Qiang Wang *Editor*

Microbial Photosynthesis

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Preface

As the largest scale chemical reaction, photosynthesis supplies all of the organic carbon and oxygen for life on Earth. It is estimated that more than 50% of the primary production and molecular oxygen on Earth are by the photosynthetic activity of microorganisms.

This book highlights recent breakthroughs in the multidisciplinary areas of microbial photosynthesis. The chapters feature the most recent developments in microbial photosynthesis research, from bacterial to eukaryotic algae, from theoretical biology to structural biology and biophysics. Furthermore, the book also features the latest advancements in artificial photosynthetic. Contributed by leading authorities in photosynthesis research, Microbial Photosynthesis will offer a valuable resource for graduate students and researchers in the field. Furthermore, it will be of great significance for the study of biological evolution and the origin of life.

Kaifeng, Henan, China — Qiang Wang

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Part I
Photosynthesis and Energy Transfer

Molecular Mechanism of Photosynthesis Driven by Red-Shifted Chlorophylls

Artur Sawicki and Min Chen

Abstract Photosynthesis is the process of light-driven production of organic molecules needed as starting components for whole cellular processes and as the energy source. It is carried out by primary producers including land plants, algae, and oxygenic/anoxygenic photosynthetic bacteria. Oxygenic photosynthesis involves two stages: light-dependent reactions generating NADPH and ATP molecules with oxygen as a by-product and light-independent reactions involving utilization of NADPH and ATP as the energy source to convert carbon dioxide into organic molecules. Light-dependent processes require light-absorbing pigments categorized as carotenoids, bilins, and chlorophylls. Chlorophylls and carotenoids are ubiquitous to all photosynthetic organisms, while bilins in phycobiliprotein complexes are specific to cyanobacteria, rhodophytes, glaucophytes, and cryptophytes. Each pigment has several variations, and a particular type of organism has a specific set optimized for light-capture and photosynthetic efficiency in the given environment. The common chlorophyll to each oxygenic photosynthetic organism is chlorophyll *a*, with chlorophyll *b*, *c*, *d*, or *f* synthesized depending on the organism. Chlorophyll *d* (3-formyl-chlorophyll *a*) and chlorophyll *f* (2-formyl-chlorophyll *a*) have far-red long-wavelength absorption features, namely, red-shifted chlorophylls. These chlorophyll molecules enable some cyanobacterial species to grow in shaded environments and establish unique habitats. *Acaryochloris marina* is predominantly a chlorophyll *d*-containing cyanobacterium with minor ($\leq$ 5%) amounts of chlorophyll *a* under all culture conditions, while all chlorophyll *f*-producing cyanobacteria constitute chlorophyll *f* as a minor chlorophyll ~2–15% of the total chlorophyll pool and only produced under far-red light conditions. This chapter will focus on summarizing the current knowledge of the light-dependent processes with particular attention on structures and functions of far-red absorbing pigments and pigment-binding protein complexes.

Keywords *Acaryochloris marina* · Biochemistry of chlorophylls · Chlorophyll *d* · Chlorophyll *f* · Light-harvesting protein complexes · Photosystem

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Abbreviations

APC	Allophycocyanin
CBP	Chlorophyll *a*-/*b*-binding protein
Chl	Chlorophyll
CHLF	Chlorophyll *f* synthase
FaRLiP	Far-red light-induced photoacclimation
FRL	Far-red light
IsiA	Iron-stress induced chlorophyll-binding protein
LHC	Light-harvesting protein complex
PAR	Photosynthetically active radiation
PBP	Phycobiliprotein
PBS	Phycobilisome
PC	Phycocyanin
Pcb	Prochlorophyte chlorophyll *a*-/*b*-binding protein complexes
PE	Phycoerythrin
PEC	Phycoerythrocyanin
Pheo *a*	Pheophytin *a*
PS	Photosystem
Q_A	Plastoquinone A
RC	Reaction center

1 General Knowledge of Photosynthesis

Photosynthesis is the conversion of light energy into chemical energy stored in organic molecules. It occurs through two stages: light-dependent and light-independent reactions. Light-dependent processes involve (1) the absorption of light by pigments and energy delivery by antenna/light-harvesting complexes (LHCs), (2) primary electron transfer in reaction centers (RCs), and (3) electron transport processes facilitating the synthesis of NADPH and ATP molecules. Light-independent processes involve utilization of NADPH and ATP for carbon (CO_2) fixation by the Calvin-Benson cycle including carboxylation, reduction, and ribulose 1,5-bisphosphate (RuBP) regeneration. Sucrose is an example of the stable photosynthetic product that is used to power a variety of cellular processes.

The light-gathering antenna system functions to absorb light and to transfer the energy in the light photons to RCs. Various photopigments are used in the antenna system, including chlorophylls (Chls) and carotenoids in integral membrane LHCs, and linear tetrapyrroles bilins complexed with biliproteins organized as a phycobilisome (PBS). The three photopigments have particular absorption wavelength maxima to facilitate photosynthetically active radiation (PAR) between 400 and 700 nm. With the aid of red-shifted Chls (Chl *d* and Chl *f*) and the red-shifted phycobiliproteins (PBPs), some cyanobacteria are able to extend their photosynthetically active

spectral region into the long-wavelength region of 700–760 nm, which extends the coverage of the PAR availability in a local habitat (Chen and Blankenship 2011). Increasing the PAR cross section beyond 700 nm has promoted the photoautotrophic growth in conditions where far-red light (FRL) predominates.

Two photosystems (PSs) are essential for conducting sustained oxygenic photosynthesis, PSI and PSII. Each PS has a special pair of Chl molecules otherwise known as primary electron acceptors which consist of Chl *a* and Chl *a′*, which are bound to the RC core peptides of PsaA/PsaB and PsbA/PsbD for PSI and PSII, respectively. Photosynthesis begins with the collective absorption of photons by antenna photopigments, and the collective energy can be transferred from one pigment to another following an energy gradient established by pigments, from higher absorbed light energy to lower absorbed light energy in the RCs. Reaction centers are the location where light-driven charge separation and electron transfer occur and Chls in RC are excited and readily pass electrons to the primary electron acceptor (A) causing a P^+A^- pair (Caffarri et al. 2014). In the unique FRL-absorbing cyanobacterium *Acaryochloris marina* (*A. marina*), Chl *d* replaces functions of Chl *a* in the RCs of PSI and PSII (Mimuro et al. 2004), while in *Prochlorococcus marinus*, Chl *a* is replaced by divinyl Chl *a* (Chl a_2), which coexists with divinyl Chl *b* (Chl b_2) (Partensky et al. 1993). Meanwhile Chl *f* is present in isolated PSI complexes from *Halomicronema hongdechloris* (*H. hongdechloris*) (Li et al. 2018a) and also present in isolated PSI and PSII in *Chroococcidiopsis thermalis* (Nürnberg et al. 2018).

In PSII, the primary electron acceptor is pheophytin *a*, while the secondary electron acceptor is plastoquinone A (Q_A). Each photochemical reaction requires two electrons to be fed into the electron transport chain. The four electrons lost from two water molecules generate one oxygen molecule and four protons (H^+). Similar to PSII, Chl molecules in the RC of PSI are excited, and the separated electrons are readily passed to electron acceptor Chl molecules (A_1), followed by delivery to the next electron acceptors: phylloquinone; FeSx, FeSb, and FeSA; ferredoxin; and $NADP^+$ reductase. The coupling of PSI and PSII via cytochrome b_6f complexes in oxygenic photosynthetic organisms is called noncyclic photophosphorylation. Therefore, PSII and PSI are connected with equal electron currents coupling to production of ATP and NADPH.

Cyclic electron transport involves transfer of electrons from ferredoxin back to cytochrome b_6f complex, plastoquinone, and plastocyanin and onto the RC Chl of PSI. This involves only PSI and couples ATP production without NADPH synthesis (Arnon 1984). Anoxygenic photosynthetic bacteria often only have a cyclic electron transport photosynthetic apparatus. The photosynthetic cyclic electron transport means no direct need for the initial electron donor or terminal electron acceptors. The ATP and NADPH molecules generated from the light-dependent reactions are utilized by the “dark” (light-independent) reactions of the Calvin-Benson cycle which involves the fixing of CO_2 into organic carbon, namely, the production of three-carbon sugar (glyceraldehyde 3-phosphate), and eventually converts into starch or sucrose.

2 Photosynthetic Organisms

Chlorophyll-driven oxygenic photosynthesis occurs in the primary producers: plants, algae, and cyanobacteria. Bacteriochlorophyll-driven anoxygenic photosynthesis occurs only in bacteria under aerobic and anaerobic conditions. Oxygenic photosynthesis facilitated conditions favorable for oxygen-dependent species. Cyanobacteria are the only bacteria that perform oxygenic photosynthesis, which thrive in various environments, including some extreme environmental conditions. For example, cyanobacteria containing FRL-absorbing pigments Chl *d* and Chl *f* can be found from deprived visible light conditions where the FRL is enriched (Zhang et al. 2019; Kühl et al. 2005).

