATP is synthesized (see Section 2.4), according to the chemiosmotic hypothesis of Peter Mitchell (e.g., see Jagendorf [91]).

Thus the result of the light-induced electron transport in all oxygenic photosynthesizers is water splitting concomitant with O_2 evolution (see Chapter 3 by Gernot Renger for details), production of the reducing power (NADPH), and phosphorylation of ADP to ATP (see Section 2.4 and Fig. 2.2). With the energy stored in NADPH and ATP, carbon dioxide can be converted to carbohydrates through a complicated carbon-fixation cycle (the Calvin–Benson–Bassham cycle) (see Section 2.5).

2.3.2 Components Associated with P680 and P700 and the Entry into the Electron Transfer Chain

As mentioned above, the energy of light becomes converted into chemical energy upon formation of the cation radicals of the RC Chls, $P680^{\bullet+}$ (in PSII) and $P700^{\bullet+}$ (in PSI). These cation radicals are formed due to stable and directed charge separation that occurs upon absorption of photons or excitation energy transfer to P680 and P700 molecules. However, there are still debates as to the definition of P680 and P700 and the detailed steps involved in the primary photochemistry [92–96].

The term P680 was first used in 1965 by Rabinowitch and Govindjee [97]; Döring et al. [98] showed its existence experimentally, and the primary charge separation, within 3 ps, between it and a nearby pheophytin (Pheo) molecule (the primary electron acceptor) was first measured by Wasielewski et al. [99] (also see Greenfield et al. [100], and for historical overview on Pheo discovery, see Klimov [101]). Excitation energy reaching the PSII RC complex leads to the formation of the singlet excited state of P680, $^1P680^*$; what one calls P680 needs to be specified; some consider P_{D1} and P_{D2} as P680, whereas others include two other nearby Chl *a* molecules (Chl_{D1} and Chl_{D2}) (e.g., see Durrant et al. [102] and Fig. 2.6). The long-wavelength peak of P680 is at 680 nm ($E_{680} = 1.83$ eV), whereas the short-wavelength peak is at ~440 nm [103]. It appears that we have two alternate primary steps: the primary charge separation is either from P_{D1} to $Pheo_{D1}$, or from Chl_{D1} to $Pheo_{D1}$. If the latter occurs first, then the oxidized Chl_{D1} oxidizes P_{D1}. However, there is now evidence that we have mixed electron transfer states [104]. The primary charge separation steps are over within a few picoseconds (3–7 ps), with the fastest steps being faster than 0.3–0.7 ps. As a result, the electron available on the $Pheo^{\bullet-}$ molecule enters into the ETC via a one-electron acceptor primary plastoquinone Q_A within PSII and then a two-electron acceptor Q_B, also within PSII.

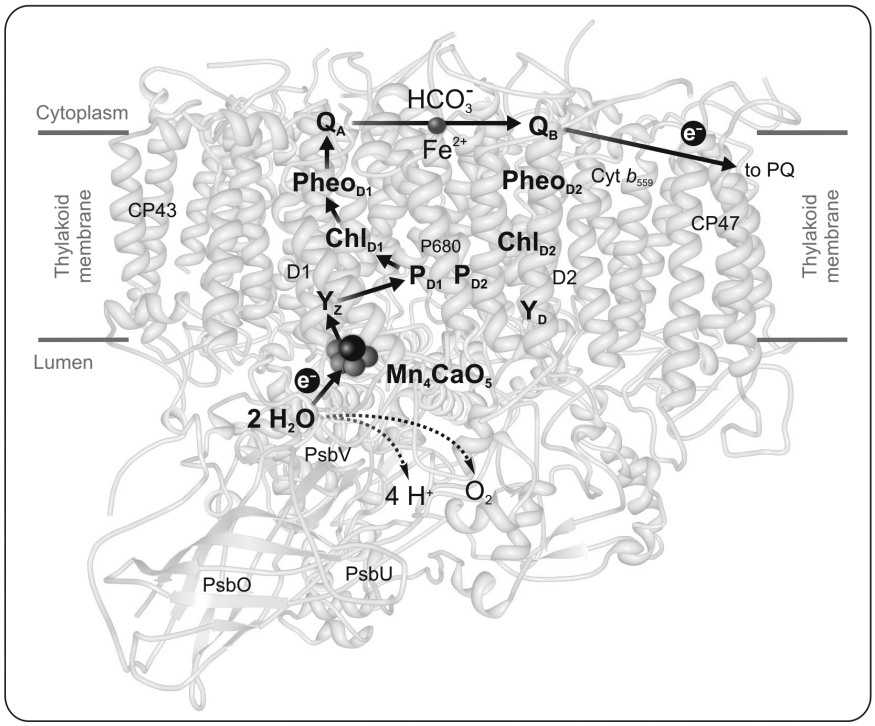

FIGURE 2.6 A side-on view of cyanobacterial PSII monomer and schematic arrangement of its central redox cofactors. Black solid arrows indicate the direction of electron transfer. Only a few proteins of PSII are symbolized. For further discussion and abbreviations, see text. Adapted from Shevela et al. [249].

P700 was discovered by Kok in 1957 [105]. In contrast to PSII, PSI complex has not only the RC Chl *a*–Chl *a*′ heterodimer P700, but also a large number of core PSI antenna molecules [106–108]. The long-wavelength absorption peak of P700 is at ∼700 nm ($E_{700} = 1.77$ eV). In contrast to P680, P700 has a much lower redox potential (ranging from +400 to +470 mV) (see chapters in Ke [103] and Golbeck [109]), but the primary charge separation in PSI is also over in a few picoseconds. Initially, it was thought that the primary charge separation is when P700 A_0 is converted to $P700^{\bullet+}$ $A_0^{\bullet-}$, where A_0 is a nearby Chl *a* molecule, previously known as the first electron acceptor (see Rutherford and Heathcote [110] and relevant chapters in Golbeck [109]; for a new proposed nomenclature of the redox active cofactors of the ETC in PSI, see Section 2.3.4 and Redding and van der Est [111]); however, recent data indicate that the primary charge separation may involve oxidation of another neighboring Chl *a* molecule, denoted as A and reduction of A_0 (see Fig. 2.7 and Refs. [94, 112, 113]). Thus these data suggest that the primary charge separation in PSI begins with the generation of a primary radical pair $A^{\bullet+}A_0^{\bullet-}$, followed by fast reduction of $A^{\bullet+}$ by P700 and the formation of the secondary radical pair $P700^{\bullet+}A_0^{\bullet-}$. The electron

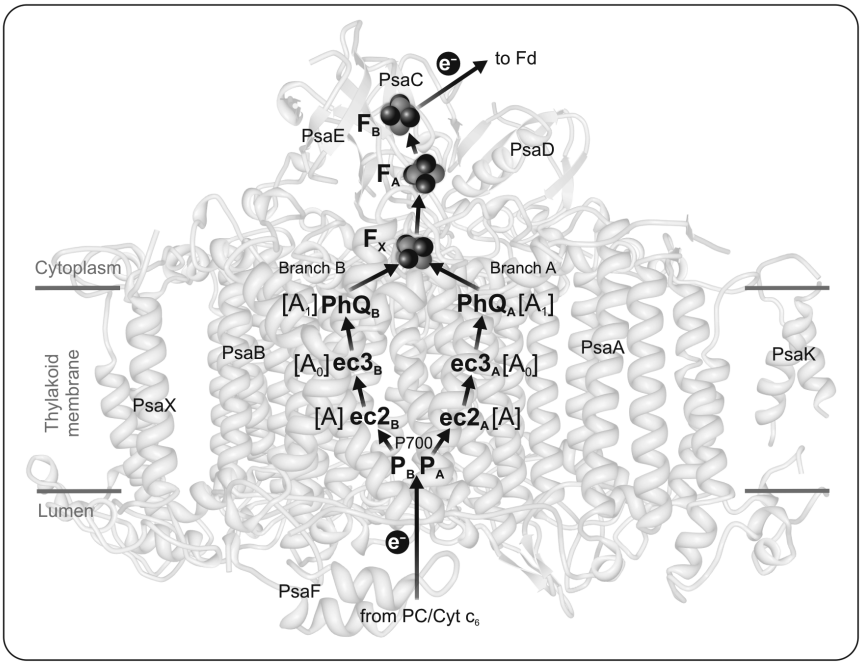

FIGURE 2.7 A side-on view of cyanobacterial PSI monomer and schematic arrangement of its central redox cofactors. Black solid arrows indicate the direction of electron transfer. Only a few proteins of PSI are shown. For further discussion and abbreviations, see text. Adapted from Shevela et al. [249].

on $A_0^{\bullet -}$ that had been formed during charge separation in PSI enters the ETC, and through a series of intermediates, reaches mobile Fd. While oxidized $P680^{\bullet +}$ receives electrons from water, oxidized P700 receives electrons from PC (or alternately a Cyt c_6). For overview of the primary photochemistry in photosynthesis, see Renger [93].

2.3.3 Photosystem II: Function and Electron Transfer Pathway

All oxygenic photosynthetic organisms have PSII, a large membrane-integral pigment–protein complex, which exists as a dimer with a total mass of ∼700 kDa. In this unique enzyme, light-induced charge separation drives oxidation of water to molecular oxygen on its electron-donor side and the reduction of PQ to PQH_2 on its electron-acceptor side:

$$2\,H_2O + 2\,PQ + 4\,H^+_{\text{stroma}} \xrightarrow{h\nu} O_2 + 2\,PQH_2 + 4\,H^+_{\text{lumen}} \qquad (2.1)$$

Thus PSII complex functions as a light-driven *water:plastoquinone oxidoreductase* (for reviews on PSII, see Refs. [114–116]). The processes performed by PSII (charge separation, water oxidation, and PQ reduction) are fully discussed in

Chapter 3 by Renger. Therefore we only briefly describe the main structural features, redox-active cofactors, and the electron transfer pathway of this PSII complex.

