REVIEW

Advances in the members and biosynthesis of chlorophyll family

N.W. QIU[*,†], D.C. JIANG[*,**,†], X.S. WANG[*], B.S. WANG[***], and F. ZHOU[#,+]

College of Life Sciences, Qufu Normal University, Qufu, 273165 Shandong, China[*]
Shanghai Institute of Nutrition and Health, Shanghai Institutes for Biological Sciences, University
of Chinese Academy of Sciences, Chinese Academy of Sciences, 200031 Shanghai, China[**]
Shandong Provincial Key Laboratory of Plant Stress, Shandong Normal University, 250014 Jinan, China[***]
School of Food Science, Nanjing Xiaozhuang University, 211171 Nanjing, China[#]

Abstract

Chlorophylls are vital for photosynthesis, allowing plants to absorb energy from light for photosynthesis. More than one hundred species of chlorophyll have been identified. Among them, chlorophylls *a*, *b*, *c* (c_1, c_2, and c_3), *d*, and *f* exist in oxygenic photosynthetic organisms (*e.g.*, higher plants, algae, and cyanobacteria), whereas anoxygenic photosynthetic bacteria possess bacteriochlorophylls *a*, *b*, *c*, *d*, *e*, and *g*. These chlorophylls have different chemical structures and properties that enable photosynthetic organisms to perform photosynthesis in different environments. All of the chlorophylls are biosynthesized from 3,8-divinyl-protochlorophyllide *a* by a series of enzymes. The synthetic pathways of chlorophylls have now been basically clarified. This review succinctly summarizes the structures, properties, and synthetic pathways of the chlorophylls.

Additional key words: absorption spectrum; chemical bond; porphyrin; side chain; synthetase.

Introduction

Chlorophylls are the most important and most common pigment molecules in nature and are essential for photo-synthesis. They play a central role in the absorption, transmission, and transduction of light energy in photosynthesis. Chlorophylls are widely distributed in green plants, algae, photosynthetic bacteria, and some animals [*e.g.*, *Euglena viridis* (Bennett and Triemer 2015)]. More than 100 chlorophylls are known today, with the majority discovered in the green bacteria (Scheer 2006).

According to the distribution of chlorophylls in photo-synthetic organisms, the chlorophylls can be divided into two categories: the chlorophylls in oxygenic photosynthetic organisms (abbreviated Chls) and the chlorophylls (bacte-riochlorophyll) in anoxygenic photosynthetic bacteria (abbreviated BChls) (Chew and Bryant 2007, Harada *et al.* 2014). Chls *a*, *b*, *c* (c_1, c_2, c_3), *d*, and *f* exist in oxygenic

photosynthetic organisms (*e.g.*, higher plants, algae, and cyanobacteria), whereas anoxygenic photosynthetic bacteria (*e.g.*, green sulfur bacteria, purple bacteria, filamentous anoxygenic phototrophs, acidobacteria, and heliobacteria) possess BChls *a*, *b*, *c*, *d*, *e*, and *g* (Harada *et al.* 2014). For a long period of time, it was believed that there are only four chemically distinct chlorophylls in oxygenic photosynthetic organisms, namely Chls *a*, *b*, *c*, and *d*. In 2010, the fifth Chl was discovered from a cyanobacterium in stromatolites from Shark Bay, Western Australia, and named as Chl *f* (Chen *et al.* 2010). In 1948, Harold H. Strain reported a new chlorophyll, namely Chl *e*, in his unpublished work. Since then, Chl *e* has been mentioned in a number of monographs from 1950 to 1970 (Allen 1966). However, the properties of Chl *e* are not clear, and the chemical structure and function are still uncertain. Chl *e* has not been acknowledged by the scientific community. In order to retain the name for

the yet poorly characterized Chl *e*, the newly discovered chlorophyll in stromatolites is named chlorophyll *f*. At the same time, more and more BChls have been discovered, including BChls *a*, *b*, *c*, *d*, *e*, and *g*. The tentative name and structure of BChl *f* were proposed in 1975 (Gloe *et al.* 1975). However, BChl *f* is only biosynthesized in the mutants of BChl *e* producing bacteria (*Chlorobaculum limnaeum*, a green sulfur bacteria) (Vogl *et al.* 2012) or prepared by modifying Chl *b* (Tamiaki *et al.* 2011). In theory, BChl *f* should be synthesized in the green sulfur bacteria, but has yet to be discovered in nature.

Although there are many types of chlorophyll, a large part of the synthetic pathways of chlorophylls is the same. All of the chlorophylls are biosynthesized from 3,8-divinyl-protochlorophyllide *a* (DV-PChlide *a*) by a series of enzymes (Chew and Bryant 2007, Harada *et al.* 2014). DV-PChlide *a* can be transformed into different chlorophylls through different biochemical pathways. The main pathway of chlorophyll synthesis was discovered in 1948 (Granick 1948). Some chlorophyll synthesis pathways have only been discovered in recent years (Harada *et al.* 2014, Thweatt *et al.* 2017), and some are still unclear. This paper summarizes the research on chlorophyll from the aspects of structure, property, function, distribution, and biosynthesis in order to better understand this complex bio-pigment and photosynthesis.

Family members and structure of the chlorophylls

In addition to Chls and BChls, plants also contain small amounts of pheophytin, protochlorophyllide, *etc*. Chlorophylls are defined as cyclic tetrapyrroles carrying a characteristic isocyclic five-membered ring (porphyin ring), which are functional in light harvesting or charge separation in photosynthesis (Moss 1988). The properties of chlorophyll members vary with their different structures, so that different photosynthetic organisms can adapt to different environments. Rings and carbon atoms on a chlorophyll structure are commonly numbered according to the IUPAC-IUB nomenclature (Moss 1988) (Fig. 1). The structural characteristics of all chlorophylls are described as follows.