2.1 Photosynthetic Eukaryotes

Eukaryotic photosynthesis occurs in a specialized compartment (organelle) called the chloroplast. It is thought chloroplasts originally derived from ancient cyanobacteria through primary endosymbiosis, a process whereby the cyanobacteria was engulfed by a non-photosynthetic eukaryotic organism (a protist) (Archibald 2015). The engulfing event eventually led to the development of the plastid and may have occurred multiple times; however it appears likely that rhodophytes, glaucophytes, and green algae (division chlorophytes or euglenophytes) derived from a common ancestor of primary endosymbiotic origin (Reyes-Prieto et al. 2007). Primary endosymbiotic green algae further evolved into higher plants. Evidence for secondary endosymbiosis of red and green algae exist through the presence of three or four layers of plastid envelope found in the photosynthetic organisms. A common ancestor of engulfed red algae is proposed to have developed into Chl *c*-producing algae, dinoflagellates, diatoms, brown algae, haptophytes, and cryptophytes, while green algae underwent two separate endosymbiotic events forming euglenophytes and chlorarachniophytes.

Algae are unicellular or multicellular organisms with the latter either microscopic or macroscopic, and all species are Chl *a*-dominant. Green algae have Chl *a* and Chl *b*, and an intrinsic light-harvesting system which is structurally similar to that of higher plants compared with other algal divisions. Red algae, glaucophytes, and cryptophytes have PBPs as peripheral light-harvesting systems. Red algae consist of Chl *a* and PBSs consisting of phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC). Glaucophytes have PBS consisting of only PC and APC (Chapman 1966; Giddings et al. 1983). Meanwhile cryptophytes contain a Chl *a*/*c* LHC and atypical PBP complexes with unique α-PBP and rhodophyte-like β-PBP subunits, which use either Cr-PE or Cr-PC as bound chromophores. The most striking feature of atypical PBP complexes in cryptophytes is that they are localized in the thylakoid lumen spaces (Glazer and Wedemayer 1995). The remaining Chl *c*-containing algae have a Chl *a*/*c* LHC without PBSs, which include chromophytes

(heterokonts and ochrophytes), brown algae, diatoms, dinophytes (dinoflagellates), and haptophytes. Chlorophyll *a*/*c* LHC complexes are structurally similar to Chl *a*/*b* LHC complexes in higher plants, but mostly with different bound carotenoids (Büchel 2019). Higher plants are considered to be the most advanced photosynthetic eukaryotes and have a conserved LHC composed of Chl *a*/*b* with variable carotenoid molecules (Neilson and Durnford 2010).

2.2 Photosynthetic Prokaryotes

There are five groups of photosynthetic bacteria: purple, green sulfur, green non-sulfur, heliobacteria, and cyanobacteria. The first four groups are anoxygenic photosynthetic organisms that use infrared-absorbing bacteriochlorophylls in the photosynthetic process (Scheer 1991). Anoxygenic photosynthetic bacteria have only one type of photosynthetic RC, either type I (PSI-like) or type II (PSII-like) RC, which drives only cyclic electron transport surrounding the RC (Allen 2014). Various electron donors use other than water, such as hydrogen, sulfur (H_2S), Fe^{2+}, and reduced organic compounds (Bryant and Frigaard 2006).

Cyanobacteria are oxygenic photosynthetic prokaryotes that use Chl *a* as the main light-absorbing Chl in the PSs, and most of cyanobacteria use PBS as the main part of the antenna system. The photosynthetic oxidation of water provides the electron and the free oxygen molecules like the photosynthesis conducted in higher plants.

2.2.1 Anoxygenic Photosynthetic Prokaryotes

Purple sulfur and non-sulfur bacteria are bacteriochlorophyll *a*- and bacteriochlorophyll *b*-containing organisms that conduct photosynthesis under anaerobic conditions (Frigaard and Dahl 2008; McEwan 1994). Anaerobic conditions trigger the synthesis of additional lipids leading to the invagination of cytoplasmic membrane providing an ultrastructure for the photosynthetic machinery (Madigan and Jung 2009). These intracytoplasmic membranes are comparable to the thylakoid membranes of cyanobacteria. The electron donors are either sulfide, sulfur, or hydrogen; hence no oxygen is produced as a by-product. Some species of purple bacteria are known as aerobic anoxygenic photosynthetic bacteria and carry out photosynthetic processes only under aerobic conditions (Yurkov and Beatty 1998). The purple bacteria use type II RC, which is comparable with PSII of plants, algae, and cyanobacteria. The two RC core subunits are named as L and M with the H subunit stabilizing the dimeric RC complex and enhancing the activity (Allen and Williams 2011). The light-harvesting complex has α- and β-subunits (encoded by *pufA* and *pufB*) forming a ring structure consisting either 9 subunits or the multi-metric complex of 15–16 subunits (Overmann and Garcia-Pichel 2013).

Green sulfur and green non-sulfur bacteria are obligate anoxygenic photosynthetic organisms with specialized extrinsic chlorosomes as the main antenna system

thriving in the extreme low-light environments. Chlorosomes contain bacteriochlorophyll *a*/*c*/*d*/*e* and attach to the cytoplasmic membrane via Fenna-Matthews-Olson (FMO) bacteriochlorophyll *a*-binding protein complexes. The ratio of bacteriochlorophylls/protein within chlorosomes is the highest out of any pigment-binding protein complexes reported, providing the advantages for this anoxygenic photosynthetic bacterial group to efficiently capture low-intensity light (Overmann and Garcia-Pichel 2013). Chlorosomes pass the energy to FMO and then to the RC (Hauska et al. 2001; Overmann and Garcia-Pichel 2013). The type I RC resembles that of PSI from oxygenic photosynthetic organisms, with the exception that one polypeptide (dimer) constitutes the structure (Overmann and Garcia-Pichel 2013).

Heliobacteria have a simple type I RC that localized in the cytoplasmic membrane and can only perform photoheterotrophic growth under anaerobic conditions with bacteriochlorophyll *g* as the major photopigment (Tang et al. 2010).

2.2.2 Oxygenic Photosynthetic Prokaryotes (*Cyanobacteria*)

Oxygenic photosynthetic bacteria refer to phylum *Cyanophyta* or *Cyanobacteria* that are sometimes called blue-green algae although they are true prokaryotes with no chloroplast. Cyanobacteria inhabit diverse environments including marine, freshwater, and terrestrial niches. Their morphology varies from coccoid and filamentous to colonial and unicellular with diverse thylakoid membrane architecture (Mareš et al. 2019). Certain species can tolerate high temperatures, periods of desiccation, and high or low light intensities through the ability to produce specific pigments, to modify the chemical structures in order to change the optical properties of the pigment suitable for the given light qualities (Ho et al. 2017b; Rockwell et al. 2016; Gomelsky and Hoff 2011; Kehoe and Gutu 2006).

Cyanobacteria are typically photoautotrophs with some species having the ability to live photoheterotrophically if nutrient conditions are available. Chlorophyll *a* is the major Chl pigment; however, there are some exceptions. *Acaryochloris marina* uses Chl *d* as the major Chl pigment under all light conditions with traces of Chl *a* present and an atypical phycobiliprotein complex (PBP) structure (Chen et al. 2009; Marquardt et al. 2000; Miyashita et al. 1996). All reported *A. marina* spp. phylogenetically belong to a single genus, *Acaryochloris marina* spp. Chlorophyll *f*-producing cyanobacteria phylogenetically are classified into five subsections of cyanobacteria with related morphological differences (Zhang et al. 2019). Chlorophyll *f* extends PAR further into the FRL region, and synthesis of this pigment is induced under PAR conditions up to ~2–15% of the total Chl content along with major Chl, Chl *a* (Zhang et al. 2019; Chen and Blankenship 2011; Chen et al. 2012).

Prochlorophytes are unusual cyanobacteria which synthesize Chl *a* and Chl *b*, and three genera have been identified: *Prochloron*, *Prochlorothrix*, and *Prochlorococcus* (Shih et al. 2013). *Prochloron* is a symbiotic prokaryote associated with marine invertebrates (Lewin 1977), while *Prochlorothrix* is a filamentous free-living genus (Burger-Wiersma et al. 1989; Burger-Wiersma et al. 1986).

Both these genus use Chl *a* and Chl *b* as that in higher plants. *Prochlorococcus marinus* spp. are the smallest free-living unicellular cyanobacteria using Chl a_2 and Chl b_2 instead of the typical Chl *a* and Chl *b* (Partensky et al. 1999; Chisholm et al. 1988). Prochlorophytes have no PBS and instead rely upon membrane-bound intrinsic Chl *a*-/*b*-binding protein complexes (Pcbs) (Chen et al. 2008). These proteins are similar to the core antenna protein of PSII, CP43, and CP47; hence this antenna complex differs from the membrane-bound LHCs of plants and green algae (La Roche et al. 1996).