According to recent crystallographic models of PSII with a resolution ranging from 2.9 to 1.9 Å [19, 26], each monomer of PSII contains 20 proteins (17 of those are transmembrane subunits and 3 are peripheral subunits on the lumenal side of the complex) and about 90 cofactors. Some of these proteins and major redox cofactors as well as their spatial arrangement are shown in Fig. 2.6. All electron transfer cofactors of PSII are bound to two central transmembrane proteins known as D1 (or PsbA) and D2 (or PsbD). These proteins exist as a D1/D2 heterodimer, which, together with a few other proteins, forms the core of PSII RC. As indicated in Fig. 2.6, the D1/D2 heterodimer binds two branches of redox cofactors related by a pseudo *C*2 symmetry. However, the electron transfer within PSII occurs via cofactors located mainly on the D1 side of the D1/D2 heterodimer. Thus this "active branch" contains two Chl *a* molecules assigned to P680 (P_{D1} and Chl_{D1}), the primary electron acceptor $Pheo_{D1}$, and the primary PQ electron acceptor Q_A; the latter is located on the D2 side (see Refs. [21, 26, 117], Chapter 3 by Renger, and Fig. 2.6). The "inactive branch" of PSII contains symmetrically related cofactors bound mainly on the D2 protein (P_{D2}, Chl_{D2}, and $Pheo_{D2}$), and only the secondary PQ electron acceptor, Q_B, located on the D1 protein, is a part of the ETC, and thus an exception here. The nonheme iron (Fe^{2+}) and an associated (bi)carbonate ion (HCO_3^-/CO_3^{2-}) are located between the quinones Q_A and Q_B.

The reactions of photosynthetic water oxidation in PSII are catalyzed by the so-called oxygen-evolving complex (OEC) located on the lumen side of the thylakoid membrane. The "heart" (inorganic core) of this catalytic complex of water splitting is a cluster of four Mn ions and one Ca ion with five bridging oxygen atoms (denoted as Mn_4CaO_5 cluster) [11, 26, 118]. Three extrinsic proteins, called PsbO (33 kDa), PsbP (23 kDa), and PsbQ (17 kDa), surround and stabilize the Mn_4CaO_5 cluster. In cyanobacteria, however, the two smaller subunits are substituted by PsbV (also known as Cyt c_{550}; 17 kDa) and PsbU (12 kDa) proteins (see Fig. 2.6; for recent reviews on the extrinsic proteins, see Bricker et al. [119] and Fagerlund and Eaton-Rye [120]).

As mentioned above, the end result of the primary charge separation in PSII is the formation of the radical pair $P_{D1}^{\bullet+}Pheo_{D1}^{\bullet-}$. The cation radical $P_{D1}^{\bullet+}$ (traditionally indicated as $P680^{\bullet+}$), which has a very high midpoint potential of ~1.25 V [121,122], is able to sequentially withdraw electrons from the charge-accumulating Mn_4CaO_5 cluster, a catalyst that couples the slow (1–2 ms) four-electron oxidation chemistry of two water molecules with ultrafast (a few ps) one-electron photochemistry. This happens, in turn, via the redox active tyrosine residue of the D1 protein (generally labeled as Y_Z) located between the Mn_4CaO_5 cluster and $P_{D1}^{\bullet+}$. On the acceptor side of PSII, the formed $Pheo_{D1}^{\bullet-}$ very rapidly (within ~200–300 ps) transfers the electron to a tightly bound Q_A molecule that acts as one-electron acceptor; this results in the formation of $P_{D1}^{\bullet+}Q_A^{\bullet-}$. Then the electron is further transferred from $Q_A^{\bullet-}$ to a loosely bound Q_B molecule that acts as a two-electron acceptor (sometimes called a "two-electron gate" of PSII). After a second light reaction followed by charge separation and the formation of $Pheo_{D1}^{\bullet-}$, and further of $Q_A^{\bullet-}$, the once-reduced Q_B^- accepts a second electron. The negative charge of the doubly reduced Q_B (Q_B^{2-}), on

the electron-acceptor side of PSII, is further stabilized by protons derived from the stroma side of the thylakoid membrane, and PQH_2 is formed [115, 123]. Bicarbonate ion, tightly bound to the nonheme iron between Q_A and Q_B is known to play an important role in this protonation reaction (recently reviewed in Shevela et al. [124]). It takes ∼400 μs to form $Q_B{}^{2-}$ and almost 1 ms to form PQH_2. PQH_2 then leaves the complex and the empty Q_B binding site is filled by fresh PQ from the PQ pool in the thylakoid membrane. Meanwhile, PQH_2 diffuses in the membrane toward the Cyt b_6f complex: the electrons from PSII have thus entered the intersystem ETC (Figs. 2.2, 2.5, and 2.6).

2.3.4 Photosystem I: Function and the Electron Transfer Pathways

Just like PSII, PSI is a membrane-integral protein complex that is present in all oxygenic photosynthesizers. Similar to PSII, PSI is also a key player in the utilization of light energy for driving the redox reactions of the ETC. Thus PSI uses the energy of light to power the electron transfer from one mobile electron carrier PC (or alternatively Cyt c_6) to another carrier, Fd, and thereby provides the electrons for the reduction of $NADP^+$. It therefore acts as a *light-driven plastocyanin:ferredoxin oxidoreductase* (see Golbeck [109]). In cyanobacteria, an oligomeric from of PSI exists mainly as a trimer having a molecular weight of ∼1100 kDa. However, in plants, PSI always exists as a monomeric complex (for reviews on PSI, see Refs. [21, 125] and chapters in Golbeck [109]). Each monomer of PSI is known to contain 12 proteins and about 130 noncovalently bound cofactors [106, 126, 127].

The core of PSI complex is formed by two proteins, PsaA and PsaB. These two proteins coordinate the majority of the cofactors of the ETC within PSI, as well as most of the antenna Chls and the Cars of PSI. Moreover, PsaA/PsaB heterodimer is known to be involved in the docking of the mobile electron carrier (PC or Cyt c_6) to be near P700 on the electron donor (lumenal) side of PSI (see Fig. 2.7 and Grotjohann et al. [24]). On the other hand, three proteins (PsaC, PsaD, and PsaE) on the electron-acceptor side of PSI form the docking site of PSI for harboring the mobile electron acceptor Fd (see Fig. 2.7 and Refs. [106, 107]).

As in PSII, the cofactors of the ETC in PSI are arranged in two branches (A and B). These branches contain six Chls, which are P700 the "special pair" of Chl *a* and Chl a' heterodimer, also denoted as P_A and P_B according to Redding and van der Est [111], a pair of neighboring Chl *a* molecules ($ec2_A$ and $ec2_B$) also called A, and another pair of Chl *a* molecules ($ec3_A$ and $ec3_B$) denoted as A_0 in addition to two phylloquinone molecules A_1 (PhQ_A and PhQ_B) (Fig. 2.7). However, unlike in PSII, both branches in PSI are active for the electron transfer [128, 129]. Interestingly, the branch A in PSI of cyanobacteria was found to be more active than B, while in algae the branch B is known to be more active than A [130–133]. The components of two branches are followed by three iron–sulfur centers ([4Fe–4S] clusters) termed F_X, F_A, and F_B (see Fig. 2.7).

As described above, the primary and the secondary charge separation(s) lead to the formation of the secondary radical pair $P700^{\bullet+}\,A_0{}^{\bullet-}$ (also see Müller et al. [94] and Holzwarth et al. [112]). The generated $P700^{\bullet+}$ is then reduced by electrons obtained

from the external electron donor PC (or Cyt c_6). On the other hand, the electron available on $A_0^{\bullet -}$ is transferred via a series of intermediates (including A_1, F_X, F_A, and F_B) toward the Fd docking site, where it finally reduces the external redox carrier Fd. Reduced Fd then provides electrons to the FNR, which catalyzes the production of NADPH, using $NADP^+$ and protons from the stroma (for more information, see Medina [88] and Aliverti et al. [89]). It takes about 200 ps for the electrons to reach A_1, and about a few ms to reach $NADP^+$ (see Fig. 2.5). The reduction of $NADP^+$ to NADPH completes the sequence of the linear ETC.

2.3.5 Intersystem Electron Transfer

2.3.5.1 Cytochrome b_6f Complex and the "Q"-Cycle Light-induced electron transfer between the two photosystems is mediated by the membrane-integrated Cyt b_6f complex; here, this complex catalyzes the transfer of electrons from lipophilic PQH_2 to the soluble PC (see Figs. 2.2 and 2.5). For its property to oxidize PQH_2 and to reduce PC (or Cyt c_6), this complex is often also called the *plastoquinone:plastocyanin oxidoreductase* (for reviews, see Refs. [22, 134, 135]). Moreover, the intersystem electron transport network performed by the Cyt b_6f complex is coupled with transport of protons from the stroma to the lumen, thus contributing to the generation of a proton gradient, and an electrochemical potential across the thylakoid membrane that is further utilized by the ATP synthase for ATP synthesis.