Chlorophyll *a* and chlorophyll *b*: The molecular structure of Chl *a* and Chl *b* (Fig. 1) is made up of a dihydroporphyrin ring 'head' (known as a chlorin) and a phytol 'tail' (20-carbon atoms hydrocarbon). The chlorin ring is a macrocycle formed by four pyrrole rings and four methene bridges (=CH–). The magnesium atom is located in the center of the chlorin ring and non-coordinatively bonded to the nitrogen atoms on the pyrrole ring A and the pyrrole ring C. The magnesium atom carries positive charge, while the nitrogen atoms connected to it carry negative charges. So the 'head' is hydrophilic. The phytol is composed of four isoprene units, which are bonded with the propionic acid on the side chain of the pyrrole ring D by an ester bond. Therefore, the 'tail' is lipophilic. The only difference between Chl *a* and Chl *b* is that there is a methyl group (–CH$_3$) at the C-7 position on the pyrrole ring B of Chl *a*, while it is a formyl group (–CHO) on
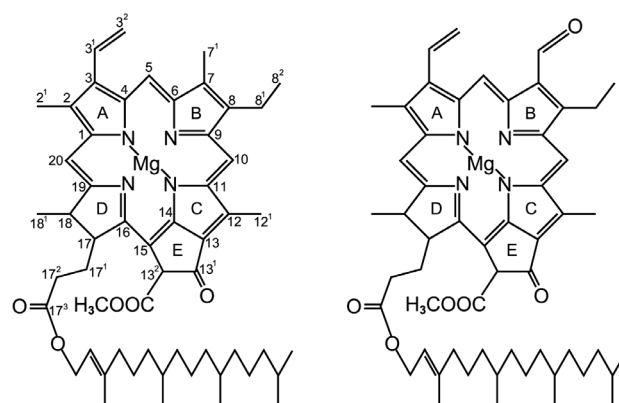

Fig. 1. The structures of chlorophyll (Chl) *a* (*left*) and Chl *b* (*right*). Numbering of rings and carbon atoms according to the IUPAC-IUB nomenclature (Moss 1988).

the same position of Chl *b* (Fig. 1).

Chlorophylls *c*: Most members of the Chl *c* family are porphyins with no phytol 'tail', while the other Chls are dihydroporphyrins (chlorins) with a propionic acid side chain esterified with phytol (Helfrich *et al.* 2003). There is a carbon-carbon double bond (C=C) between C-17^1 and C-17^2 on the side chain of the pyrrole ring D of Chls *c*, while a carbon-carbon single bond is present at the same position of the other chlorophylls. The second structural feature of Chls *c* is that there is a double bond between C-17 and C-18 carbons instead of a single bond for the other chlorophylls.

At present, the number of known Chls *c* has increased to more than ten (Zapata *et al.* 2006, Xu *et al.* 2016), among which Chl c_1, Chl c_2, and Chl c_3 are the common types of Chls *c* (Fig. 2). Protochlorophyllide *a* (PChlide *a*) and DV-PChlide *a*, the biosynthetic precursors of chlorophyll, are classified as Chls *c* in some studies (Helfrich *et al.* 2003). However, there is a carbon-carbon single bond between C-17^1 and C-17^2 in their structures, which is the only difference between DV-PChlide *a* and Chl c_2 or PChlide *a* and Chl c_1. Chl c_1 differs from Chl c_2 in the structure of the C-8 side chain of the pyrrole ring B, having an ethyl group (–CH$_2$CH$_3$) instead of a vinyl group (–CH=CH$_2$). Chl c_3, also known as 7-methoxycarbonyl-Chl c_2, is formed by the substitution of a methyl group (–CH$_3$) at C-7 on the porphyin ring B of Chl c_2 by a methoxycarbonyl group (–COOCH$_3$) (Fookes and Jeffrey 1989).

Chlorophyll *d* and chlorophyll *f*: Both Chl *d* and Chl *f* have similar structures to Chl *a* (Fig. 3). The replacement of the vinyl group (–CH=CH$_2$) at C-3 on the pyrrole ring A of Chl *a* with a formyl group (–CHO) becomes Chl *d* (Manning and Strain 1943). The replacement of the methyl group (–CH$_3$) at C-2 on the pyrrole ring A of Chl *a* with a formyl group (–CHO) produces Chl *f*. Therefore, the structural differences of Chl *a*, *d*, *f* lie in the side chains on the pyrrole ring A. Chl *f* and Chl *b* are isomers, only the positions of the formyl and methyl group are different (Chen *et al.* 2010).
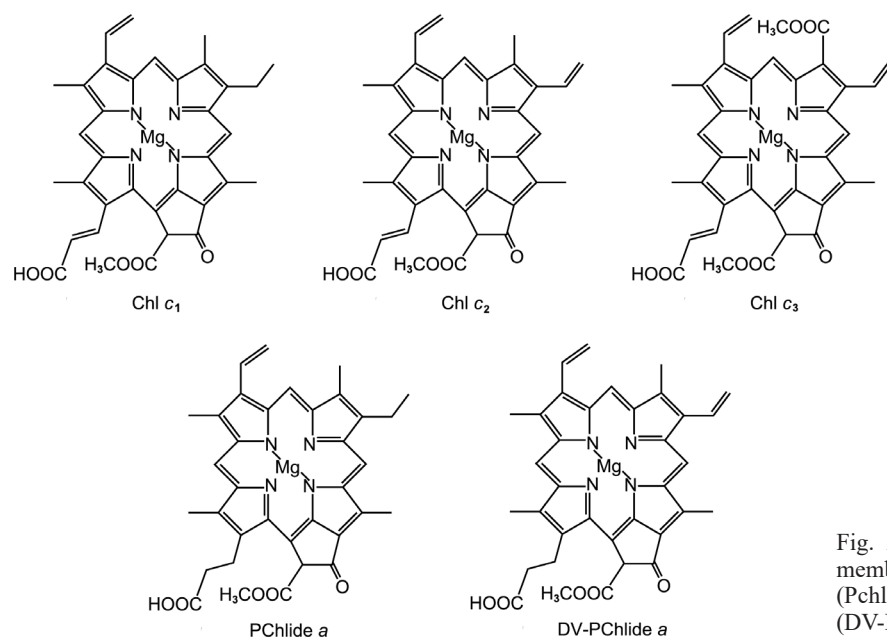
975

Fig. 2. The structures of chlorophyll (Chl) *c* members: Chls *c₁*, *c₂*, *c₃*, protochlorophyllide *a* (Pchlide *a*) and 3,8-divinyl-protochlorophyllide *a* (DV-Pchlide *a*).
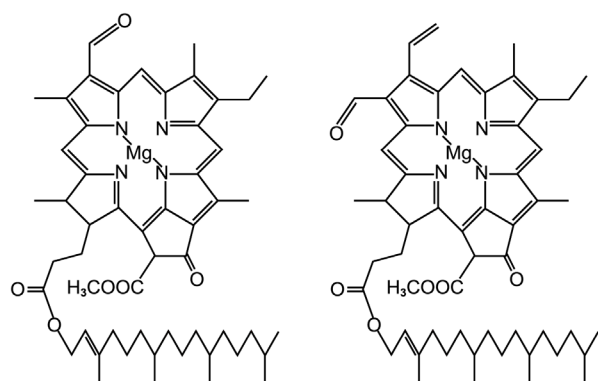

Fig. 3. The structures of chlorophyll (Chl) *d* (*left*) and Chl *f* (*right*).