3 Photopigments

The main light-absorbing photopigments include carotenoids, bilins, and Chls. Bilins capture light energy between approximately 520 and 670 nm that is poorly absorbed by Chls (Fig. 1) (Chen and Scheer 2013). However, Chl molecules are critical for photochemical reactions in the RCs. Together, various photopigments provide the entire PAR between 400 and 700 nm, which is made of 47% of solar photon inputs. The FRL-absorbing Chls (Chl *d* and *f*) extend the PAR beyond 700 nm that is enriched in shade and low-light environments.

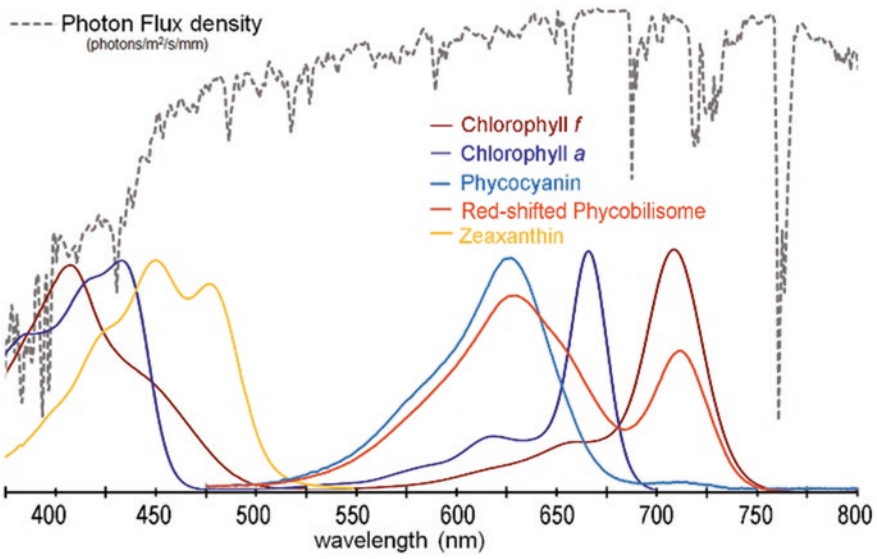


Fig. 1 Solar irradiance and absorption spectrum of some typical common light-absorbing pigments involved in photosynthesis. The spectra of carotenoids (zeaxanthin), chlorophyll *a*, and *f* were recorded in 100% methanol, and the spectra of isolated phycobilisome and red-shifted allophycocyanin were recorded in phosphate buffer. Solar irradiance spectra downloaded from https://www.nrel.gov/grid/solar-resource/spectra-am1.5.html

3.1 Carotenoids

Carotenoids are colored linear tetraterpenoids containing a polyene chain of conjugated double bonds which absorb light mainly in the blue light region, 400–530 nm (Hashimoto et al. 2016). They are involved in light harvesting and also absorption of excessive light energy and quenching of singlet oxygen generated by photosynthetic reactions. Carotenoids may be classified as nonpolar oxygen-lacking structures called carotenes and polar oxygen-containing xanthophylls. There are approximately 30 different carotenoid structures reported in photosynthetic organisms with their own specific pigment composition (Takaichi 2011). Cyanobacteria typically have β-carotene and different xanthophyll structures, such as zeaxanthin, echinenone, myxoxanthophyll (myxol glycosides), and canthaxanthin (Zakar et al. 2016; Takaichi 2011; Takaichi and Mochimaru 2007). In some genus such as *A. marina* spp. and *Prochlorococcus* spp., α-carotene is present instead of β-carotene (Takaichi et al. 2012).

Carotenoid contents and compositions generally change in response to light stress conditions, for example, zeaxanthin concentration is increased under high-light irradiation. In green algae and plants, a rapid epoxidation and de-epoxidation cycle among zeaxanthin, antheraxanthin, and violaxanthin reflects the changed light conditions, which refer as the xanthophyll cycle (Demmig-Adams 1990; Goss and Jakob 2010). Although this cycle is not present in cyanobacteria, zeaxanthin may accumulate in high-light conditions through oxidation of β-carotene by β-carotene hydroxylase (E.C.1.14.13) (Masamoto and Furukawa 1997). Other cyanobacteria produce excess myxoxanthophyll under high-light conditions (Steiger et al. 1999), while a cyanobacteria-specific orange carotenoid protein bound with echinenone is upregulated in high light and can quench the excessive energy generated from the PBS (Schagerl and Müller 2006; Domonkos et al. 2013; Kirilovsky and Kerfeld 2012). The localization of xanthophyll in photosynthetic apparatus has not been determined; however, it plays an important role in photosynthetic reactions. A xanthophyll-less mutant of *Synechocystis* sp. PCC6803 showed a globally negative effect on the photosynthetic apparatus including reduced PBS rod length, monomerized PSI, and irregularly assembled PSII (Tóth et al. 2015).

3.2 Phycobiliprotein Complexes

Bilins are linear tetrapyrroles derived from the oxidative ring opening of heme and are chromophores for bilin-binding PBPs. The bilin-binding site is usually on $C3^1$ of ring A through a cysteine residue of the apoprotein, with some bilins additionally attaching to $C18^1$ of ring D (Fig. 2). In cyanobacteria and some algae (rhodophyte, cryptophyte, glaucophyte), PBS represents an assembly structure of bilin-binding protein complexes (for details, see Sect. 4.4). Allophycocyanin is at the core of PBS, with rods projecting outward as an external antenna, and may be composed of PC,

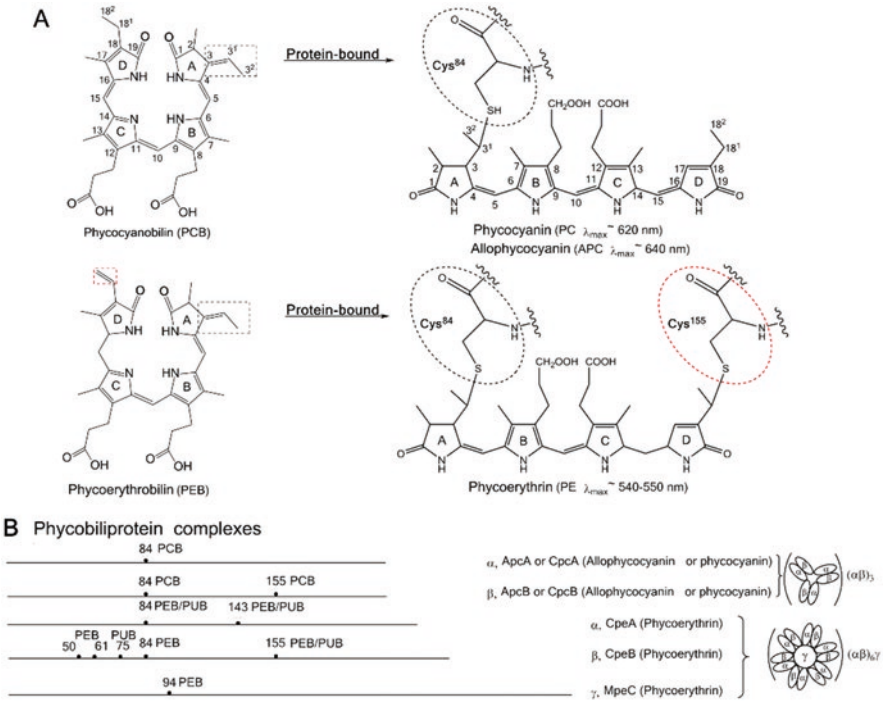


Fig. 2 Chemical structure of PCB and PEB and their association into phycobiliproteins. (**a**) Structure of phycocyanobilin (PCB) and phycoerythrobilin (PEB). The dashed boxes highlight the sites where covalent attachment of bilins occurs with a cysteine residue of the apoprotein. The numbering of carbon atoms is following the IUPAC system. Cys84 and Cys155 represent the relative position of cysteine in apo-phycobiliprotein. (**b**) Primary binding position of bilins on the phycobiliproteins and the quaternary structural model of the phycobiliprotein. The length of the lines represents the approximate relative lengths of the polypeptides

PE, or phycoerythrocyanin (PEC), with PE/PEC at the periphery and PC closer to the core. The basic disc structure of PBP typically has a αβ trimeric $(\alpha\beta)_3$ or $(\alpha\beta)_6\gamma$ structure, and each subunit is structurally related with a variable number of bilin-binding sites. Genes encoding apo-PBP subunits are often clustered together and contain *apc*s, *cpc*s, and *cpe*s, which encode apo-APC, apo-PC, and apo-PE peptides, respectively.