In both cyanobacteria and plants, the Cyt b_6f complex exists as a dimer and contains several prosthetic groups [135, 136]. Each monomeric form of the Cyt b_6f consists of four large protein subunits: *c*-type cytochrome *f* (Cyt *f*), the Rieske iron–sulfur protein (FeS), cytochrome b_6 (Cyt b_6), and subunit IV (suIV). When PQH_2 is oxidized by the FeS protein, protons are released into the lumen. One of two available electrons delivered by PQH_2 passes along a linear ETC to the Cyt *f* subunit and further to a small mobile PC (or Cyt c_6), which then carries the electron toward PSI. The other electron is transferred through the two Cyt b_6 hemes, which then reduces a PQ to PQ^- (semiquinone). Upon receiving one of the two electrons from a new PQH_2 molecule (oxidized by the FeS), PQ^- is reduced to PQ^{2-} at the same time picking up two protons (and forming PQH_2), participating thereby in a cyclic process called the *Q-cycle*. Thus the Q-cycle increases the number of protons pumped across the membrane (Fig. 2.2). Overall, for every two electrons that reach PSI, four protons are translocated across the thylakoid membrane. For more detailed information on the Cyt b_6f complex and its electron transfer, see Refs. [22, 134–138].

2.3.5.2 Linear Versus Cyclic Electron Transfer When the two photosystems, along with the Cyt b_6f complex, drive the electron transfer from water to $NADP^+$, we have the *linear electron transfer* (also referred to as *noncyclic* electron transfer) (see Figs. 2.2 and 2.5). The end result of such an electron transfer is the production of both NADPH and ATP required for the dark reactions of carbon fixation (see Section 2.5). However, under some conditions, electrons from the reducing side of PSI may cycle back toward the Cyt b_6f complex and/or PQ pool rather than to $NADP^+$ and then again back to the oxidizing side of PSI, performing the *cyclic*

electron transfer (see Bendall and Manasse [139] and Joliot et al. [140] and Figs. 2.2 and 2.5). As a result, no NADPH is produced, but the available energy may be used for ATP synthesis. This "extra" ATP can be utilized not only for carbon fixation reactions but also for other processes (e.g., for starch synthesis).

2.3.6 Water as a Source of Electrons for the Photosynthetic Electron Transfer Chain

An important issue here is to realize the unique role of the catalytic Mn_4CaO_5 cluster in the removal of electrons from water for the ETC. Water is a very poor electron donor and its oxidation into O_2 and protons requires a strong driving force. Although the oxidizing potential of cation radical $P_{D1}^{\bullet +}$ ($P680^{\bullet +}$) is very high (about 1.25 V) [121, 122], it is not strong enough to directly "extract" electrons from water. However, it has the ability to split water *involving* the Mn_4CaO_5 cluster. This is because this unique catalyst has the ability to first store four oxidizing equivalents and then to use them for a dovetailed four-electron water-splitting chemistry. Here, one-electron photochemistry is linked to the "extraction" of four electrons from two water molecules. See Chapter 3 by Renger for all aspects of photosynthetic water oxidation and O_2 evolution, its mechanisms and energetics.

2.3.7 Can the Rate Limitation of O_2 Production by Photosystem II Be Improved in Future Artificial Water-Splitting Systems?

The bottleneck reaction of the entire process of light-induced reactions of oxygenic photosynthesis is the diffusion and oxidation of PQH_2, and this could be as slow as 20 ms (see Fig. 2.5). Any improvement in the overall efficiency of electron transport would require that we engineer the system to make this reaction faster.

Interestingly, data obtained on PSII membrane fragments show that at high flashing rates the electron-acceptor side of PSII is rate limiting for O_2 evolution (water oxidation) [141, 142]. Such limitation of O_2 evolution by the reactions on the electron-acceptor side may indicate that there was no evolutionary pressure to make the relatively "slow" 1–2 ms turnover frequency of the OEC faster. Therefore one can assume that there is a chance to develop in future water-splitting artificial catalysts that have higher turnover rates than the "natural" Mn_4CaO_5 cluster of PSII.

2.4 PHOTOPHOSPHORYLATION

2.4.1 Overview

Photophosphorylation was discovered independently by Albert Frenkel in 1954 [143] in chromatophores of photosynthetic bacteria, and in the same year by Arnon et al. [144] in chloroplasts of higher plants. In addition, Strehler [145] had earlier observed photosynthetic ATP production, using the luciferase–luciferin system. The overall process is the production of ATP, on the ATP synthase, from ADP and inorganic

phosphate (P_i), using the pmf, the sum of the ΔpH and $\Delta\psi$, the membrane potential, formed during light-induced electron flow in photosynthesis, on the thylakoid membrane [146] (see Section 2.3); for a historical perspective, see Jagendorf [91]. We note at the very outset that about 42 kJ of converted light energy is stored in each mole of ATP, and ATP is dubbed the *energy currency of life*.

In view of the earlier controversies between the chemical and the chemiosmotic mechanisms for ATP synthesis, we refer readers to a review of opinions that had, interestingly, all the parties as coauthors [147]. In 1978, Peter Mitchell received the 1978 Nobel Prize in Chemistry for the chemiosmosis hypothesis, and in 1997, Paul Boyer and John Walker shared the Nobel Prize, also in Chemistry, on how the ATP synthase converts this pmf to chemical energy via a rotary mechanism [148–150].

The pmf is formed during the noncyclic electron flow from water to $NADP^+$, involving PSII, Cyt b_6f, and PSI, as well as during the cyclic electron flow involving PSI and Cyt b_6f (see Figs. 2.2 and 2.5). As mentioned above (see Section 2.3.5.1), there is also a so-called Q-cycle around Cyt b_6f (for a perspective on the Q-cycle, see Crofts [151]). Oxidation of two water molecules, in PSII, is accompanied by the release of four protons into the lumen (the *p*-side); and consequent reduction and oxidation of two molecules of PQ leads to the transfer of another four protons from the stromal (the *n*-side) to the lumen. When PSI and the Q-cycle are involved, a much larger number of protons are available (Fig. 2.2).

2.4.2 Mechanism of ATP Synthesis

When Jagendorf and Uribe [152] suspended thylakoids in an acidic medium and then transferred them to an alkaline medium, and that too in darkness, but in the presence of ADP and P_i, ATP was produced, as if ΔpH was responsible for ATP synthesis! Furthermore, Witt et al. [153] found that an applied voltage in the medium was able to drive ATP synthesis. These and other early discoveries are thus the backbone of Mitchell's chemiosmotic hypothesis, which is simply that it is the energy available from the dissipation of the pmf, through the thylakoid membrane, from the *p*-side (lumenal) to the *n*-side (stromal) that makes ATP from ADP and P_i [146].

The ATP synthase, which has a molecular mass of ~600 kDa, is ~15 nm long and ~12 nm wide. It is made up of F_0 (a hydrophobic part, embedded in the membrane) and F_1 (a hydrophilic part that protrudes into the stroma) (see Fig. 2.8). The F_0 has three subunits: a, b, and c (several copies, up to 15 in some cases), whereas F_1 has 5 subunits: α (3 copies); β (3 copies), γ (1 copy), δ (1 copy), and ε (1 copy). The ATP synthase is actually a rotary motor, with most of the F_0 units rotating, after protons are bound to its "c" subunits; the binding energy of protons, which are being translocated, is converted into mechanical energy leading to rotation (see Refs. [20, 150, 154]). Thus protons on the *p*-side of the thylakoid membrane begin the process of ATP synthesis [155]. The number of protons used for the synthesis of one molecule of ATP is not a constant number; in many cases four protons per ATP have been observed (see van Walraven and Bakels [156]).

In the stator (α, β, and δ) part of F_1, the mechanical energy in the above-mentioned rotary motion is converted into chemical energy needed to make the *high-energy*

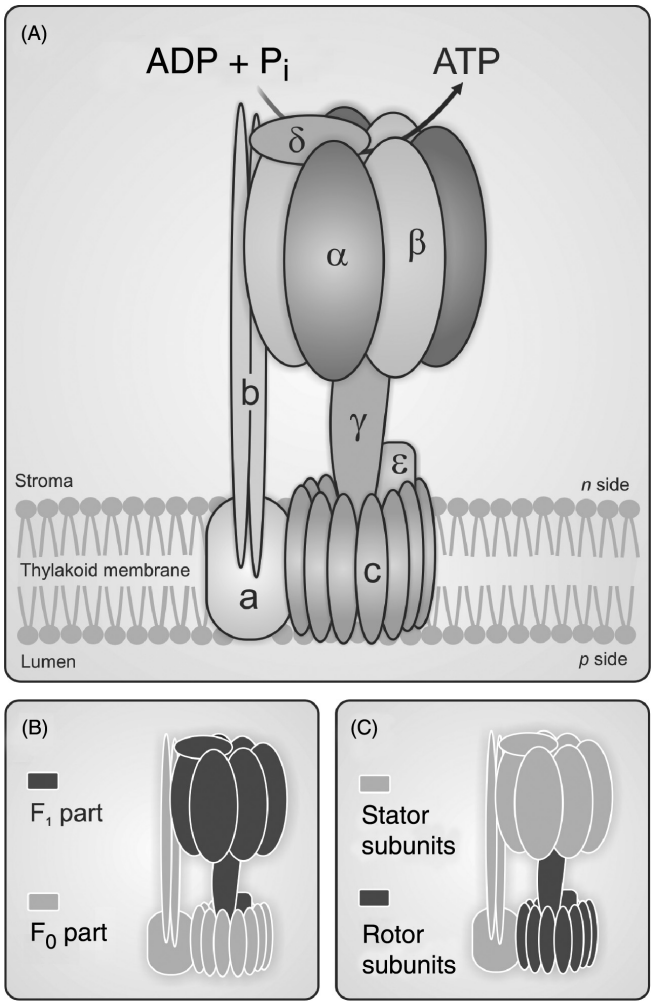

FIGURE 2.8 A schematic view of ATP synthase and its subunits. (A) Overall view of subunits of ATP synthase. (B) Two basic parts of ATP synthase: a membrane-integrated F_0 part and peripheral F_1 part (shaded individually as depicted in the figure). (C) Rotor and stator parts of ATP synthase (also shaded individually as depicted in the figure). For further details and abbreviations, see text. Adapted from Shevela et al. [249].

phosphate bonds in ATP. On the α and β subunits, ADP and P_i are converted into ATP (for details, see Spetzler et al. [157]; also see a self-explanatory Fig. 2.8).