Pheophytin (Pheo): Chlorophylls contain Mg^{2+} encased in their porphyrin ring structure. This Mg^{2+} is active and easily replaced by H^+ to form pheophytin (Fig. 4). Mg^{2+} also can be replaced by Cu^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, and Ni^{2+} in heavy metal-stressed plants (Taniguchi and Lindsey 2017, Li and Bridwell-Rabb 2019). Pheo can be produced from chlorophyll by the treatment with a weak acid. Pheo serves as the first electron carrier in the electron transfer pathway of PSII in plants.

Bacteriochlorophyll (BChls): According to the number of reduced pyrrole rings, BChls can be divided into two types (Fig. 5). BChls *a*, *b*, and *g* have two reduced pyrrole rings (B, D), and BChls *c*, *d*, *e*, and *f* have one reduced pyrrole ring (D) (Chew and Bryant 2007, Vogl *et al.* 2012). So the 'head' of BChls *a*, *b*, and *g* belongs to bacteriochlorins (tetrahydroporphyrins) and the 'head' of BChls *c*, *d*, *e*, and *f* belongs to chlorins. In addition, the side chains on the porphyrin ring of each BChl are also different. For instance, the only difference between BChl *a* and *b* is that BChl *b* has an ethylidene ($=CH–CH_3$) instead of an ethyl

($–CH_2CH_3$) at the C-8 side chain. The structures of BChl *c* and *e*, *d*, and *f* are almost the same, except that BChls *c* and *d* have a methyl group ($–CH_3$) at the C-7 side chain of the porphyrin ring, and BChls *e* and *f* have a formyl group ($–CHO$) at the same position. BChls *c* and *e* have one methyl group ($–CH_3$) at C-20 of the porphyrin ring, while BChls *d* and *f* do not. The porphyrin ring of BChl *g* is similar to BChls *a* and *b*; but its 'tail' is the same as that of BChls *c*, *d*, *e*, and *f*. The predominant esterifying alcohol 'tail' of BChls *c*, *d*, *e*, *f*, and *g* is farnesol (15-carbon atoms), 5-carbon atoms less than the phytol 'tail' of BChls *a* and *b* (20-carbon atoms) (Niedzwiedzki and Blankenship 2010). Many derivatives of BChls *c*, *d*, *e*, and *g* with different side chains and other esterifying alcohols have been identified (Glaeser *et al.* 2002, Frigaard *et al.* 2003, 2006; Chew *et al.* 2007), which are not described here.

Properties, function, and distribution of chlorophylls

Chl *a* and Chl *b*: Chl *a* (molecular formula: $C_{55}H_{72}O_5N_4Mg$; molecular mass: 893.48 Da; color: blue-green) is the most widely distributed form in all terrestrial plants and oxygenic photosynthetic algae, which can be also found in very small quantities in green sulfur bacteria (an anaerobic photoautotroph) (Eisen *et al.* 2002). However, Chl *b* (molecular formula: $C_{55}H_{70}O_6N_4Mg$; molecular mass: 907.46 Da; color: yellow-green) is distributed in Cyanophyta II, Dinophyta (Dinoflagellates), Euglenophyta, Chlorarachniophyta, Prasinophyceae, Chlorophyceae, green plant plastids (Green *et al.* 2003). Chl *a* absorbs light within the violet, blue, and red wavelengths. Its absorption peak wavelengths in 90% acetone are 430 and 664 nm. Chl *b* also absorbs light within the violet, blue, and red wavelengths, but its absorption peak in the blue region is longer than that of Chl *a*, and the absorption peak wavelength in the red region is shorter than that of Chl *a*. The absorption peak wavelengths of Chl *b* in
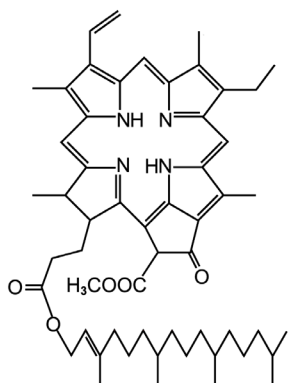
Fig. 4. The structure of pheophytin *a*.

90% acetone are 460 and 647 nm. Accessory Chl *b* and other photosynthetic pigments broaden the spectrum of light absorbed, increasing the range of wavelengths that can be used in photosynthesis (Raven *et al.* 2005). The addition of Chl *b* next to Chl *a* can extend the absorption spectrum. In low light conditions, plants produce a higher ratio of Chl *b* to Chl *a*, increasing the photosynthetic yield (Björkman 1981, Esteban *et al.* 2015). The Chl *b*-deficient wheat mutant has a decreased photosynthetic function and is more sensitive to heat stress (Brestič *et al.* 2016). Both Chl *a* and Chl *b* can transfer the captured light from one pigment to the next as the resonance energy, until reaching the dimeric Chl *a* molecule in the reaction center (RC). These dimeric Chl *a* molecules are located in both PSII and PSI, known as P680 for PSII and P700 for PSI (Ishikita *et al.* 2006). Chl *a* is required for most photosynthetic organisms (except for some anaerobic photosynthetic bacteria), because it is the only pigment in their RCs that converts light energy into electrical energy and participates in photosynthetic electron transport (Björn *et al.* 2009).

Chl *c*: Chls *c* are blue-green, absorbing red light and blue-violet light. Compared to Chl *a* and *b*, the light absorption peak wavelength of Chls *c* in the red region is shorter, the absorption peak wavelength in the blue region is between that of Chl *a* and Chl *b*. The members of the Chls *c* family are widely distributed in the golden-brown eukaryotic algae and the chromophytes. Together with Chl *a* and carotenoids, Chls *c* function as light-harvesting pigments (Zapata *et al.* 2006).

Chl c_1 (molecular formula: $C_{35}H_{30}O_5N_4Mg$; molecular mass: 610.94 Da) is a common form of Chls *c*. Its absorption peak wavelengths in 90% acetone are 442 and 630 nm. There are three absorption peak wavelengths in pure diethyl ether and acetone, which are 444, 577, 626 nm and 447, 579, 629 nm, respectively (Fawley 1989).

Chl c_2 (molecular formula: $C_{35}H_{28}O_5N_4Mg$; molecular mass: 608.92 Da) is the most common form of Chls *c*. Its absorption peak wavelengths in 90% acetone are 444 and 630 nm. The absorption peak wavelengths in pure diethyl ether and acetone are 447, 580, 627 nm and 450, 581, 629 nm, respectively (Fawley 1989).