Phycocyanins consist of CpcA/CpcB subunits with PC bound to Cys84 of each subunit and also to Cys84 and Cys155 for the beta-subunit (Fig. 2) (Schirmer et al. 1985). There are various ways which apoproteins bind with bilins: C-PC contains only one bound PC, and R-PCII binds one PCB and two PEB, while R-PCIII binds two PCBs and one PEB (Sidler 1994). C-PC absorbs light in the wavelength of ~610–620 nm (Glazer 1977), while R-PC absorbs at 530–550 and 615 nm (Ong and Glazer 1987). Allophycocyanin has only one PC bilin attached to each Cys84 residue of ApcA and ApcB, and the different chromophore-protein interactions lead to ~30-nm red-shifted absorption maxima (640–670 nm) compared with that of PC

(Brejc et al. 1995). In general, the shorter-wavelength photon-absorbing subunits assembled in PBS surrounding structure and the longer-wavelength photon-absorbing subunits are located in the core of assembled PBSs (Stomp et al. 2007).

Phycoerythrin (PE) contains CpeA/B subunits and the bound PEB (C-PE) (Sonani et al. 2018) or the bound phycourobilin (PUB) and PE (R-PE) (Ritter et al. 1999; Nagy et al. 1985), which covers mainly absorption range of 520–570 nm. There are two PEB-binding sites for the α-subunit and three PEB to each β-subunit in C-PE of the cyanobacterium. PEC is an uncommon PBP present in heterocyst-containing cyanobacteria and is composed of α-subunits (PecA) and β-subunits (PecB) bound to one phycoviolobilin (PVB) at Cys84 and two PCB pigments at sites of Cys82 and Cys153, respectively (Schmidt et al. 2006), and it uses green light in the wavelength range of 570–595 nm (Bryant 1982). Organisms which have PEC do not have PE and vice versa (Bryant 1982).

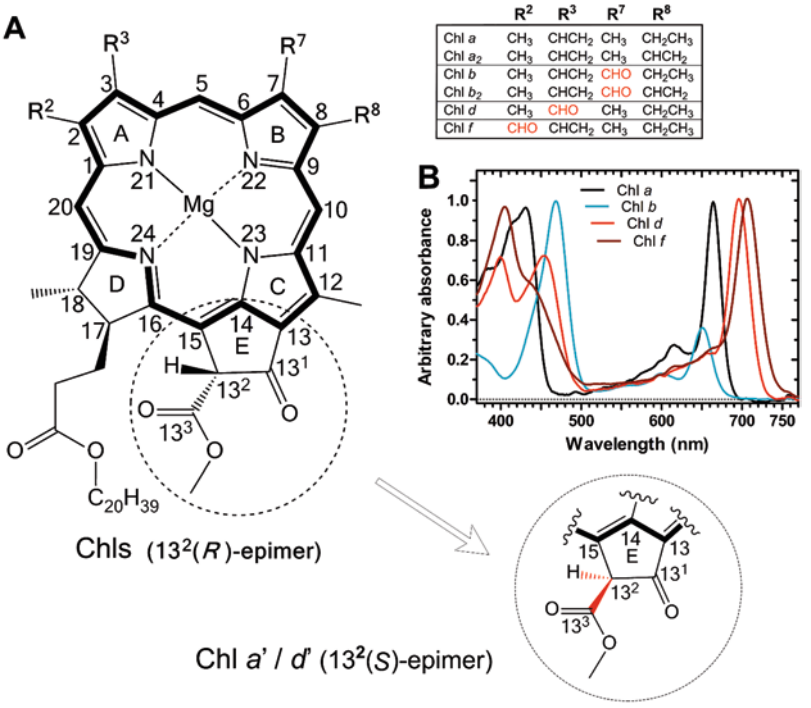
Linker proteins play important roles for PBS assembly and energy transfers. There are different linkers that are named after their functions: the linkers within rod, L_R, encoded by *cpcC*s; the linker at the rod-core interface, L_{RC}, encoded by *cpcG*s; the linker core-membrane subunits, L_{CM}, encoded by *apcE*; and the rod-capping linker protein, which is encoded by *cpcD* (Six et al. 2007).

3.3 Chlorophylls

Chlorophylls are major photopigments in oxygenic photosynthesis and are structurally described as cyclic tetrapyrroles containing a distinctive five-membered ring (Fig. 3). They absorb light strongly in the blue and red light regions and reflect the green light which explains their inherent green color. There are three possible fates for the absorption of light: (1) emitted as heat or fluorescence through redistribution into atomic vibrations within the molecule (non-photochemical quenching), (2) transfer of light energy to nearby photopigments via resonance, and (3) photochemical reduction/oxidation and the electron transferred to a new molecule. The first process occurs under saturating light conditions and is required to quench the potentially damaging Chl excitation energy which may lead to free radicals. The second and third occur under optimal conditions and lead to photosynthetic light reactions.

There are five types of Chl characterized in oxygenic photosynthetic species, Chl *a*, Chl *b*, Chl *c*, Chl *d*, and Chl *f*, named in the order of discoveries (Fig. 3, Chen 2019). While Chls *a*, *b*, *d*, and *f*, including Chl a_2 and Chl b_2, have a phytyl chain esterified at position C17[3], Chl *c* has the free acid and double bond at C17–C18, which is similar to protochlorophyllide. There are several Chl *c* derivatives with variations in side chains, and Chl c_1 – c_3 are the most common species reported (Fig. 4) (Myśliwa-Kurdziel et al. 2019; Zapata et al. 2006).

The absorbance maxima of Chls depend on the distribution of electron density within the Chl structure, and alterations in the Chl peripheral structure lead to redistribution of the electron density. Chl *a* has Soret absorption maxima of ~433 nm and Q_y absorption at 665 nm in methanol that is taken as a reference point for all other Chl



	R^2	R^3	R^7	R^8
Chl *a*	CH_3	$CHCH_2$	CH_3	CH_2CH_3
Chl a_2	CH_3	$CHCH_2$	CH_3	$CHCH_2$
Chl *b*	CH_3	$CHCH_2$	CHO	CH_2CH_3
Chl b_2	CH_3	$CHCH_2$	CHO	$CHCH_2$
Chl *d*	CH_3	CHO	CH_3	CH_2CH_3
Chl *f*	CHO	$CHCH_2$	CH_3	CH_2CH_3

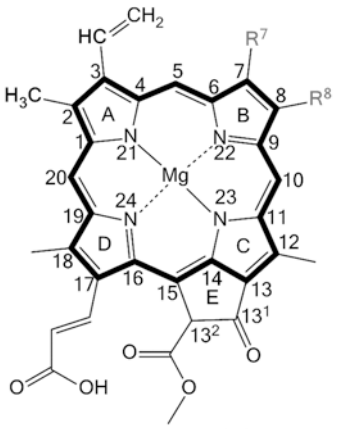
Fig. 3 Structure and spectral properties of chlorophylls present in cyanobacteria. (**a**) Chemical structure of chlorophylls (Chls) *a*, *b*, *d*, and *f* with highlighted 20 π electrons. The different chlorophylls have variations at position C2, C3, C7, and C8 that are summarized in the inserted table. The formyl group substitutions are highlighted in red. The dashed circles highlight the changes from *R*-configuration at position $C13^2$ to the epimer (*S*-stereoisomer) of Chl *a*′/Chl *d*′. (**b**) Absorption spectrum of purified Chl *a*, Chl *b*, Chl *d*, and Chl *f* dissolved in methanol. The absorbance maxima of each of the Chls mentioned here are summarized in Table 2

molecules. Chl *b* has the maxima of Soret and Q_y at 468 and 652 nm, while that of Chl *d* is at 456 and 697 nm and that of Chl *f* at 407 and 707 nm, respectively (Fig. 3b).

Chlorophyll molecules have either singular or dual roles in photosynthesis, namely, (1) accessory light harvesting and energy transfer and (2) photochemical charge separation in the RC (Table 1). Chlorophyll *a* and Chl *d* are involved in all aspects of photosynthesis, and Chl *b* and Chl *c* are strictly light-harvesting pigments, while Chl *f* appears to be light harvesting with an unusual long-lived fluorescent species facilitating uphill energy transfer (Zamzam et al. 2019; Kaucikas et al. 2017).