In conclusion, the pmf is used by the c subunits of F_0 to be converted into a mechanical rotation energy (a torque), which is then subsequently used by the α and β subunits of F_1, where this rotation energy is converted into chemical energy, and ATP is formed from ADP and P_i. Furthermore, ε and γ subunits are used to regulate this ATP synthase activity [158, 159].

2.4.3 Concluding Remarks

The ATP produced by the ATP synthase has many functions; it is used for the biosynthesis of many compounds, particularly in the steps leading to carbon fixation (see Section 2.5). It is also used, for instance, for protein synthesis in the chloroplast, while the ATP necessary for the synthesis of proteins and other compounds, outside of the chloroplast, is generated by respiration.

2.5 CARBON DIOXIDE TO ORGANIC COMPOUNDS

2.5.1 Overview of Carbon Dioxide Assimilation Systems in Oxygenic Organisms

Over the ages, many pathways have evolved both in photoautotrophic and chemoautotrophic organisms (Fig. 2.9). Figure 2.9A shows the photosynthetic carbon reductive pathway, known as the Calvin–Benson–Bassham cycle, which is the assimilation pathway in oxygenic organisms, as well as in some nonoxygenic ones. Figure 2.9B depicts a reductive tricarboxylic acid (TCA) cycle (known as the Arnon–Buchanan cycle, in some bacteria); it shares a common evolutionary origin with the well-known respiratory TCA cycle (the Krebs cycle), as it is essentially the same cycle running in reverse. The other two cycles occur in various prokaryotes: the reductive acetyl-CoA pathway (of methanogenic archaea) (Fig. 2.9C), and the 3-hydroxypropionate cycle (of some bacteria) (Fig. 2.9D).

During 2007–2008, two additional pathways for the assimilation of CO_2 were discovered in Archaea: the 3-hydroxypropionate/4-hydroxybutyrate cycle [160] and the dicarboxylate/4-hydroxybutyrate cycle [161]. Figure 2.10 represents the proposed reactions of the autotrophic 3-hydroxypropionate/4-hydroxybutyrate cycle in the thermoacidophilic archaeon *Metallosphaera sedula* (for further details, see Berg et al. [160]). For overviews of "classical" and "novel" metabolic pathways, see Refs. [162–165]. For thermodynamic constraints of carbon fixation pathways, see Bar-Even et al. [166].

In plants there exist two main pathways for CO_2 assimilation, the C3 (Calvin–Benson–Bassham cycle, mentioned above), and C4 (Hatch–Slack pathway) cycles. They are discussed below.

2.5.2 C3 Pathway Versus C4 Pathway

In C3 plants CO_2 is bound to the enzyme ribulose bisphosphate carboxylase oxygenase, often referred to as RuBisCO (or Rubisco). This enzyme converts it to the three-carbon compound phosphoglyceric acid, hence the term C3. Phosphoglyceric acid is then further processed in the Calvin–Benson–Bassham cycle, shown on the right side of Fig. 2.11. For a description of its discovery, see Benson [167] and Bassham [168].

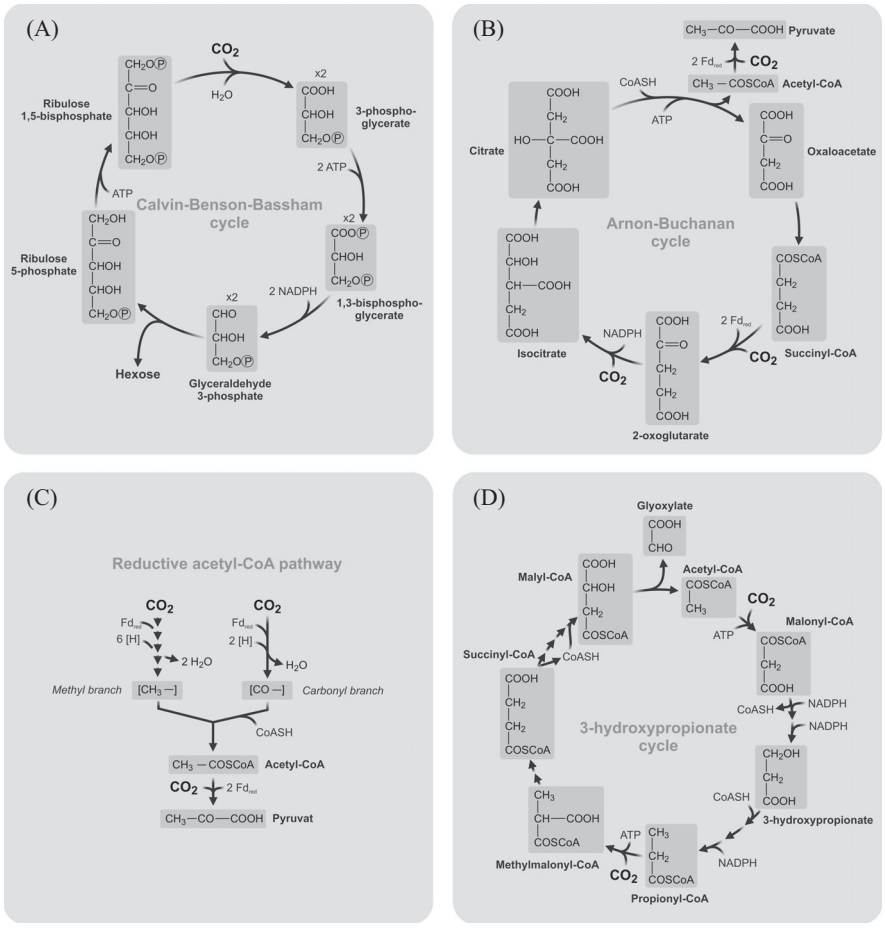

FIGURE 2.9 Metabolic cycles for the assimilation of CO_2 present in various organisms: (A) The Calvin–Benson–Bassham cycle. (B) The reductive TCA cycle (also called Arnon–Buchanan cycle). (C) The reductive acetyl-CoA pathway. (D) The 3-hydroxypropionate cycle. Of these, only the Calvin–Benson–Bassham cycle is present in oxygenic organisms (cyanobacteria, algae, and plants). Symbols in the cycles are: C, assimilated carbon; H, reduction equivalents; Fd_{red}, reduced ferredoxin; P or P within a circle, phosphate groups; CH_3-, enzyme-bound methyl group; CO−, enzyme-bound carbon monoxide. The above schemes are reproduced from Sato and Atomi [163] and Strauss and Fuchs [164] with permission of John Wiley & Sons, Inc.

At low CO_2/O_2 ratios, C3 plants are at a disadvantage because Rubisco does not bind CO_2 tightly, and because O_2 competes with CO_2 at the Rubisco surface, and results in photorespiratory carbon loss. High temperature is also to the disadvantage of C3 plants, because photorespiration increases sharply with temperature. (Photorespiration is an oxygen-consuming process resulting from the fact that the CO_2-binding enzyme, Rubisco, also binds O_2, starting a complicated reaction sequence.) Finally,

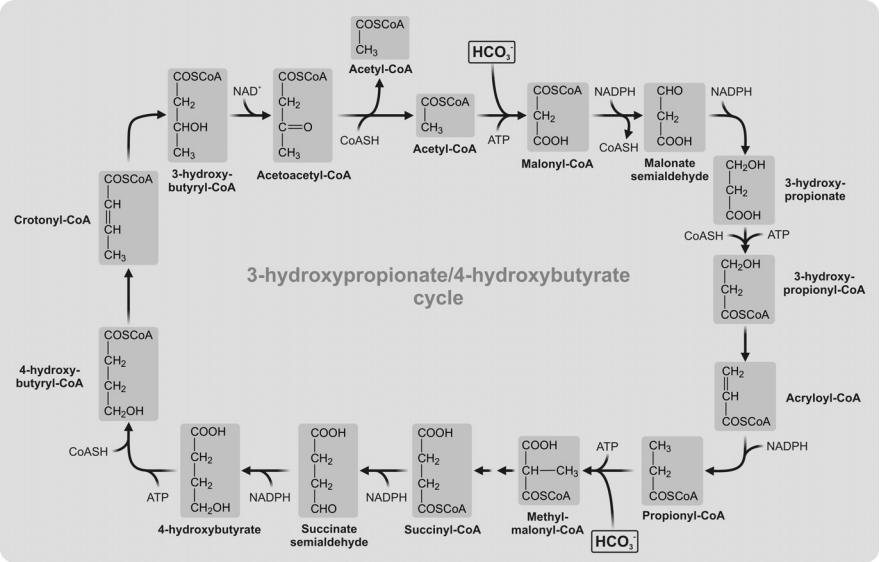

FIGURE 2.10 Proposed autotrophic 3-hydroxypropionate/4-hydroxybutyrate cycle in the archaeon *Metallosphaera sedula*. This scheme is reproduced from Sato and Atomi [163] with permission of John Wiley & Sons, Inc.

arid conditions disfavor C3 plants, because they have to keep their stomata more open, since they cannot maintain an equally steep diffusion gradient for CO_2 across the stomatal pores as do the C4 plants. Stomata are the tiny pores in plant leaf surfaces which let carbon dioxide in and oxygen and water vapor out. C4 plants are apparently at a disadvantage relative to C3 plants at high CO_2/O_2 ratios because of the additional energy expense needed to concentrate CO_2 in their bundle sheath cells (left side of Fig. 2.11). However, at low CO_2/O_2, C4 plants can achieve a high quantum yield of photosynthesis by suppressing photorespiration. For details on C4 photosynthesis and related CO_2 concentrating mechanisms, see chapters in Raghavendra and Sage [169].