Chl c_3 (molecular formula: $C_{36}H_{28}O_7N_4Mg$; molecular mass: 652.93 Da) is a form of Chl *c* found in the microalga *Emiliania huxleyi* (Fookes and Jeffrey 1989). Its absorption peak wavelengths in diethyl ether and acetone are 452, 585, 625 nm and 452, 585, 627 nm, respectively (Fawley 1989).
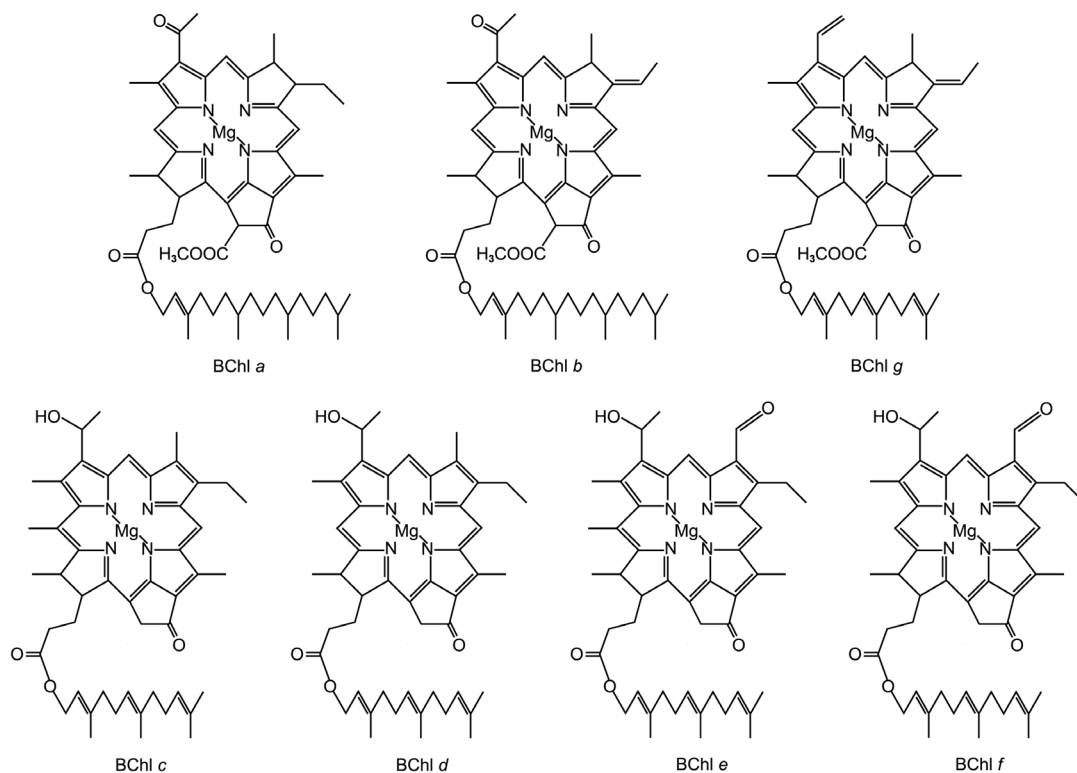

BChl *a*

BChl *b*

BChl *g*

BChl *c*

BChl *d*

BChl *e*

BChl *f*

Fig. 5. The structures of bacteriochlorophylls *a*, *b*, *c*, *d*, *e*, *f*, and *g*.

Chl *d* and Chl *f*: Both Chl *d* and Chl *f* are yellow-green. Chl *d* (molecular formula: $C_{54}H_{70}O_6N_4Mg$; molecular mass: 895.5 Da) has now been identified as the major pigment in several oxygenic prokaryotes, including *Acaryochloris marina* (Miyashita *et al.* 1997). Red algae do not have Chl *d*; the source of this was determined to be contaminating *Acaryochloris* attached to the surface of the red alga (Manning and Strain 1943). The absorption peak wavelengths of Chl *d* in 90% acetone are 401, 455, and 696 nm (Chen 2014). Chl *d* can absorb longer wavelengths of far-red light and shorter wavelengths of violet-blue light compared with those by Chls *a*, *b*, and *c*. Due to the strong transmission of far-red light in water, photosynthetic organisms containing Chl *d* are suitable for living in the water layers with overlying organisms living on Chl *a* photosynthesis, where it can use far-red light (up to 710 nm in wavelength) for photosynthesis (Larkum and Kühl 2005). There is a layer of Chl *a*-containing organisms in the shallow water. So there is very little red light below due to the absorption by Chl *a*, while far-red light is still present. Under these conditions, Chl *d* is favorable for photosynthesis. In the past, only Chl *a* was thought to be indispensable for energy transduction in the RCs (Björn *et al.* 2009), until Chl *d* was shown to constitute up to 99% of all chlorophylls in the cyanobacterium *Acaryochloris marina*. In this cyanobacterium, Chl *d* can replace Chl *a* in the RCs, harvesting excited state energy from the light-harvesting complex (Kühl *et al.* 2007, Allakhverdiev *et al.* 2016). There is a Chl *a* monomer in PSI that participates in electron transport. Chl *d* is likely involved in electron transport in *A. marina*.

Chl *f* (molecular formula: $C_{55}H_{70}O_6N_4Mg$; molecular mass: 907.46 Da) was discovered from cyanobacteria (Chen *et al.* 2010, Gan *et al.* 2014), which has the most red-shifted absorption among all Chls because of the formyl group at C-2 of Chl *f* (Garg *et al.* 2017). The absorption peak wavelengths of Chl *f* in methanol are 407 and 706 nm. Its absorption spectrum is extended to the near-infrared region (Chen and Blankenship 2011). Although there is almost no visible light in the stromatolites, cyanobacteria can also perform photosynthesis.

Pheophytin (Pheo): Pheo *a* is demetallated Chl *a* that serves as the first electron carrier in the PSII RC in plants. Demetallation of Chl *a* easily occurs under acidic conditions. The absorption peak wavelength of Pheo *a* in the red region is 665 nm in 90% acetone. Pheo *a* was first postulated to be the primary electron acceptor in PSII by Van Gorkom (1974). Subsequently, the presence of two Pheo *a* molecules in the RC of PSII was established, and one of them was shown to function as the primary electron acceptor (Hastings *et al.* 1992, Klimov 2003). In addition to the controversy in *A. marina* (Pheo *d* may be the primary acceptor in *A. marina*.), the primary electron donor of PSII in other oxygenic photosynthetic organisms is Pheo *a*. While bacteriopheophytin *a* (BPheo *a*) is found in the RCs of almost all purple bacteria and green filamentous bacteria, it functions as an intermediary electron acceptor. Other purple bacteria, whose major pigment is BChl *b*, use BPheo *b* as they all employ type-2 RCs

(Kobayashi *et al.* 2006).

Bacteriochlorophyll (BChls): BChls are distributed in various photosynthetic bacteria and are capable of absorbing longer wavelength red light and shorter wavelength near ultraviolet light than other chlorophylls. With an absorption wavelength range of 350–1050 nm of BChls, photosynthetic bacteria can carry on photosynthesis by absorbing light energy that cannot be absorbed by higher plants or green algae (Chew and Bryant 2007, Larkum *et al.* 2018). For example, the absorption peak wavelengths of BChl *a* in ethanol are 367 and 770 nm, and that of BChl *b* are 373 and 795 nm (Croce and van Amerongen 2014).