3.3.1 Chl *a* and Its Spectral Properties

The blue-green-colored Chl *a* molecule is the most ubiquitous pigment in oxygenic photosynthetic organisms, plants, algae, and cyanobacteria, with the exception being Chl a_2 in *Prochlorococcus marinus* that has no detectable Chl *a*



	R7	R8	λmax (nm) Soret	Qy	Solvent
Chl c_1	CH_3	CH_2CH_3	444-5	633-4	Methanol[a]
			444-5	628	Diethyl ether[a]
			442-4	630-1	Acetone[a]
Chl c_2	CH_3	$CHCH_2$	451-2	635	Methanol[a]
			448-9	628-9	Diethyl ether[a]
			444-5	630-1	Acetone[a]
Chl c_3	$COOCH_3$	$CHCH_2$	451	626	Diethyl ether[b]
			452	627	Acetone[b]

Fig. 4 Chemical structure of chlorophyll *c* family. Structure of the three main Chl *c*s found in nature: Chl c_1, Chl c_2, and Chl c_3. The structural differences presented in the inserted table. The respective absorbance maxima are cited from[a] (Jeffrey 1969)[b], (Jeffrey and Wright 1987)

Table 1 The role of chlorophyll molecules in light-dependent processes in photosynthetic organisms

Chlorophylls	Role in photosynthesis	Organisms
Chlorophyll *a*	LH/RC	All oxygenic photosynthetic organisms except *P. marinus* spp. and *A. marina* spp.
Chlorophyll a_2	LH/RC[a]	*P. marinus* spp.
Chlorophyll *b*	LH[b]	Plants, green algae, *Prochlorothrix* spp., *Prochloron* spp., *A. marina* sp. RCC1774
Chlorophyll b_2	LH (PCB)[a,c]	*P. marinus* spp.
Chlorophyll *c*	LH(FCP)[d]	Cryptophyte, dinophytes, haptophytes, some heterokonts, diatoms, Prasinophyceae
Chlorophyll *d*	LH/RC[e]	*A. marina* spp.
Chlorophyll *f*	LH/RC[f,g]	Some cyanobacteria

References: (a) Goericke and Repeta (1992), (b) Scheer (2006), (c) La Roche et al. (1996), (d) Zapata et al. (2000), (e) Loughlin et al. (2013), (f) Ho et al. (2016), and (g) Chen et al. (2019)

(Goericke and Repeta 1992). Chlorophyll *a* is involved in each aspect of light-dependent photosynthetic reactions including light-harvesting processes and photochemical reactions. Different solvents change the Chl spectral maxima to varying degrees (Table 2). The Chl in Chl-binding protein complexes demonstrates a red-shifted absorbance depending on the interaction and influences of surrounding protein structure and residues of amino acid. PsaA/PsaB and PsbA/PsbD are RC subunits and bound Chl *a* P_{700} of PSI and Chl *a* P_{680} of PSII II, respectively.

Table 2 Summary of optical properties of different chlorophyll (Chl) pigments dissolved in selected organic solvents, including their absorbance maxima and molar extinction coefficients (ε)

	λ_{max} (nm)			
	Soret	Q_y	Solvent	ε (L.mol^{-1}.cm^{-1}) × 10^3
Chl *a*	432	665	Methanol[ab]	$\varepsilon_{665.5nm}$ = 70.02[a]; $\varepsilon_{665.2nm}$ = 79.24[b]; $\varepsilon_{431.8nm}$ = 77.05[b]
	428	660	Diethyl ether[b]	
	430	662	Acetone[b]	
Chl a_2	442	668	Aq. methanol*[c]	
	435	660	Diethyl ether[d]	
	438	664	80% acetone[e]	
Chl *b*	469	652	Methanol[b]	$\varepsilon_{652.1nm}$ = 38.87[b]; $\varepsilon_{469.2nm}$ = 105.36[b]
	452	642	Diethyl ether[b]	
	456	645	Acetone[b]	
Chl b_2	478	658	Aq. methanol*[c]	
	460	644	Diethyl ether[d]	
	468	651	80% acetone[e]	
Chl *d*	456	697	Methanol[a]	ε_{697nm} = 63.68[a]; $\varepsilon_{455.5nm}$ = 44.41[a]; ε_{400nm} = 45.74[a]
	446	686	Diethyl ether[a]	
	447	688	Acetone[a]	
Chl *f*	407	707	Methanol[a]	ε_{707nm} = 71.11[a]; $\varepsilon_{406.5nm}$ = 66.92[a]
	396	695	Diethyl ether[a]	
	397	698	Acetone[a]	

References: (a) Li et al. (2012), (b) Lichtenthaler (1987), (c) Goericke and Repeta (1993), (d) Goericke and Repeta (1992), and (e) Shedbalkar and Rebeiz (1992)
*Aq. methanol refers to the HPLC elution buffer which was c.a. 94% methanol in c.a. 31 mM ammonium acetate

3.3.2 Formyl Substitution in Chl *b*, Chl *d*, and Chl *f*

Modifications at C7, C3, and C2 side chains of Chl *a* to formyl (CHO) groups, individually, lead to Chls *b*, *d*, and *f*, respectively (Fig. 3). Chlorophyll *b* is an accessory pigment of plants, green algae, and prochlorophytes (Castenholz 2001; Burger-Wiersma et al. 1986) and recently a Chl *d*-less species of *A. marina* sp. RCC1774 (Partensky et al. 2018). The formyl group substitution at position C7 of Chl *b* shifts the Soret band toward a longer wavelength (469 nm) and the Q_y peak toward the shorter wavelength (652 nm) in comparison with Chl *a*. A formyl group at position C7 in Chl *b* shifts electron densities within the macrocycle, resulting into an increased intensity of the Soret band simultaneously weakening the Q_y dipole moment, resulting in the Soret/Q_y ratio of 2.71 compared with 0.97 in Chl *a* (Hoober et al. 2007).

For Chl *d*, the formyl group substitution at position C3 in Chl *d* strengthens the Q_y dipole moment and results into a red-shifted Q_y band to 697 nm. Chl *f* retains the vinyl group at C3 and has a formyl group replacing the methyl group at C2 position; the Q_y peak of Chl *f* is even further red-shifted to a maximum of 707 nm (Chen et al. 2010). Both Chl *d* and Chl *f* are called longer-wavelength light-absorbing Chls or

red-shifted Chls (Chen and Scheer 2013; Chen and Blankenship 2011). Each of these formyl derivatives of Chl *a* is biosynthesized from oxidation, utilizing molecular oxygen as a substrate (Garg et al. 2017; Schliep et al. 2010; Porra et al. 1994).

3.3.3 Diformyl Variants

Chlorophyll *a* oxygenase (CAO) converts Chl *a* and Chlide *a* to Chl *b* and Chlide *b* (Oster et al. 2000). Transformation of the Chl *d*-containing *A. marina* with CAO of *Prochlorothrix hollandica* resulted in the unexpected formation of 7-formyl-Chl *d* or 3,7-diformyl-Chl *a* with absorption maxima at 470 nm and 667 nm in acetone (Tsuchiya et al. 2012a, b). Surprisingly the total amount of Chl *a* in the Δ*CAO*-expressing *A. marina* mutant does not change, but Chl *d* levels in isolated PSII complexes decrease.

The 3,8-diformyl-Chl *a* has not been detected from any natural sources but synthesized in vitro using 3,8-divinyl-Chl *a* as a substrate. Its absorption with the maxima of 484 nm and 674 nm in methanol; hence the Soret and Q_y bands are shifted toward longer wavelengths like that of 3,7-diformyl-Chl *a* with the maxima of 483 and 7-diformyl-Chl *a* 4 and 674 nm in methanol (Loughlin et al. 2014).

3.3.4 Chl *c* Family

Chlorophyll *c* resembles a porphyrin-like structure with 22 π electrons compared with 20 π electrons in other Chl molecules due to double bond between C17 and C18. In Chls c_1, c_2, and c_3, no phytyl chain is present at $C17^3$; however, some Chl *c* pigments are esterified at $C17^3$ with monogalactosyldiacylglycerol (MGDG), which appears to be characteristic of the Prymnesiophyceae class (Zapata et al. 2006). For example, Chl c_2-MGDG was isolated from *Emiliania huxleyi*, which has a blue-shifted Q_y peak at 625–632 nm (Garrido et al. 2000). Conjugation of the entire porphyrin ring in Chl *c* results in weakening of the Q_y dipole strength, resulting in ε = 150,000 $mol^{-1}.L.cm^{-1}$ at 625–632 nm and approximately 5–13-fold reduced intensity compared with ε = 20,000 $mol^{-1}.L.cm^{-1}$ at Soret band of 445–454 nm (Scheer 2006). Several Chl *c* structures have been characterized, and up to 11 different types of Chl *c* pigments have been identified (Zapata et al. 2006).

Chl *c* is an accessory pigment present mainly in the algae, including diatoms and dinoflagellates, Prasinophyceae, Xanthophyceae, Phaeophyceae, cryptomonad, and chrysomonad (Cavalier-Smith 2018) (Scheer 2006; Jeffrey 1976; Jeffrey 1972; Jeffrey 1969). The best studied Chl *c*-containing LHC is from diatoms that have specific bound carotenoid, fucoxanthin, called fucoxanthin Chl *a*-/*c*-binding protein (FCP) (Büchel 2019).