2.5.3 C3 Versus C4 Plants During Glacial/Interglacial Periods

During the ice ages, the CO_2 content of the atmosphere was lower than during the interglacial periods. During the last glacial maximum, it was only half of the present content. The climate was also drier. This would favor C4 plants. But it was also colder. This would favor C3 plants. The outcome of these opposing tendencies is that C4 plants were more competitive against C3 plants during ice ages than they are now. But the total primary production of plants was only about 63% of that at the present [170].

The balance between C3 and C4 plants has shifted over time in a complex way and has varied from place to place. Greater C4 plant abundance only occurred when low CO_2 pressure coincided with increased aridity, as observed during the last glacial

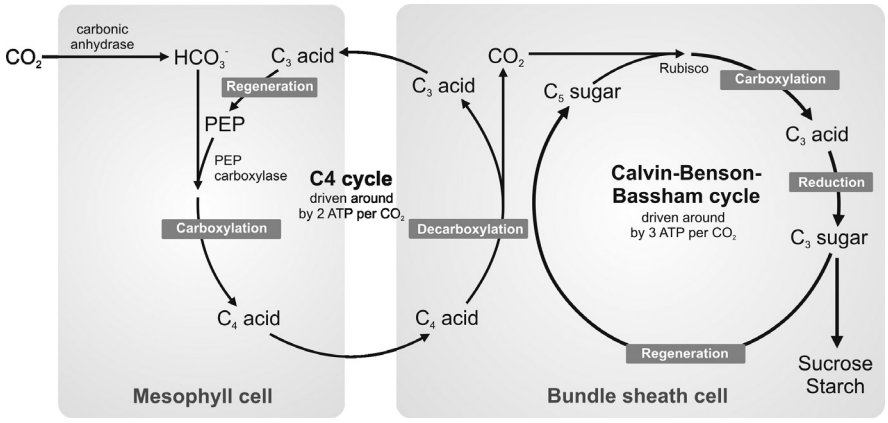

FIGURE 2.11 Carbon assimilation in C4 plants takes place in two cycles. The first cycle acts as a pump to deliver carbon dioxide at a high concentration to the second cycle, which is identical to the Calvin–Benson–Bassham cycle in C3 plants. The specific C4 cycle, driven by the energy in ATP, is slightly different in different groups of C4 plants, and the diagram here is much simplified.

maximum in Guatemala and in tropical Africa and India. Even at the minimum atmospheric CO_2 pressure during the last glacial maximum, the combined effect of high winter precipitation and low temperatures led to an expansion of C3 plants [171]. An investigation of the still arid Chinese loess plateau shows that the highest percentage of C4 plants did not occur there at the last glacial maximum (about 20,000 years ago), but much later, about 7000 years ago [172]. In the Ganges-Brahmaputra drainage area (including the Himalayas), the percentage of C4 plants, as deduced from marine sediments in the Bay of Bengal, declined almost continuously from the last glacial maximum to the present day [173, 174].

2.5.4 Concluding Remarks: Can the Natural Assimilation Pathways Be Improved to Help Solve the Energy Crisis?

The Calvin–Benson–Bassham cycle was the first assimilation pathway to be discovered, and it was first thought that it was the only one. Later, we learned that there were many other possibilities, each one adapted for a specific situation. A take-home lesson is that, since our needs are different from those of any other organism, we should not try to uncritically copy nature. There are indications that the natural fixation pathways can be "improved" in order to better comply with our specific needs. Bar-Even et al. [175] have proposed several artificial cycles. As an example we show the simplest one (Fig. 2.12).

We wish to aim at a future where less of our energy needs are met by burning fuel, but still there will probably always be a need for some fuel burning, and the fuel should be produced by us, and not from fossils. The ideal fuel from a climate-friendly aspect is hydrogen gas, which does not cause emission of CO_2 when burned, but transport issues must be solved. The kind of fuel we primarily want should be fluid (i.e., liquid

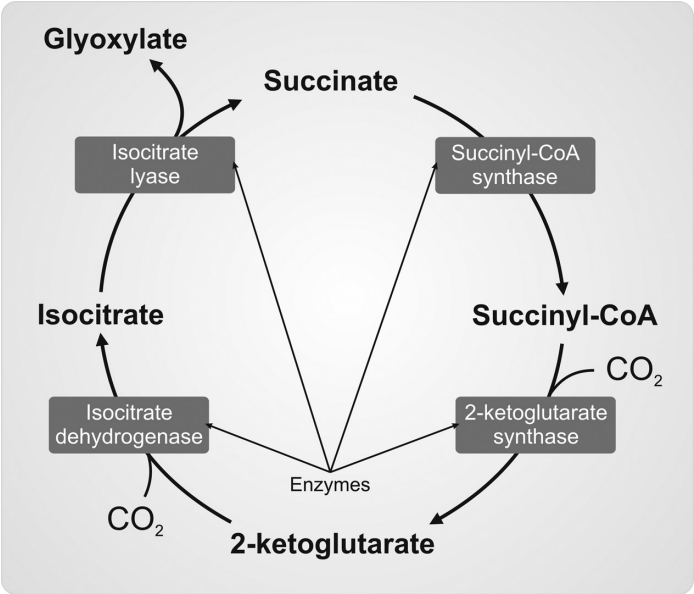

FIGURE 2.12 The shortest possible carbon-fixation cycle and the involved enzymes. Two CO_2 molecules are fixed to give glyoxylate, a two-carbon compound, as the cycle product. In fact, we are of the opinion that this cycle is not thermodynamically feasible and does not represent a viable alternative for carbon fixation. Adapted from Bar-Even et al. [175].

or gaseous), not carbohydrate, which plants mostly produce as primary assimilation products. To produce fluid fuels we need to handle carbon, but not necessarily in a process resembling the assimilation by photoautotrophic organisms.

2.6 EVOLUTION OF OXYGENIC PHOTOSYNTHESIS

2.6.1 Overview

Oxygenic photosynthesis utilizes two photosystems, PSI and PSII, coupled in series via many redox intermediates (see Section 2.3). Clearly this very complex machinery has evolved from simpler bacterial systems using a single photochemical reaction. Many bacteria that are not closely related are capable of anoxygenic photosynthesis; in fact, variants of this process take place in species belonging to most major groups of bacteria. For this reason it was for some time believed that the last common ancestor of bacteria was a photoautotroph. More recent insight showing that horizontal gene transfer is very common even between distantly related bacteria has changed this opinion. It is now believed that chemoautotrophy preceded photoautotrophy during biological evolution. Before molecular oxygen became common, other chemical species such as trivalent iron [176] and/or nitric oxide (NO) [177] would have served as electron sinks for respiration. Nevertheless, photosynthesis is a very ancient process, and its beginning is obscure.

2.6.2 Two Photosystems for Oxygenic Photosynthesis

As mentioned earlier, oxygenic photosynthesis depends on two photochemical reactions (by PSII and PSI), connected via the Cyt b_6f complex, and mobile electron carriers. PSI has a great similarity to a homodimeric type I RC that is present in modern green sulfur bacteria, and PSII has a great similarity to a type II RC that is present in purple bacteria. The differences between PSI and PSII are pronounced, and their amino acid sequences are very different. Yet the structural similarities are great enough to make it very plausible that they both have evolved from the same primitive type of RC [178] resembling a present-day type I RC of Chlorobiaceae [179, 180], and that in turn would have evolved from some component of a respiratory electron transport chain, probably a precursor of Cyt *b* [181, 182].

We do not know whether the present-day organisms, which have only one kind of RC, have evolved by gene loss from an early kind of prokaryote(s) with RCs of both type I and type II. The competing hypothesis is that organisms with both RCs have evolved from symbiosis between one organism with a type I RC and another one with a type II RC [25, 183, 184]. The current opinions seem to have been leaning more and more toward the former hypothesis. Pierson and Olson in 1987 [185], and, with new arguments, Nitschke et al. in 2010 [186] came to the conclusion that Heliobacteria (having only a type I RC) were derived from organisms with two RCs. In 1981 Olson [187] described how the two RCs evolved in a Chl *a*-containing organism carrying out anoxygenic photosynthesis. From this, three evolutionary paths diverged. One led to oxygenic photosynthesis. In the other two paths, one or the other RC was lost, and new RC Chls evolved. In 2007, Allen and Martin [188] gave a new twist to this and described how even an anoxygenic organism could profit from having two kinds of RC in order to be able to adapt to environments with varying redox potentials.

The very early appearance of cyanobacteria suggests that two photosystems must have evolved a long time ago. The last common ancestor for cyanobacteria and Chloroflexi is estimated to have lived about 2.6 billion years (Ga) ago [189, 190]. Whether this ancestor was oxygenic or not is not known, but the ability to evolve oxygen is thought to have originated at least 2.4 Ga ago. Nisbet et al. [191], based on carbon isotopic evidence for early appearance of type I Rubisco, conclude that "oxygenic photosynthesizers first appeared ∼2.9 Ga ago, and were abundant 2.7–2.65 Ga ago." Czaja et al. [192, 193], based on isotopic data for both iron and molybdenum, assume that free O_2 existed in the oceans 2.5–2.7 Ga ago; while the O_2 content of the atmosphere could still have been very low, they believe that their work "provides strong support for the development of oxygenic photosynthesis by at least 2.7 Ga, because anoxygenic photosynthesis will not produce the coupled variations in the measured Fe and Mo isotope compositions." Schwartzman et al. [194], based on atmospheric and climatic history, believe that oxygenic photosynthesis with bicarbonate, instead of water, as electron donor, and primitive cyanobacteria, existed already 2.8 Ga ago.