Biosynthesis of chlorophyll

Biosynthesis of metabolic precursors: In recent years, new progress has been made in several investigations of chlorophyll biosynthesis, which are the focus of this section. The synthesis of chlorophylls in organisms begins with the synthesis of δ-aminolevulinic acid (ALA). ALA can be synthesized in two pathways, the C5 pathway utilizing glutamate/2-oxoglutarate and the C4+C1 pathway utilizing succinate and glycine (Porra 1997). And then protoporphyrin IX (Proto-IX) is synthesized from eight molecules of ALA by six enzymes. The synthesis of ALA and Proto-IX has been reviewed comprehensively in the articles by Beale (2005, 2006). Proto-IX is the metabolic precursor of all Chls, BChls, heme, and heme derivatives, such as the chromophores (phycobilins) of the phycobilisomes (Chew and Bryant 2007).

Mg-chelatase (a multisubunit enzyme) catalyzes the insertion of Mg^{2+} into Proto-IX to form Mg-Proto-IX, which is the first committed step in Chl biosynthesis (Walker and Willows 1997, Bollivar 2006, Tamiaki *et al.* 2016). This reaction takes place in two ATP-dependent steps, activation and chelation, and requires the hydrolysis of about 15 ATP per inserted Mg by Mg-chelatase (Reid and Hunter 2004). Mg-Proto-IX is subsequently methylated to form Mg-Proto-IX-monomethylester by Mg-proto-IX methyltransferase (ChlM or BchM). Then, the propionic acid chain at C-13 is oxidized and cyclized to form an isopentanone fifth ring (E ring) by Mg-proto-IX monomethylester cyclase (BchE or ChlA). The product of this step is DV-PChlide *a* (Willows 2003). All Chls and BChls are biosynthesized from DV-PChlide *a* by a series of enzymes (Fig. 6). ChlA is an oxygen-dependent, diiron oxygenase found in the organisms that synthesizes Chls in oxic environments, whose catalytic subunit is termed the aerobic cyclization system Fe-containing subunit (AcsF) (Pinta *et al.* 2002). BchE is an oxygen-sensitive, radical-SAM enzyme found in the organisms that live in anoxic environments. Some purple bacteria have both ChlA and BchE, and they live in anoxic and microaerophilic conditions (Oh *et al.* 2000).

The next step of chlorophyll biosynthesis includes two pathways (Fig. 6). One pathway is to reduce the C-17/C-18 double bond on the pyrrole ring D of DV-PChlide to produce 8-vinyl-chlorophyllide *a* (8V-Chlide *a*) by protochlorophyllide oxidoreductases (PORs), and then
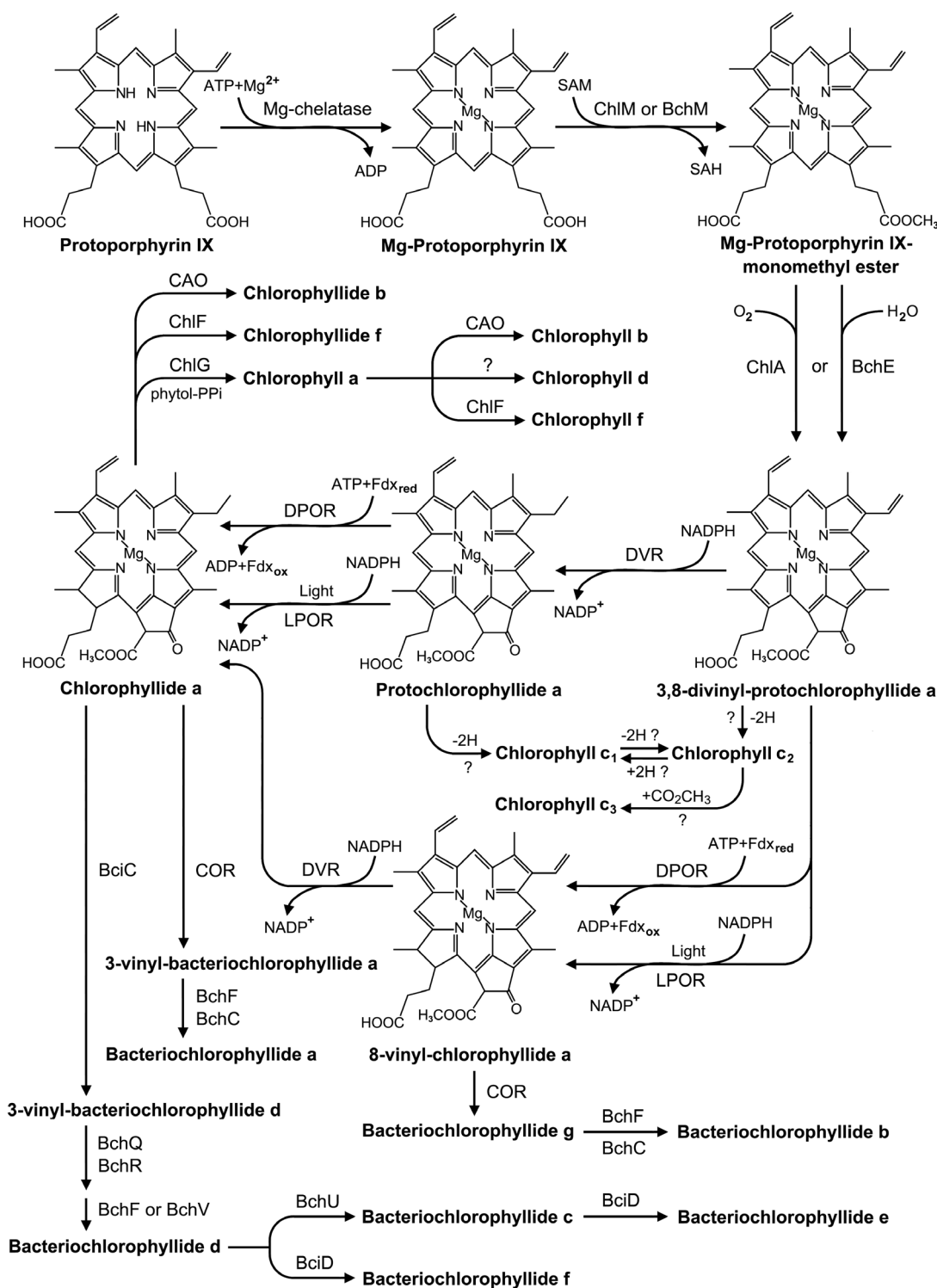
Fig. 6. The biosynthesis pathway of chlorophyllides and bacteriochlorophyllides from Protoporphyrin IX.

reduce the C-8 vinyl group of 8V-Chlide a to produce chlorophyllide a (Chlide a) by divinyl reductases (DVRs) (Tsukatani et al. 2013a). Three members of DVRs have been discovered, namely BciA, BciB, and chlorophyllide a oxidoreductase (COR) (Harada et al. 2014). Another pathway is to reduce the C-8 vinyl group to produce

PChlide a by DVRs first (Saunders et al. 2013), and then the C-17/C-18 double bond of PChlide a is reduced to produce Chlide a (Wang et al. 2010). Studies have shown that the preferred substrates for both pathways of DVRs are 8V-Chlide a, with DV-PChlide being reduced only under conditions in which this pigment accumulates as

979

a result of the alternative formation of Chlide *a* (Canniffe *et al.* 2014).