In addition Chl *c*-like pigment Mg-divinyl phaeoporphyrin a_5 (MgDVP) or 3,8-divinyl protochlorophyllide is present in some cyanobacteria and functions as light-harvesting pigment (Larkum et al. 1994). There are trace amounts detected in *A. marina* (Schliep et al. 2007; Miyashita et al. 1997). The biosynthesis of Chl *c*

most likely is via dehydrogenation of C17[1] of protochlorophyllide (Green 2011; Myśliwa-Kurdziel et al. 2019).

3.3.5 Other Chlorophyll Variants (Including Chemically Modified)

Chlorophyll a_2 and Chl b_2 with a double bond at C8 are present in some photosynthetic organisms including a *Zea mays* mutant ON 8147 (Bazzaz and Brereton 1982), *Prochlorococcus marinus* spp. (Chisholm et al. 1992; Chisholm et al. 1988), and a dinoflagellate (Rodríguez et al. 2016). It has 435 and 660 nm maxima for Chl a_2 and 460 and 644 nm maxima for Chl b_2, slightly red-shifted Soret band and blue-shifted Q_y band compared with that of Chl *a* and Chl *b*, respectively Table 2. Both *Prochlorococcus marinus* spp. and the dinoflagellate are low-light-adapted organisms and have lost the 8-vinyl reductase (DVR, EC 1.3.1.75) gene through evolution (Rodríguez et al. 2016).

The biosynthesis of Chl *b* can be described as two-step reactions, oxygenation and hydroxylation. The intermediate Chl molecule, 7-hydroxymethyl Chl *a*, is generated by oxygenation of the methyl group catalyzed by CAO (EC 1.14.13.122), and this intermediate is not detected in nature (Ito et al. 1996). Currently it is unknown if Chl *d* and Chl *f* biosyntheses proceed via intermediate Chl molecules, such as 3-hydroxymethyl Chl *a* and 2-hydroxymethyl Chl *a*, respectively (Fig. 5). Both putative intermediates have similar spectral feature with more polar retention times using reversed-phase high-performance liquid chromatography compared with that of Chl *a* (Sawicki et al. 2019).

An acidophilic aerobic anoxygenic photosynthetic bacterium consists of zinc bacteriochlorophyll *a* as the major light-harvesting and RC pigment together with bacteriopheophytin *a* (Tomi et al. 2007; Wakao et al. 1996). It appears that the low pH triggers loss of magnesium, which yields zinc-bacteriochlorophyll. No naturally occurring zinc-Chl molecule has been detected in oxygenic photosynthetic organisms.

Two iso-epimer forms of Chl *a* are present in vivo, $13^2(R)$ (Chl *a*) and $13^2(S)$ (Chl *a*′) (Fig. 3). $13^2(R)$ (Chl *a*) is the most common epimer, named as Chl *a*. $13^2(S)$-Chl *a* is the *S*-stereoisomer, named as Chl *a*′, which is found in almost all PSI. The special pair of Chl in PSI is made of Chl *a*/Chl *a*′, which is a feature of one of the two primary electron acceptors (P_A) bound to PsaA of the RC of PSI (Sect. 4.1) (Blankenship 2002). Epimerization of extracted Chl in organic solvent in vitro may occur, particularly, when there are accessible Lewis bases (Hynninen 1991). Epimerization of Chl *a* and *a*′ has identical absorbance maxima of 433 nm and 665 nm in methanol, but with slightly changed nonpolar retention time (Loughlin et al. 2015).

Demetallized Chl is called pheophytin, and pheophytin *a* (Pheo *a*) is present as the primary electron acceptor (A_0) of PSII. However, no pheophytin *d* is detected in *A. marina*; even Chl *d* is the main Chl in PSI and PSII. In addition, no pheophytin *b* and *f* are reported to be involved in photochemical reactions (Miyashita et al. 2014).

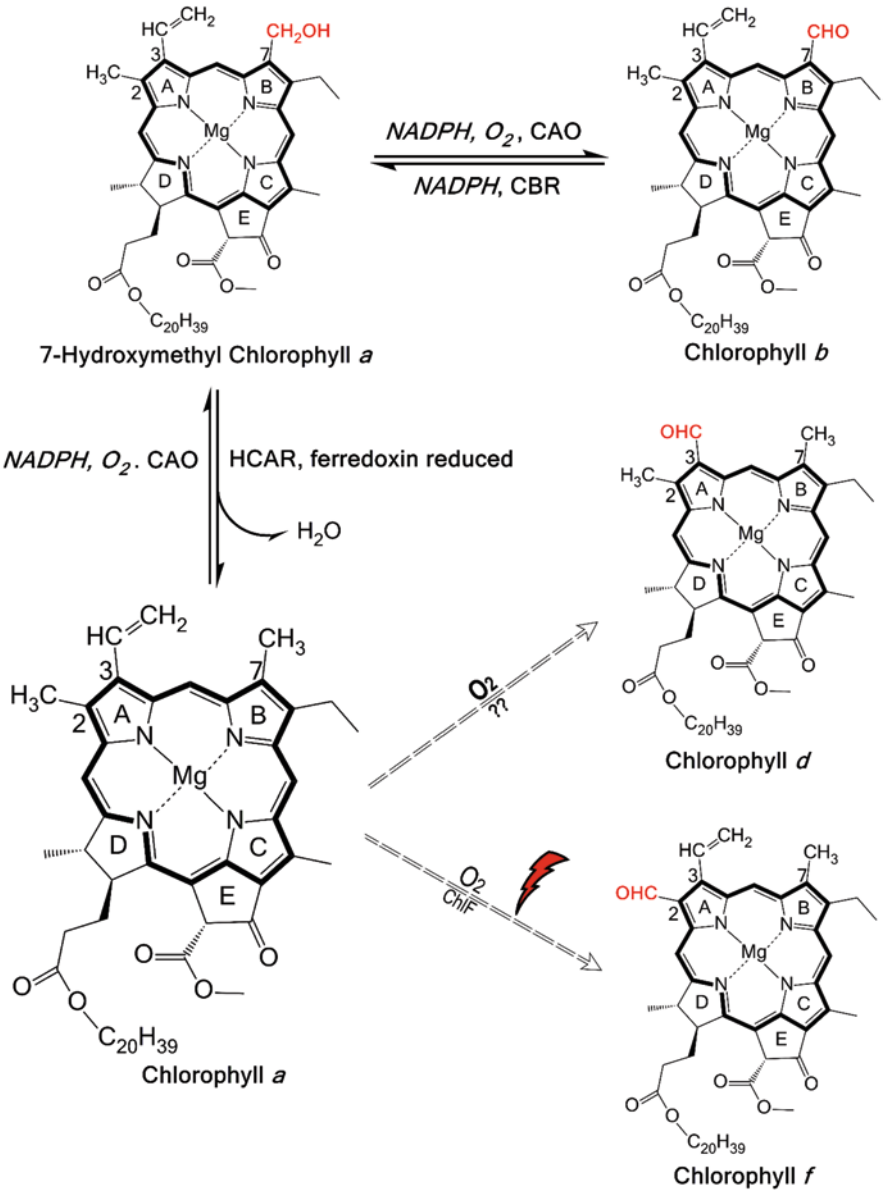


Fig. 5 Oxidation pathway of chlorophyll *a* to produce formyl-substituted chlorophyll. Chlorophyll *b* is synthesized by chlorophyll *a* oxygenase (CAO, EC 1.14.13.122) through the intermediate 7-hydroxymethyl Chl *a*. Oxygen (O_2) and NADPH are required. The Chl *b* cycle is catalyzed by chlorophyll *b* reductase (CBR, EC 1.1.1.294) and 7-hydroxymethyl chlorophyll *a* reductase (HCAR, EC 1.17.7.2). The oxygen atom of formyl group from Chl *b*, *d*, and *f* is derived from molecular oxygen. The question mark represents the uncertainty of Chl *d* synthase. ChlF is proposed the chlorophyll *f* synthase

Pheophytin *a* is also the important breakdown product of Chl degradation, and further breakdown proceeds to numerous breakdown colored or colorless products (Hörtensteiner et al. 2019; Kräutler 2016; Hörtensteiner and Kräutler 2011). Pheophytin *b* is not produced as a breakdown product because Chl *b* is firstly reduced by Chl *b* reductase (CBR, EC 1.1.1.294) (Scheumann et al. 1996) and 7-hydroxymethyl Chl *a* reductase (HCAR, EC 1.17.7.2) (Meguro et al. 2011) into Chl *a* prior to entering the degradation pathway (Christ and Hörtensteiner 2014).

Removal of the phytol chain from Chl is an important Chl degradation, which is catalyzed by chlorophyllase (EC 3.1.1.14) and produces chlorophyllide and phytol molecule. Although not normally prevalent in vivo, in *Synechocystis* PCC6803, N^{15} labeling studies revealed the increased chlorophyllase activity was coordinated with quick turnover of photodamaged PsbD2. Therefore, de novo synthesis of Chl and the recycling of chlorophyllide are avoided under stress conditions (Vavilin and Vermaas 2007).