Thus the evidence is strong, and comes from various kinds of data that oxygenic photosynthesis took place already about 2.7 Ga ago, half a billion years before the "great oxygenation event." There are indications for the presence of primitive morphological forms of cyanobacteria (about 3.5 Ga ago) [195, 196]. However, there

is no evidence that these first cyanobacteria were oxygenic. Other groups including photosynthetic bacteria, such as Chlorobia, must have split off from the cyanobacterial ancestors much earlier than 2.7 Ga ago, but their photosynthetic members could have acquired photosynthesis genes by horizontal gene transfer.

The next major step in the evolution of photosynthesis is the endosymbiotic event in which chloroplasts arose from cyanobacteria [197]. According to Falcón et al. [198], the clade leading to chloroplasts split off from extant cyanobacteria a little over 2 Ga ago.

2.6.2.1 Photosystem I: From Cyanobacteria to Angiosperm Although the PSI core complexes are very similar in cyanobacteria and higher plants, this does not hold for the structure at the periphery [199, 200]. Another difference is that the cyanobacterial PSI core is trimeric heterodimer, while the plant PSI core appears as a monomeric heterodimer. The heterodimer has evolved from an ancestral homodimer. Trimerization of the cyanobacterial variant occurs via a polypeptide called PSaL [201]. The monomeric state of the plant PSI core allows for more peripheral proteins to be included in the system, and in particular for the constitutive LHCI antenna, and for the mobile LHCII antenna, which migrates from the grana and PSII during state transition [202]. The plant PSI–LHCI supercomplex contains 19 known protein subunits and approximately 200 noncovalently bound cofactors. The simpler PSI of cyanobacteria has no LHCI and only 12 subunits and 127 cofactors [106, 126]. In unicellular green algae, on the other hand, whose path of evolution separated from that of the land plants over 1 billion years ago, LHCI is about three times larger than that of higher plants [203, 204]. Both this larger antenna in aquatic green algae and the trimeric state in cyanobacteria may reflect the lower light levels in many aquatic environments.

On the reducing (electron-acceptor) side of PSI, changes have taken place during evolution from cyanobacteria to higher plant chloroplasts. Cyanobacterial PSI has only one docking site for Fd, which binds this cofactor loosely, and there is no docking site for FNR. The Fd thus has to diffuse away from the PSI complex to reduce $NADP^+$. Plant PSI has one loosely binding and one firmly binding Fd site, and also a site for the binding of the FNR. This allows a direct electron transfer in PSI, and thus there is less risk for competing reactions, such as reduction of O_2 to O_2^-. On the other hand, cyanobacteria have greater flexibility in case of nutrient deficiency. In the case of iron deficiency, they can use flavodoxin in place of the iron-containing Fd (Fig. 2.7). Flavodoxins occur in a wide range of bacteria and are thus not an invention of cyanobacteria. Although higher plants do not naturally contain flavodoxin, bioengineering can produce such plants, and they are more tolerant than natural plants to iron deficiency [205, 206]. The gene for flavodoxin may have been lost when plants first made their way onto land in an iron-rich environment [207].

In the case of copper deficiency, cyanobacteria (as well as red and brown algae) can use Cyt c_6 instead of PC. Plants and green algae (as well as some cyanobacteria) contain the related Cyt c_6 with unknown function, but this has a too low midpoint redox potential to be able to function in the same way. It is thought that Cyt c_6

preceded PC in evolution and was the electron carrier to PSI in the first cyanobacteria. *Geobacter sulfurreducens* has a *c*-type Cyt closely resembling Cyt c_6 [208]. PC was probably introduced when oxygenic photosynthesis had already begun, and copper became more easily available than iron [209].

2.6.2.2 Photosystem II: Its Light-Harvesting Proteins and Water-Splitting Site During evolution from bacterial type II RCs to plant PSII, several remarkable changes took place. The redox potential on the oxidizing side had to be increased to allow for the oxidation of water, and protection from the destructive action of molecular oxygen and its high-potential precursors had to be achieved [210,211]. Regulation mechanisms, the so-called state transitions, were developed to make PSI and PSII work "in step." Light-harvesting, pigment-carrying proteins and the remarkable water-oxidizing enzyme that collects the four charges for creation of a molecule of oxygen were added.

The primitive cyanobacterium *Gloeobacter violaceues* lacks thylakoids and does not have well-developed state transitions [212]. The absorption capacity (absorption cross section) of PSII can be varied by only a few percent [213]. In contrast, other cyanobacteria can acclimate to light and vary their distribution of energy to the photosystems both by movement of the phycobilisomes in the thylakoid membranes [214] and by chromatic acclimation ("chromatic adaptation"), that is, change of the pigment composition of the phycobilisomes. In higher plants the light-harvesting proteins of PSII have the major role of capturing light. The steps in the evolution of light-harvesting proteins of PSII have been described by Ballotari et al. [215].

As mentioned above, the OEC contains four atoms of manganese and one atom of calcium. It is natural to assume that it may have evolved from a precursor that is also a precursor to another extant manganese protein. Raymond and Blankenship [216] have explored this idea and found a structural similarity between the OEC and manganese catalase, but the latter enzyme contains only two manganese atoms. Dismukes et al. [217] envisage an early form of oxygenic photosynthesis in which bicarbonate (HCO_3^-), which is easier to oxidize to oxygen, rather than water served as the electron source. Bicarbonate under the early conditions with more reducing condition and higher concentration of CO_2 in the atmosphere and more bicarbonate in the ocean would have led to the formation of manganese bicarbonate complexes, $Mn_2(HCO_3)_4$. Could this have contributed the other two manganese atoms? For a review on the roles of bicarbonate in PSII, most of which have been shown to be on its electron-acceptor side, see Shevela et al. [124].

2.6.3 Evolutionary Acclimation to Decreasing CO_2 Availability

2.6.3.1 Is Rubisco a "Bad" Enzyme? Rubisco has often been described as a "bad" enzyme, because it is relatively slow, it does not bind CO_2 very tightly, and O_2 competes with CO_2 at the binding site. Many pathways have evolved for the assimilation of CO_2, but Rubisco is the only CO_2-binding enzyme at which O_2 can compete with CO_2. Therefore it seems a bit surprising that it is Rubisco that has come

to completely dominate the entrance to CO_2 assimilation in the oxygenic organisms. The explanations for this are the following:

1. The CO_2 concentration was much higher and the O_2 concentration negligible when water oxidation was "invented."
2. Various mechanisms evolved for concentrating CO_2 at the Rubisco surface.
3. Rubisco is perhaps not that bad after all.

2.6.3.2 Rubisco Properties We start the discussion with Rubisco being not that bad after all. Tcherkez et al. [218] explained that, for Rubisco, there exists an intrinsic and inescapable conflict between the rate constant for carboxylation, k^c_{cat}, and the CO_2/O_2 specificity, $S_{c/o}$ (see Fig. 2.13): the higher the specificity, the lower the rate. They argued that all Rubiscos are almost perfectly tuned to the requirement of different organisms in their environment. From Fig. 2.13 it seems as if the cyanobacterial Rubiscos are superior to the eukaryotic ones, but they are, in fact, inferior with respect to another important property: they bind CO_2 less tightly with a Michaelis–Menten constant of around 300 μM as compared to 10–80 μM for the

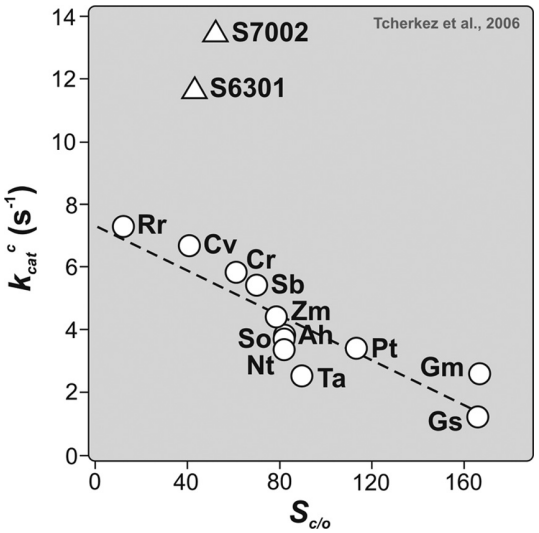

FIGURE 2.13 Rate constant for carboxylation as a function of the CO_2/O_2 specificity for Rubiscos from various species. The regression line is drawn neglecting the two cyanobacterial outliers (triangles). The Rubiscos were isolated from the following organisms: Ah, *Amaranthus hybridus* (C4 dicot); Cr, *Chlamydomonas reinhardtii* (green alga); Cv, *Chromatium vinosum* (bacterium); Gm, *Griffithsia monilis* (red alga); Gs, *Galdieria sulfuraria* (red alga); Nt, *Nicotiana tabacum* (C3 dicot); Pt, *Phaeodactylum tricornutum* (diatom); Rr, *Rhodospirillum rubrum* (bacterium); Sb, *Sorghum bicolor* (C4 monocot); So, *Spinacia oleracea* (C3 dicot); S6301, *Synechococcus* PCC 6301 (cyanobacterium); S7002, *Synechococcus* PCC 7002 (cyanobacterium); Ta, *Triticum aestivum* (C3 monocot); and Zm, *Zea mays* (C4 monocot). Adapted from Tcherkez et al. [218], copyright © 2006 National Academy of Sciences, U.S.A.

eukaryotes [219]. The relation between the properties of Rubisco is further discussed by André [220].