There are two structurally unrelated PORs found in photosynthetic organisms, light-dependent protochlorophyllide oxidoreductase (LPOR) and dark-operative protochlorophyllide oxidoreductase (DPOR) (Suzuki and Bauer 1995). LPOR only has one nuclear-encoded subunit, which needs light to operate and is ubiquitously distributed in cyanobacteria and all eukaryotic photosynthetic organisms (Heyes *et al.* 2006). In contrast, DPOR is a protein complex composed of three subunits (L, B, and N), which is ubiquitously distributed in prokaryotic photosynthetic organisms and some eukaryotic photosynthetic organisms except angiosperms (Chen 2014). DPOR catalysis depends on ATP and dithionite or reduced ferredoxin (Nomata *et al.* 2006), which is highly sensitive to free oxygen and is deactivated when the concentration of free oxygen exceeds 3% (Yamazaki *et al.* 2006). Angiosperms do not have the DPOR-encoding genes, so the conversion of PChlide *a* to Chlide *a* is light-dependent in angiosperm plants, and such plants are etiolated if grown in darkness. PChlide exists mostly in the complex form with proteins in etioplast, which mainly absorbs near-red and blue-violet light and has strong fluorescence properties. However, under light conditions, PChlide acts as a photosensitizer, forming highly toxic free radicals. Hence, plants need an efficient mechanism of regulating the content of PChlide. Angiosperms can regulate the PChlide content at the synthetic step of ALA (Melkozernov and Blankenship 2006).

Biosynthesis of Chls and BChls: Chlorophyllides (Chlides) or bacteriochlorophyllides (BChlides) are the immediate precursors of Chls and BChls, respectively. Only BChlide *b* and *g* are synthesized from 8V-Chlide *a*. The precursor of all other types of Chlides and BChlides is Chlide *a* (Fig. 6, Table 1). In the last step of chlorophyll biosynthesis, chlorophyll synthases esterify Chlides (or BChlides) with phytyl-pyrophosphate (or farnesyl-pyrophosphate) and produce Chls (or BChls).

Biosynthesis of Chl *a*, Chl *b*, and Chl *d*: Chlide *b* is synthesized through the oxidation of the methyl group at C-7 of Chlide *a* to a formyl group by chlorophyll *a*

Table 1. Chemical reactions in the synthesis of chlorophylls (Chls) and bacteriochlorophylls (BChls). R_1 can be ethyl, propyl, isobutyl or neopentyl; and R_2 can be methyl or ethyl (Glaeser *et al.* 2002, Frigaard *et al.* 2003, 2006).

Substrate	Site	Substrate group	Product group	Enzyme	Product	References
Chl *a* Chlide *a*	C-7	$-CH_3$	$-CHO$	CAO	Chl *b* Chlide *b*	Tanaka *et al.* 1998, Tanaka and Tanaka 2011
Chl *a*	C-3	$-CH=CH_2$	$-CHO$	unknown	Chl *d*	Schliep *et al.* 2010
Chl *a* Chlide *a*	C-2	$-CH_3$	$-CHO$	ChlF	Chl *f* Chlide *f*	Ho *et al.* 2016, Li and Bridwell-Rabb 2019
Chlide *a*	C-7, 8	C=C	C–C	COR	3V-BChlide *a*	Nomata *et al.* 2006
3V-BChlide *a*	C-3	$-CH=CH_2$	$-COCH_3$	BchF, BchC	BChlide *a*	Porra *et al.* 1996, Lange *et al.* 2015
8V-Chlide *a*	C-8 C-7, 8	$-CH=CH_2$ C=C	$=CHCH_3$ C–C	COR	BChlide *g*	Tsukatani *et al.* 2013a,b
BChlide *g*	C-3	$-CH=CH_2$	$-COCH_3$	BchF, BchC	BChlide *b*	Bollivar *et al.* 1994, Suzuki *et al.* 1997
Chlide *a*	C-13^2	$-COOCH_3$	$-H$	BciC	3V-BChlide *d*	Liu and Bryant 2011, Teramura *et al.* 2016a
3V-BChlide *d*	C-8 C-12 C-3	$-CH_2CH_3$ $-CH_3$ $-CH=CH_2$	R_1 R_2 $-CH(OH)CH_3$	BchQ BchR BchF or BchV	BChlide *d*	Chew *et al.* 2007, Frigaard *et al.* 2003, Teramura *et al.* 2016b
BChlide *d*	C-7	$-CH_3$	$-CHO$	BciD	BChlide *f*	Thweatt *et al.* 2017
BChlide *d*	C-20	$-H$	$-CH_3$	BchU	BChlide *c*	Maresca *et al.* 2004, Harada *et al.* 2005
BChlide *c*	C-7	$-CH_3$	$-CHO$	BciD	BChlide *e*	Thweatt *et al.* 2017

Chlorophyll synthase	Substrate	Product	References
ChlG	Chlide *a*, *b* + phytyl-pyrophosphate	Chl *a*, *b*	Chen 2014
BchG	BChlide *a*, *b* + phytyl-pyrophosphate	BChl *a*, *b*	Garcia-Gil *et al.* 2003
BchG	BChlide *g* + farnesyl-pyrophosphate	BChl *g*	Chew and Bryant 2007
BchK	BChlide *c*, *d*, *e*, *f* + farnesyl-pyrophosphate	BChl *c*, *d*, *e*, *f*	Frigaard *et al.* 2002

oxygenase (CAO) (Tanaka *et al.* 1998, Tanaka and Tanaka 2011, Tsuchiya *et al.* 2012, Li and Bridwell-Rabb 2019). Chlide *a* and *b* are esterified with phytyl-pyrophosphate by ChlG (Chl specific synthase) to form Chl *a*, *b* (Chen 2014). Experiments have validated that Chl *a* is the biosynthetic precursor of Chl *d*, and that the formyl group at C-3 of Chl *d* is formed *via* an oxidative reaction (Schliep *et al.* 2010, Chen 2014). However, the related enzymes that convert Chl *a* to Chl *d* are yet to be discovered.