4 Photopigment-Binding Protein Complexes

All carotenoids, bilins, and Chls are carefully arranged inside the pigment-binding protein complexes. The major Chl-binding protein complexes in oxygenic photosynthetic organisms are PSI and PSII which are associated with either thylakoid membrane-embedded Chl-binding LHCs or extrinsic PBSs. Photosystems are composed of an RC core, surrounding by the inner antenna and associated with either additional intrinsic membrane-bound Chl-binding antenna or extrinsic PBSs. In plants and algae, the intrinsic Chl-binding antenna is three-helical transmembrane Chl *a*-/*b*-binding light-harvesting complexes (LHCs), except for Chl *c*-containing alga, which have Chl *a*-/*c*-binding LHC. There are two main classes of LHC: LHCI and LHCII. LHCI is composed of four Lhca subunits (Lhca1–4) associated with PSI, while LHCII has three LHC subunits (Lhcb1–3) associated with PSII mainly. Multiple LHC subunits associated with PSI or PSII forming supracomplexes provide efficient energy transfer pathways (Neilson and Durnford 2010; Caffarri et al. 2014; Ballottari et al. 2012; Umena et al. 2011; Jordan et al. 2001). Some algae, such as red algae, cryptophytes, and glaucophytes, also have the extrinsic PBSs as the major antenna system, which is commonly arranged in a hemidiscoidal shape (MacColl 1998). There are three main components in assembled PBPs: PE, PC, and APC. In addition, an unusual external soluble antenna complex composed of peridinin-chlorophyll *a* protein (PCP) has been reported from dinophytes (Bautista et al. 1999). How PCP connects with membrane-bound PSs is largely unknown although the route of energy transfer was determined as PCP ➔ LHC ➔ RC (Ogata et al. 1994; Polívka et al. 2008; Bricker and Lo 2015).

Most *Cyanobacteria* have two antenna systems, PBS and Chl-binding antenna systems. The Chl-binding light-harvesting systems are six-helical transmembrane Chl-binding protein complexes, structurally different from the LHCs in eukaryotic

photosynthetic organisms, including Pcb and IsiA (Chen et al. 2008; La Roche et al. 1996). Most Pcb and IsiA will bind available Chls to form the functional CBPs. In *Prochlorococcus marina* spp., CBP contains Chls a_2 and b_2; in *A. marina* spp., CBP contains Chl *d* mainly (Chen et al. 2008). Phycobilisomes are efficient at capturing the light in the range of 520–660 nm, complementary with the light absorbed by Chls. There are several nontypical PSB structures reported from various cyanobacteria. *Acaryochloris marina* sp. MIBC11017 has a simple rod structure of PBPs, which tightly associates with the photosynthetic membrane via CpcG linkers (Chen et al. 2009). *Gloeobacter violaceus*, a cyanobacterium lacking thylakoid membranes, has bundle-shaped PBSs attached to the cytoplasmic plasma membrane (Koyama et al. 2006; Guglielmi et al. 1981). According to recent omics studies, PBSs could be remodeled depending on the light conditions (Wiltbank and Kehoe 2019). Under FRL conditions, Chl *f*-producing cyanobacteria remodel the PBS to a red-shifted PBS in order to optimize light capture efficiently (Li et al. 2016; Gan et al. 2014). The red-shifted PBSs are mainly made of APC subunits in *H. hongdechloris* and form the smallest PBS reported up to date (Li et al. 2016). A different mechanism to the red-shifted absorption feature was proposed in *Synechococcus* sp. PCC7335 (Herrera-Salgado et al. 2018; Ho et al. 2017a). Changed chromophore interaction within the PBP subunits and random reorganization of PBP might result in the red-shifted absorption feature of PBS when *Synechococcus* sp. PCC7335 is grown under FRL conditions.

Prochlorophytes use CBP that have sequences homologous to the core antenna protein, CP43 and CP47 of PSII (Bibby et al. 2001b). These proteins form a large antenna-RC supracomplexes, including CBP-PSI and CBP-PSII (Chen et al. 2008; Bibby et al. 2001a, b). Under nutrient limitation or high-light stresses, PBSs rapidly degrade, while PSs are also negatively affected, particularly PSI (Grossman et al. 1998). Different copy numbers of CBP-encoded genes are reported from various *Prochlorococcus* spp. isolated from different niches. The copy numbers of CBP reflect well with the accessible light levels at the eco-location. Multiple copies of CBP-encoded genes are reported from low-light-adapted *Prochlorococcus* sp. SS120, thriving at ~120 m below sea level, in contrast to high-light-adapted *Prochlorococcus* sp. MED4 isolated from the surface of the ocean and have one copy of CBP (Garczarek et al. 2000).

4.1 Photosystem I

Photosystem I from cyanobacteria is typically a trimer and has extrinsic CBP complexes, if present (Jordan et al. 2001; Chen et al. 2008). Each core complex of PSI mainly consists of 12 subunits which includes the RC core comprising PsaA and PsaB subunits surrounded by small transmembrane proteins of PsaF, PsaI, PsaJ, PsaK, PsaL, PsaM, and PsaX and 3 stromal subunits of PsaC, PsaD, and PsaE (Jordan et al. 2001). PsaA and PsaB are approximately 750 amino acids in size, and each contains an RC domain and a core antenna domain at the N-terminal. The six

N-terminal transmembrane helices of PsaA and PsaB show homology to CP43 and CP47 of PSII (Sect. 4.2) (Jordan et al. 2001). The direct interaction between antenna and PSI is determined after isolation of antenna-PSI supracomplexes (Toporik et al. 2019; Ihalainen et al. 2005; Bibby et al. 2001b). Some cyanobacteria show interaction between PBSs and PSI resolved by state-of-the-art cryo-EM technology (Li et al. 2019; Zhang et al. 2017). In addition to P_{700}, PsaA and PsaB host an accessory Chl *a* molecule and an electron acceptor Chl *a* molecule, plus a phylloquinone molecule in the RC domain side. Charge separation occurs when a special pair Chl passes on an electron to the acceptor Chl *a* molecule producing $P_{700}{}^{+}A_{0A}{}^{-}$ or $P_{700}{}^{+}A_{0B}{}^{-}$ which occurs within ~3.7 ps (Shuvalov et al. 2006). The electron is then transferred to electron carrier of phylloquinone within ~30 ps (Hecks et al. 1994). In *A. marina*, Chl *d* replaced Chl *a* in RCI. The special pair of Chl *d*/*d*′ has a unique absorption maxima of 740 nm and is named as P740 (Hu et al. 1998) (Sect. 5.2).

In Chl *f*-producing cyanobacterium, *H. hongdechloris*, Chl *f* production is induced under FRL conditions, and isolated PSI complexes contain about 8% of Chl *f* from a total of ~110 chlorophylls per isolated PSI from FRL-grown *H. hongdechloris* cells, while the isolated PSI complexes contain only Chl *a* from white light-cultured *H. hongdechloris* cells (Li et al. 2018a). Chl *a*/*a*′ formed P_{700} in isolated PSI from *H. hongdechloris* grown under WL and FR light conditions. The uphill energy transfer was further confirmed by decay fluorescence measures (Schmitt et al. 2018) (Sect. 6.2).

4.2 Photosystem II

Photosystem II is a multiple protein subunit complex containing RC and intrinsic core antenna and typically arranges as a dimer (Eaton-Rye and Sobotka 2017). PsbA (D1) and PsbD (D2) are the core subunits in RCII and bind six Chls including the special pair of Chl *a* (P680). CP43 and CP47 are core antenna subunits and bind 13–16 Chls individually. The extra loop of CP43 protein with D1 subunit together forms a binding dock for oxygen evolution center including Mn_4CaO_5 complexes (Umena et al. 2011). There are also small single transmembrane proteins such as PsbE, PsbF, PsbH-N, and PsbX-Z containing a single helix, and PsbO, PsbU, and PsbV are external subunits on the periphery of PSII, which do not have transmembrane domains (Gao et al. 2018). Some small transmembrane proteins (PsbI and PsbM) are involved in dimerization and stability of the structure and function of dimeric PSII (Shi et al. 2012; Guskov et al. 2009). PsbU, PsbV, PsbP, and PsbQ are positioned around D1 and D2 proteins to shield the Mn_4CaO_5 cluster from reductants (Bricker et al. 2012).