2.6.3.3 CO_2 Concentrating Mechanisms

Cyanobacteria compensate for the high Michaelis–Menten constant (the low affinity) for CO_2 by active uptake of bicarbonate into the cytoplasm in combination with carboxysomes. Carboxysomes are "Rubisco cages" surrounded by a barrier that is impermeable to CO_2. They also contain carbonic anhydrase that converts bicarbonate to carbon dioxide, resulting in a high CO_2 concentration at the Rubisco. The plasma membrane is permeable to carbon dioxide, so it is essential that the cytoplasm lacks carbonic anhydrase. In various groups of cyanobacteria several mechanisms for the active uptake of bicarbonate have evolved [221, 222]. Further, eukaryotic algae and some higher plants are equipped with various CO_2-concentrating mechanisms [223]. We have already mentioned the C4 mechanism above.

2.6.3.4 CO_2 Levels in the Atmosphere Over Time

We do not know what the availability of CO_2 was for the first photosynthetic organisms, but the concentration was much higher than now [224, 225]. From an initially very high level, it declined sharply around 4 Ga ago, when CO_2 was partly replaced by methane, resulting from the activity of methanogenic archaea [226].

Several methods have been used to estimate the past levels of atmospheric carbon dioxide. They do not show detailed agreement but give a reasonably consistent overall picture. One method is based on a budget of carbon, sulphur, and oxygen as the atoms circulate among different chemical forms and compartments over time. Several biological proxies have also been used, the most important is based on plant stomata. It has been found that both their size and their density on the leaf surface adapt to carbon dioxide concentration in the surrounding air, and they can be measured and counted on leaf fossils. Of course, this method can be used only for times since stomata evolved, in the late Silurian, about 420 million years ago. Figure 2.14 shows a comparison of the budget method (specifically the GEOCARBSULF model of Berner; for details, see Berner [227]) and various stomata-based proxies.

2.7 SOME INTERESTING QUESTIONS ABOUT WHOLE PLANTS

2.7.1 Overview

Here we first list some questions for the readers to ponder; then we present our views on these topics.

- Why are there grana in land plants but not in algae?
- Why are leaves darker on the upper side than on the lower side?
- How much do different layers in the leaf contribute to photosynthesis?
- How does photosynthesis interact with climate-atmosphere?

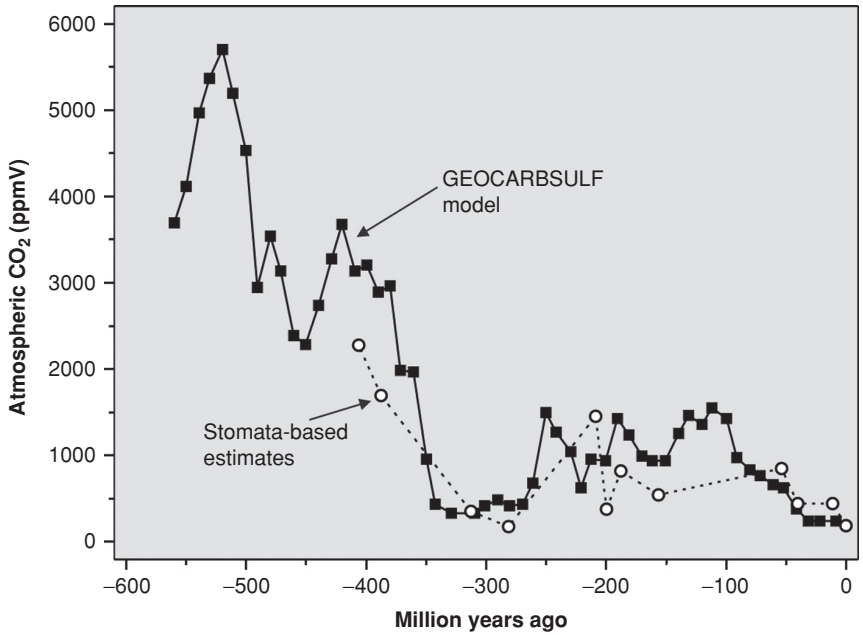

FIGURE 2.14 Atmospheric CO_2 over the Phanerozoic by the GEOCARBSULF model of Berner [227] (solid line, closed symbols), and stomata-based estimates [250–252] of van der Burgh et al. [252] (dashed line, open symbols).

- Is there photosynthesis without CO_2 assimilation (N_2 fixation in cyanobacteria, light-dependent NO_3^- assimilation in land plants)?
- How can animals carry out photosynthesis?

2.7.2 Why Are There Grana in Land Plants but Not in Algae?

A characteristic of land plants is that their chloroplasts contain grana, regions where the thylakoid membranes are appressed without any stroma regions between them (Fig. 2.1). Algae living in an aquatic environment do not have this structural arrangement.

The most likely explanation for this was advanced by Jan M. Anderson [228]. Her theory is based on the existence of different light environments in water and in air. The light environment of the red algae and the land plants is very different. Red algae live in water, often deeper than other algae. The light reaching them is filtered through a thick layer of water. This layer absorbs long-wavelength light more strongly than other visible (and photosynthetically active) light. Therefore an energy deficiency in light absorption by PSI relative to PSII could easily develop. However, extra energy can come to PSI by a process called *spillover*, from PSII [229]. Spillover of energy is possible since the light quanta absorbed by PSII are larger than needed to excite long-wavelength absorbing Chls in PSI. Furthermore, spillover is possible here

because the two photosystems are intermingled in the algal chloroplasts. Because the spectrum of available light in deep water lacks far red light, there is never a risk that there will be too much energy for PSI, as compared to that in PSII. Furthermore, since light intensity is attenuated in deep water, it is advantageous for the red algae to retain the light-harvesting phycobilisomes, which came with the cyanobacteria giving rise to chloroplasts.

For land plants, the situation is different. The first plants colonizing land were small beech organisms living without competition from larger plants, exposed to full sunlight; their forerunners, the green algae, lived in very exposed habitats [230]. Their problem was not lack of light energy, and thus they did not have much use for phycobilisomes. With time, plants grew larger and were more numerous, and started to shade one another and compete for light. The average chloroplast became more and more shaded, filtered by other chloroplasts. For an individual chloroplast, it did not matter much whether the chloroplasts shading it were located in other plants, in other leaves on the same plant, or even in the same leaf. The light hitting the chloroplast became, during the evolution of plants and ecosystems, more and more depleted in short-wavelength light, while the long-wavelength light, on the long-wave edge of the chlorophyll absorption spectrum, was not attenuated to the same extent. The spectral situation contrasted sharply against that for chloroplasts in red algae. Now we speculate that the imbalance between the photosystems could not be adjusted by spillover, since the light quanta, absorbed by the most exposed PSI, were too small. Therefore PSI and PSII had to be separated to prevent spillover, or PSII would receive even less energy. Evolution has succeeded in this by development of grana in the chloroplasts of land plants (see Fig. 2.1). Grana are regions in the chloroplasts where the thylakoid membranes are closely stacked on top of one another and are enriched in PSII. The stacking of membranes and absence of PSI gave room for larger pigment antennas, not in the form of phycobilisomes, but in the form of protein-bound pigment (Chl *a* and Chl *b*) complexes. PSI is located in the more sparsely distributed membranes between the grana. There, it is in contact with stroma between the membranes, and this is advantageous because PSI delivers reducing equivalents via ferredoxin to $NADP^+$, which are then used for the reduction of carbon dioxide in the stroma region.

2.7.3 Why Are Leaves Darker on the Upper Side than on the Lower Side?

Almost all leaves, except those positioned almost vertically, appear darker on the upper (adaxial) side than on the lower (abaxial) side. We speculate that the reason for this is that they are adapted for receiving light from the upper side. The mesophyll on the upper side, a *palisade* with oblong cells, is arranged perpendicular to the leaf surface. Light entering through the transparent upper epidermis is partly absorbed by the chloroplasts in this layer, and partly penetrates deeper into the leaf through the vacuoles and cell walls [231]. Only little light is scattered back [232].

The mesophyll in the lower part of the leaf has a different structure, with large intercellulars and cells extending in different directions in a loose, spongy way. This

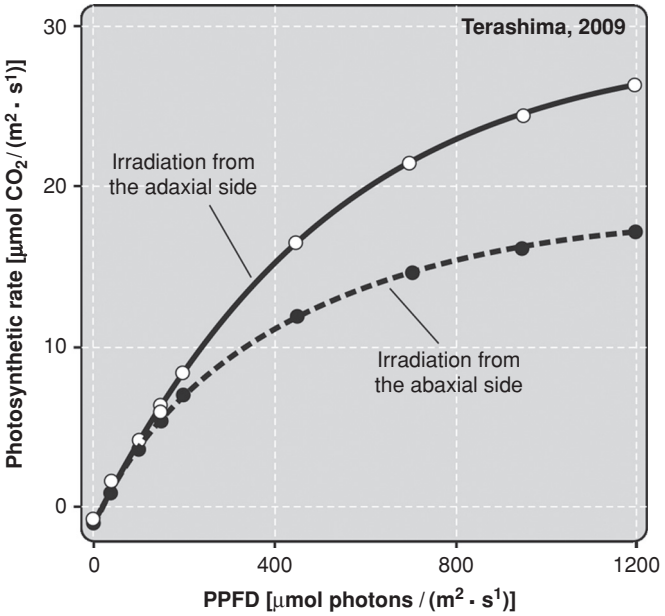

FIGURE 2.15 Net photosynthetic rate versus photosynthetic photon flux density for a leaf of *Helianthus annuus*, for the two sides of the leaf. Conditions are: 25°C, 390 μL/L CO_2, water vapor pressure deficit 0.7 kPa. Adapted from Terashima et al. [233], copyright © 2009, Oxford University Press.

layer scatters much of the light reaching it from above, back into the palisade layer (while some is absorbed in the spongy layer). Also, light hitting the lower side is to a large extent scattered back, giving the lower side of the leaf a lighter color. The greater reflectivity of the lower side of the leaf causes light hitting the lower side to have less effectiveness than light hitting the upper side (see Terashima et al. [233] and Fig. 2.15).