Biosynthesis of Chl *c*: Based on molecular structure similarities, Bogorad (1976) first suggested that PChlide and DV-PChlide may be the biosynthetic precursors of Chl c_1 and Chl c_2, respectively. Beale first proposed the biosynthetic relationship between DV-PChlide, PChlide, Chl c_1, and Chl c_2 (Beale 1984). Porra (1997) suggested that Chl c_3 is synthesized from Chl c_2. The formation of the 7-methylcarboxylate group of Chl c_3 involves 7-formyl group formation by an oxygenase, as in Chl *b* biosynthesis. Unlike Chl *a* and Chl *b*, the enzymic mechanisms involved in the biosynthesis of the Chl *c* group are still poorly understood until now.

Biosynthesis of Chl *f*: The molecular structure of Chl *f* has been confirmed to be 2-formylated Chl *a* (Willows *et al.* 2013). Experiments show that the synthesis of Chl *f* is light-dependent, which implies that Chl *f* synthase (ChlF) is the photooxidoreductase that oxidizes Chl *a* (or Chlide *a*) to form Chl *f* (or Chlide *f*) (Ho *et al.* 2016, Li and Bridwell-Rabb 2019). The oxygen atom in the C-2-formyl group on Chl *f* originates from molecular oxygen instead of water (Garg *et al.* 2017).

Biosynthesis of BChl *a*: The first step of synthesis of BChl *a* from Chlide *a* is the reduction of the double bond between the C-7 and C-8 carbons of Chlide *a* by COR to produce 3-vinyl-bacteriochlorophyllide *a* (3V-BChlide *a*) (Nomata *et al.* 2006). COR is composed of three subunits: BchX, BchY, and BchZ. BchX is assumed to be the electron-donating component. BchY and BchZ would play a role as the catalytic, substrate-binding proteins (Nomata *et al.* 2006, Tsukatani *et al.* 2013a, 2015). The C-3 vinyl group of 3V-BChlide *a* is then hydrated by BchF enzyme to introduce a C-3^1-hydroxyl group (Porra *et al.* 1996). This hydration reaction is followed by dehydrogenation on the C-3 side chain by the BchC enzyme to form the C-3-acetyl group, and the product is BChlide *a* (Bollivar *et al.* 1994, Lange *et al.* 2015). The final step in BChl *a* biosynthesis is the addition of a phytyl 'tail' to BChlide *a* by BchG, a BChl *a*-specific synthase (Addlesee *et al.* 2000, Garcia-Gil *et al.* 2003).

Biosynthesis of BChl *b* and BChl *g*: BChl *b* and *g* have an ethylidene group at the C-8 position. The COR enzymes (BvCOR or HmCOR) simultaneously catalyze the C-8-vinyl and C7=C8 double bond of 8V-Chlide *a* to form C-8-ethylidene ($CH_3CH=$), producing BChlide *g* (Tsukatani *et al.* 2013a,b). Similar to the synthesis of BChlide *a* from 3V-BChlide *a*, the C-3 vinyl group of BChlide *g* can be catalyzed by the BchF enzyme and BchC

enzyme to form a C-3-acetyl group through hydration and dehydrogenation, producing BChlide *b* (Bollivar *et al.* 1994, Suzuki *et al.* 1997). Based on genome information, BchF and BchC gene are also present in BChl *b*-producing bacteria. The ethylidene formation, therefore, has been thought to occur on BChlide *a* to form BChlide *b* (Tsukatani *et al.* 2013a). Finally, BChlide *b* and BChlide *g*, respectively, add a phytol and a farnesyl 'tail' by the synthase BchG to form Bchl *b* and Bchl *g* (Chew and Bryant 2007).

Biosynthesis of BChl *c*, *d*, *e*, and *f*: In the biosynthesis of BChl *c*, *d*, *e*, and *f*, the C13^2-methoxycarbonyl group is removed by the BciC enzyme first. After the C-13^2-methoxycarbonyl group of Chlide *a* is removed by the BciC enzyme, 3-vinyl-bacteriochlorophyllide *d* (3V-BChlide *d*) is formed (Liu and Bryant 2011, Teramura *et al.* 2016a). Then, the BchF or BchV enzyme hydrates the C-3 vinyl group to introduce the C-3^1-hydroxyl group to form BChlide *d* (Harada *et al.* 2015, Teramura *et al.* 2016b). BChlide *d* is the biosynthetic precursor of BChlide *c* and *e*.

The naturally occurring BChlide *c*, *d*, and *e* have multiple homologs that carry different side chains at the C-8 and C-12 positions. During the synthesis of BChlide *d* by 3V-BChlide *d*, these different side chains arise from methylation using S-adenosylmethionine (SAM) as the methyl donor (Huster and Smith 1990). Two types of chlorophyll methyltransferases have been found in bacteria, BchQ and BchR, which add the methyl groups to the C-8^2 ethyl carbon and C-12^1 methyl carbon, respectively. Up to three methyl groups can be added at C-8^2, and only one methyl group can be added at C-12^1. Hence, the C-8 side chain can be ethyl, propyl, isobutyl or neopentyl, and the C-12 side chain can be methyl or ethyl (Glaeser *et al.* 2002, Frigaard *et al.* 2003, 2006). Methylation of the C-8 and C-12 side chains does not affect the absorbance properties of BChl *c* (Frigaard *et al.* 2003, Chew *et al.* 2007).

BChlide *d* can be converted to BChlide *c* by transferring a methyl to the C-20 methine bridge position of BChlide *d*. The enzyme that catalyzes this step is BchU methyltransferase (Maresca *et al.* 2004, Harada *et al.* 2005). The methyl group at the C-7 position of BChlide *c* is oxidized by a radical SAM enzyme, BciD, to form the formyl group by which to form BChlide *e*. BChlide *d* can be converted to BChlide *f* through the same reaction by the BciD enzyme (Thweatt *et al.* 2017). The last specific synthase in the biosynthesis of BChl *c*, *d*, *e*, and *f* is BchK, which adds the farnesyl 'tail' to BChlide *c*, *d*, *e*, and *f* (Frigaard *et al.* 2002). In addition to the phytol 'tail' and farnesol 'tail', there are other esterifying alcohols in certain BChls, such as geranylgeranyl, dihydrogeranylgeranyl, and tetrahydrogeranylgeranyl (Frigaard *et al.* 2002, Glaeser *et al.* 2002, Tsukatani *et al.* 2015).