The RCII uses Pheo *a* as the primary electron acceptors (A_0) and passes the electrons to the secondary acceptors, A_1 and plastoquinone (Q_A/Q_B). Electrons from Pheo *a* are readily passed onto Q_A by non-heme iron Fe^{2+}. Meanwhile oxidized primary donor Chl is reduced by accepting electrons from the oxidation of water through a redox-active Tyr residue of D1, known as Yz, leading to the formation of

$P_{680}Q_AQ_B^-$ which is a stable charge separation. This process is repeated to produce $P_{680}Q_A^-Q_B^-$ which is stabilized by the uptake of H^+ ions from the stroma to form $P_{680}Q_AQ_BH_2$. Plastoquinol (Q_BH_2) readily diffuses from the Q_B-binding site of PsbD and binds with cytochrome b_6f complex. Cytochrome b_6f complex moves two protons to the lumen, and electrons, which are transferred to the electron carrier of plastocyanin, eventually transfer to PSI.

In Chl *d*-containing *A. marina*, Chl *d* represents more than 95% of total Chls. There are several mechanisms proposed for PSII in *A. marina* due to limited purified PSII complexes. Chen et al. (2005d) reported that Chl *d* is the special pair of Chl in isolated enriched PSII fraction with the proposed absorption maxima of P_{715} nm. Tomo et al. (2007) used FTIR technology to report Chl *d* in isolated PSII with the absorption maximum of 713 nm. In vivo measurements showed a maximum at 724 nm suggesting the primary electron donor is Chl *d* (Mielke et al. 2013) (Sect. 5.3).

4.3 Chlorophyll-Binding Light-Harvesting Protein Complexes (CBPs)

Cyanobacteria use intrinsic membrane-bound CBPs, including prochlorophyte Chl *a*-/*b*-binding protein (Pcb) and iron-stress induced chlorophyll-binding protein (IsiA) (Chen et al. 2008). The CBPs are six-helical transmembrane Chl-binding proteins, having homologous sequence with CP43 and CP47 of PSII but lacking the extra loop between helix 5 and helix 6 (Chen and Bibby 2005). The CBP proteins are unrelated to LHC proteins from eukaryotic photosynthetic organisms, instead having similarity to CBP with a six-transmembrane helical domain-binding 13–16 Chls (van der Staay and Staehelin 1994). The CBP proteins bind available Chls and form multiple subunit complexes associated with PSs. Under iron-stressed, light-stressed, or oxidatively-stressed conditions, IsiA proteins are upregulated and form 18-mer rings surrounding PSI trimer (Bibby et al. 2001b). Prochlorophytes use Pcb protein forming multiple subunit complexes surrounding PSI and PSII (Bibby et al. 2001b, 2003b).

In addition, there are additional families of CBP found in cyanobacteria, which have one or two helices of transmembrane domain with similarity to the membrane-spanning region of plant chlorophyll *a*-/*b*-binding proteins (LHCs) (Dolganov et al. 1995). For example, high-light-induced protein (HLIP), found in *Synechocystis* sp. PCC6803, binds Chls and carotenes and associates with PSII to protect D1 protein from degradation under high-light stress conditions (Knoppová et al. 2014).

4.3.1 Inner Antenna Complexes

Constitutively expressed CP43 and CP47 proteins encoded by *psbC* and *psbB* represent the inner antenna domains of PSII, an indispensable subunit of PSII complexes (Eaton-Rye and Putnam-Evans 2005). They function in the same manner as

that from PSII system in plants. The N-terminal domain of PsaA and PsaB protein serves as the inner core antenna and has a homologous sequence as CP43 and CP47 (Jordan et al. 2001). The inner core antenna binds 15–30 Chls and directly transfers the captured energy to the core of RC.

4.3.2 Chl-Binding Proteins in Cyanobacteria

In prochlorophytes, several types of Pcbs encoded by different genes, *pcbA–pcbH*, are reported and co-expressed under different culture conditions, including low-light stressed conditions, high-light-stressed conditions, and iron-stressed conditions (Garczarek et al. 2000; La Roche et al. 1996). In *Prochloron didemni*, isolated Pcb-PSII supracomplexes revealed 10Pcb:2PSII complex (Bibby et al. 2003b). *Prochlorococcus* sp. MED4 has a single copy of Pcb-encoded gene, which is constitutively expressed and formed antenna-PS supracomplexes (Bibby et al. 2003a). Since IsiA proteins are prevalent in many cyanobacteria and have high similarity with Pcbs from prochlorophytes, a new name for Chl-binding proteins in cyanobacteria (CBPs) was proposed (Chen et al. 2008). *Acaryochloris marina* uses Chl *d*-binding CBP as its major light-harvesting systems (Chen et al. 2002, 2005c; Chen and Bibby 2005), which will be discussed in details in Sect. 5.1.

4.3.3 Iron-Stress-Induced Chlorophyll-binding Protein A (IsiA)

IsiA protein was firstly reported as Chl-storing sites under iron-stressed condition (Burnap et al. 1993). The light-harvesting function of IsiA was confirmed after the structure of IsiA-PSI supracomplexes was solved (Bibby et al. 2001a, b; Boekema et al. 2001). IsiA binds 16–17 Chls and provides additional energy transfer network for PSs (Bibby et al. 2001a; Chen and Bibby 2005; Chen et al. 2005a; Andrizhiyevskaya et al. 2002). The crystal structure of PSI-IsiA revealed an increased surface area for light harvesting of PSI and concentration of Chl *a* molecules to the stromal side of the membrane suggesting an interaction with the remaining PBSs (Toporik et al. 2019).

4.4 Phycobilisomes (PBSs)

Phycobilisomes are the main light-harvesting structure for most cyanobacteria, red algae, and glaucophytes (Overmann and Garcia-Pichel 2013). They are composed of PBPs and linker proteins extending the light-absorbing surface area and particularly the increased light-capture cross section, especially the green-orange light (520–670 nm). Phycobiliproteins consist of apoproteins covalently bound to bilins, mainly at the A ring of bilins through thioether (C-S-C) bonds formed by cysteine residues of the apoprotein and ethylidene of the bilin (Fig. 2) (MacColl 1998).

These bonds are formed and catalyzed by lyase enzymes (Scheer and Zhao 2008). Meanwhile the linker proteins usually do not bind chromophores and function for integrating PBP subunits to PBS. This may involve rod extension (L_R/CpcCs), distal termination of rods (L_{RT}/CpcD), rod and core association (L_{RC}/CpcG), assembly of the APC core (L_c) and attachment of the core membrane linker APC with thylakoid membranes (L_{CM}/ApcE) (Guan et al. 2007). Despite usually not binding bilins, linker proteins influence the absorption maximum of a given PBP through different organizations of PBS (Glazer 1985).

The four different types of bilins in cyanobacteria are PCB (blue), PVB (purple), PEB (pink), and PUB (yellow), in which PCB and PEB are the common chromophores in cyanobacteria. There may be either a single or more bilins bound with various apoproteins, resulting in the different optical properties of APC, PC, PE, and PEC (Fig. 2). The basic structure of PBS is comprised of a αβ heterodimeric, which forms a trimer of heterodimeric disc structure and then interacts with linker proteins to form a cylindrical structure. APC has the lowest light energy (650–660 nm) followed by PC (615–640 nm) and PE (520–575 nm) (Overmann and Garcia-Pichel 2013), so this arrangement facilitates higher light energy captured by PE or PC efficiently to transfer to the lower light energy traps of APC cores (Grossman et al. 1993).

The typical PBS structure is hemidiscoidal that may have bicylindrical, tricylindrical, or pentacylindrical APC core along the thylakoid membranes (Fig. 6a). The two bottom APC core cylinders are bound to the thylakoid, composed of four different APC/linker trimer-like protein complexes, while the top cylinder is composed of four trimer/trimer-like complexes consisting of two different complexes. Each complex is composed of variable APC proteins with or without linker proteins (MacColl 1998). A tricylindrical core structure hosts six PC or PC + PE rods depending on the light conditions. Low light requires maximum rod structures for increased light absorption surface area, while high light has a more compact rod structure; even APC core without the rod structure attached was observed from FRL-adapted *H. hongdechloris* (Fig. 6c) (Li et al. 2016).

The energy transfer funnel was built through the structure of PBS. The APC core has the lowest energy of 650–660 nm, followed by PC absorbing 615–640 nm light, and then the PE using light of 520–575 nm (Overmann and Garcia-Pichel 2013). Phycobilisomes transfer the energy to Chls in PSs, mainly associated with PSII (Chang et al. 2015). A PBS-PSI-PSII supracomplex was recently isolated by pull-down experiments combining with aids of cross-linking agent and proteomic analysis (Liu et al. 2013). An atypical PBS structure lacking APC cores was isolated from *Anabaena* and reported to interact with tetrameric PSI (Watanabe et al. 2014).

5 *Acaryochloris marina*

Acaryochloris marina is the only cyanobacterial genus that uses Chl *d* as the major pigment with the trace amount of Chl *a* of ~1–5% (Loughlin et al. 2013). Chlorophyll *d* was first reported by Manning and Strain (1943) through the identification of a