2.7.4 How Much Do Different Layers in the Leaf Contribute to Photosynthesis?

This question is not easy to answer. Although very sophisticated methods have been employed to answer this question, the results remain difficult to understand. In particular, there seems to be a difference between how CO_2 assimilation and how O_2 evolution are distributed throughout the thickness of the leaves. Experiments have been conducted on leaves of different species, but we limit ourselves here to the situation in a spinach leaf, which can be regarded as a leaf with rather ordinary structure, not adapted to any kind of extreme environment. Such a leaf is about 456 μm thick when shade-adapted (grown in weak light) and 631 μm thick when grown in strong light [234].

Light entering from the adaxial (upper) side of a leaf is gradually attenuated as one goes deeper into the leaf tissue. If the Chl molecules were uniformly distributed throughout the leaf, one would assume that the rate of light absorption and of photosynthesis would vary in proportion to the light available at various depths. Measurements of CO_2 assimilation in white light, however, indicate that the rate rises to a maximum, about 35% of the total thickness into the leaf, and then slowly decreases to about 40% of the maximum value near the abaxial (lower) side [232, 235]. The spongy mesophyll fixes about 40% of the total, a much higher proportion than what would be expected from a simple light-intensity model. The explanation may be that light in the spongy mesophyll is scattered back and forth simulating the situation in a "hall of mirrors" in an amusement park [232] so that the chloroplasts have enhanced chance of absorbing light coming from various directions.

Another strange result is that the evolution of O_2 does not show the same pattern as described above for CO_2 assimilation [236]. This conclusion was obtained by using a photoacoustic technique. Oxygen evolution was much more equally distributed across the whole leaf, nowhere going below about 60% of the maximum rate, which occurred at a depth of about 12% of the leaf thickness.

2.7.5 How Does Photosynthesis Interact with Climate-Atmosphere?

Photosynthetic organisms and their photosynthesis affect climate in several ways. What first comes to mind is that CO_2 is an important greenhouse gas, and its removal from the atmosphere keeps the Earth cool enough for life. Plants affect the CO_2 content of the atmosphere not only directly, but by converting CO_2 to organic carbon. Plants also increase weathering and conversion of silicate rock to carbonate. A drastic example of this is at the time (Ordovician) when vegetation first invaded land [237]. CO_2 was then removed from the atmosphere to such an extent that a series of glaciation periods followed.

There are, however, other ways in which plants and other oxygenic organisms affect the climate. The ozone produced from their "waste product" O_2 is also a greenhouse gas that affects the temperature of the Earth's surface, but it is also important in heating the stratosphere, and in fact creating it [238]. It is the heating by the absorption of ultraviolet (UV) radiation in ozone that makes the temperature increase with altitude in the higher parts of the atmosphere (in contrast to the lower atmosphere, the troposphere), and in this way, it makes the stratosphere stratified (without the strong vertical movement of the lower atmosphere) [238]. This is important for the total atmospheric circulation, and thus for climate.

Plants need to have openings, stomata, in their outer cell layer, the epidermis, in order to be able to absorb CO_2. This leads to transpiration, release of water vapor. Water vapor is an important greenhouse gas too, and land vegetation greatly increases the transfer of water from the surface to the atmosphere. This also affects cloudiness, an important climate determinant. Plants in various ways also contribute to condensation nuclei for the formation of water droplets in the atmosphere, which is important for cloud formation and precipitation [239]. Also, some unicellular photosynthetic algae in the oceans are thought to contribute to the formation of these

nuclei by secreting sulfur compounds, which are converted, by bacteria, to dimethyl sulfide, $(CH_3)_2S$. This, in the atmosphere, is oxidized to sulfuric acid that attracts water and forms droplets [240].

On the other hand, climate affects photosynthesis. Photosynthesis is decreased by aridity and also by very low and very high temperatures, and in many cases also by cloudiness.

2.7.6 Is There Photosynthesis Without CO_2 Assimilation (N_2 Fixation in Cyanobacteria, Light-Dependent NO_3^- Assimilation in Land Plants)?

As mentioned earlier in this chapter, a type of photosynthesis exists without CO_2 assimilation. The most important case is the one tied to the assimilation of nitrogen.

Cyanobacteria have the ability to bind molecular nitrogen (N_2) by a special process. This involves the use of enzyme nitrogenase, but it is very sensitive to molecular O_2, which rapidly inactivates it if the two come together. Cyanobacteria have two strategies to keep the nitrogenase active, in spite of their own O_2. Many of them contain long cell chains with two types of cells: *vegetative cells* that carry out normal CO_2 assimilation and evolution of O_2 (using both PSI and PSII); and *heterocysts*, which assimilate N_2 using PSI, and organic compounds imported from the vegetative cells [241].

Other cyanobacteria, however, are unicellular or are filamentous but lack heterocysts and must use another strategy. In all cases of nitrogen fixation, PSI generates reduced Fd (see Section 2.3 and Fig. 2.7), which is the reductant for converting N_2 to ammonium ion, NH_4^+. One way of protecting nitrogenase from O_2 is to use some of this reduced Fd for reducing O_2 to O_2^- (superoxide ion), which can be handled further by superoxide dismutase and catalase. Another way is to separate the processes temporally and carry out the N_2 binding during night, when no oxygen evolution is taking place [242].

Higher plants too can use light for the reduction of nitrogen, but in this case only nitrogen in the form of nitrate is used. Nitrate is taken up by the roots, and in many plants, it is reduced right there, without the aid of light. But other plants transport the nitrate ions to the aboveground green parts and reduce it by NADH, which is produced indirectly from photosynthesis, via the organic photoassimilates [243], and the process is therefore stimulated by light.

2.7.7 How Can Animals Carry Out Photosynthesis?

Many animals harbor algae as endosymbionts [244]. The most important case is that of stone corals, which contain dinoflagellates of the genus *Symbiodinium*, which play an important role not only for the supply of organic compounds to supplement what the corals can catch as food, but also for the formation of the carbonate skeleton. Endosymbiotic algae that carry out photosynthesis are also very common among unicellular animals.

The most interesting examples of *photosynthetic animals* are perhaps a group of sea slugs that eat algae, but keep their chloroplasts and nucleic acid molecules; the

chloroplasts are kept in a functional state for a long time [245, 246]. Some of these sea slugs use chloroplasts from red algae, others those from green algae to carry out photosynthesis. The most studied have been the slugs of the genus *Elysia*, for example, *E. chlorotica* that feeds on the green alga *Vaucheria litorea*. *Elysia chlorotica* can keep the chloroplasts active in its body for up to 10 months in the absence of any *Vaucheria* nucleus. This is remarkable since when the chloroplasts are in the alga, they must be constantly repaired and supplied with fresh protein molecules coded for by nuclear genes. Chloroplasts are not transferred to the slug offspring, but each generation must obtain them by feeding on *Vaucheria*. Only one nuclear gene for a chloroplast protein (i.e., the phosphoribulokinase gene) has been shown to have been transferred from *Vaucheria* to *Elysia*. It is thus far a mystery how *Elysia* can maintain chloroplasts active for so long!

2.8 PERSPECTIVES FOR THE FUTURE

The problem of supplying humanity with usable energy, which is tightly coupled to climate and other environmental issues, as well as to food supply and world peace, is an enormous challenge to human ingenuity. We have little time to solve it. Use of the "know-how" accumulated in photosynthetic organisms by evolution over billions of years may provide the shortcut we need to meet the time constraint. This gives us reason to continue the exploration of how the natural systems work, and how they have been able to sustain the biosphere of our, as far as we know, unique planet.

2.9 SUMMARY

While many bacteria carry out a simple form of photosynthesis using a single photosystem, cyanobacteria, algae, and plants perform oxygenic photosynthesis using two photochemical systems, connected in series. In the latter, water is oxidized to molecular oxygen, and organic compounds are produced from inorganic carbon compounds. It is a very complex process, which has several phases starting with absorption of light by pigments in antenna complexes, and channeling of the excitation energy to the reaction centers, where positive and negative charges are separated. The positive charges, obtained from PSII, are used to oxidize water in a four-step process involving manganese in several oxidation states, whereas the negative charges are transferred over a number of electron carriers, most of which are bound to membrane proteins, but there are also carriers in the membrane lipids or in water. PSI is involved in taking the electrons coming from PSII to the next level; the negative charges are finally used to reduce $NADP^+$. The electron transfer is coupled to transfer of protons across the membranes, which together with protons from the oxidation of water leads to energy stored in the form of a pH difference across the membranes. The energy, thus transiently stored, is used for the synthesis of ATP. The ATP is used, together with NADPH, for the synthesis of carbohydrate from carbon dioxide or bicarbonate.

Oxygenic photosynthesis is the main process that provides energy to the entire biosphere of our planet. It also gives rise to the ozone layer that protects us from solar ultraviolet radiation. Furthermore, by its consumption of carbon dioxide, it has for a long time kept the climate friendly to life, and it has been an indirect cause of various geological processes. There is now hope that by learning more about natural photosynthesis we shall be able to better address humanity's energy problems by harvesting solar energy in an economically feasible, environment-friendly, and sustainable way.

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