Conclusion

More than one hundred species of chlorophylls are known in photosynthetic organisms today. The structures of the

'head' of all Chls and BChls are very similar, and there is no significant characteristic difference between the two types of chlorophylls, indicating that the 'head' is necessary for photosynthesis. Most of Chls and BChls involved in light harvesting carry an ethyl group at the C-8 position of the chlorophyll molecule (Canniffe *et al.* 2014). Different photosynthetic organisms change the light absorption spectrum by changing the single and double bonds or the side chain on the 'head', generating functional diversity for solar energy capture and conversion in phototrophs (Chew and Bryant 2007). With the exception of most *c*-type Chls, other chlorophylls have a phytol or farnesol 'tail', but the effect of 'tail' length on the properties of chlorophylls remains unclear. The stereochemistry structure of chlorophyll is helpful for understanding the function and properties of chlorophyll (Tamiaki *et al.* 2016, Harada *et al.* 2015, Teramura *et al.* 2016b), which is not covered in this paper. We mainly summarized the planar structure differences between the members of chlorophyll family (Figs. 1–5).

This paper also clarifies the synthesis pathways of various chlorophylls and their interrelationship (Fig. 6). DV-PChlide *a* is the common biosynthetic precursor of all chlorophylls, and is divided into two synthetic pathways from this substrate. DV-PChlide *a* is converted to 8V-Chlide *a* and Chlide *a* by different reduction reactions. BCh *b* and *g* are synthesized from 8V-Chlide *a*, and the other chlorophylls are synthesized from Chlide *a*. Only the synthetic pathway of Chls *c* is unclear. According to their structures, Chls *c* may also be derived from DV-PChlide *a* or PChlide *a*. Most of the steps in chlorophyll synthesis have been clarified. The enzymes involved in chlorophyll synthesis, substrates, products, catalytic sites and groups are detailed in Table 1. More chlorophylls are expected to be discovered in the future, and the biochemical pathways of chlorophyll synthesis will be fully understood. The new properties and functions of chlorophylls will be gradually revealed, helping us further understand the photosynthetic characteristics of phototrophs. This paper comprehensively reviews the biochemical processes of chlorophylls, which helps readers quickly understand the research progress of chlorophylls.

References

Addlesee H.A., Fiedor L., Hunter C.N.: Physical mapping of *bchG*, *orf427*, and *orf177* in the photosynthesis gene cluster of *Rhodobacter sphaeroides*: Functional assignment of the bacteriochlorophyll synthetase gene. – J. Bacteriol. **182**: 3175-3182, 2000.

Allakhverdiev S.I., Kreslavski V.D., Zharmukhamedov S.K. *et al.*: Chlorophylls *d* and *f* and their role in primary photosynthetic processes of cyanobacteria. – Biochemistry-Moscow+ **81**: 201-212, 2016.

Allen M.B.: Distribution of chlorophylls. – In: Vernon L.P., Seely G.R. (ed.): The Chlorophylls. Pp.511-519. Academic Press, New York 1966.

Beale S.I.: Biosynthesis of photosynthetic pigments. – In: Baker N.R., Barber J. (ed.): Chloroplast Biogenesis. Pp. 135-205. Elsevier, Amsterdam 1984.

Beale S.I.: Green genes gleaned. – Trends Plant Sci. **10**: 309-312, 2005.

Beale S.I.: Biosynthesis of 5-aminolevulinic acid. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.): Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 147-158. Springer, Dordrecht 2006.

Bennett M.S., Triemer R.E.: Chloroplast genome evolution in the *Euglenaceae*. – J. Eukaryot. Microbiol. **62**: 773-785, 2015.

Björkman O.: Responses to different quantum flux densities. – In: Lange O.L., Nobel P.S., Osmond C.B., Ziegler H. (ed.): Physiological Plant Ecology I. Responses to the Physical Environment. Pp. 57-107. Springer-Verlag, New York 1981.

Björn L.O., Papageorgiou G.C., Blankenship R.E., Govindjee: A viewpoint: Why chlorophyll *a*? – Photosynth. Res. **99**: 85-98, 2009.

Bogorad L.: Chlorophyll biosynthesis. – In: Goodwin T.W. (ed.): Chemistry and Biochemistry of Plant Pigments. Pp. 64-148. Academic Press Inc., London 1976.

Bollivar D.W., Suzuki J.Y., Beatty J.T., Dobrowolski J.M., Bauer C.E.: Directed mutational analysis of bacteriochlorophyll *a* biosynthesis in *Rhodobacter capsulatus*. – J. Mol. Biol. **237**: 622-640, 1994.

Bollivar D.W.: Recent advances in chlorophyll biosynthesis. – Photosynth. Res. **90**: 173-194, 2006.

Brestič M., Živčák M., Kunderlíková K., Allakhverdiev S.I.: High temperature specifically affects the photoprotective responses of chlorophyll *b* deficient wheat mutant lines. – Photosyn. Res. **130**: 251-266, 2016.

Canniffe D.P., Chidgey J.W., Hunter C.N.: Elucidation of the preferred routes of C8-vinyl reduction in chlorophyll and bacteriochlorophyll biosynthesis. – Biochem. J. **462**: 433-440, 2014.

Chen M., Blankenship R.E.: Expanding the solar spectrum used by photosynthesis. – Trends Plant Sci. **16**: 427-431, 2011.

Chen M., Schliep M., Willows R.D. *et al.*: A red-shifted chlorophyll. – Science **329**: 1318-1319, 2010.

Chen M.: Chlorophyll modifications and their spectral extension in oxygenic photosynthesis. – Annu. Rev. Biochem. **83**: 317-340, 2014.

Chew A.G.M., Bryant D.A.: Chlorophyll biosynthesis in bacteria: The origins of structural and functional diversity. – Annu. Rev. Microbiol. **61**: 113-129, 2007.

Chew A.G.M., Frigaard N.U., Bryant D.A.: Bacteriochlorophyllide *c* C-8[2] and C-12[1] methyltransferases are essential for adaptation to low light in *Chlorobaculum tepidum*. – J. Bacteriol. **189**: 6176-6184, 2007.

Croce R., van Amerongen H.: Natural strategies for photosynthetic light harvesting. – Nat. Chem. Biol. **10**: 492-501, 2014.

Eisen J.A., Nelson K.E., Paulsen I.T. *et al.*: The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. – P. Natl. Acad. Sci. USA **99**: 9509-9514, 2002.

Esteban R., Barrutia O., Artetxe U. *et al.*: Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. – New Phytol. **206**: 268-280, 2015.

Fawley M.W.: A new form of chlorophyll *c* involved in light-harvesting. – Plant Physiol. **91**: 727-732, 1989.

Fookes C.J.R., Jeffrey S.W.: The structure of chlorophyll *c₃*, a novel marine photosynthetic pigment. – J. Chem. Soc. Chem. Comm. **23**: 1827-1828, 1989.

Frigaard N.U., Chew A.G.M., Li H. *et al.*: *Chlorobium tepidum*: Insights into the structure, physiology, and metabolism of a green sulfur bacterium derived from the complete genome sequence. – Photosynth. Res. **78**: 93-117, 2003.

Frigaard N.U., Chew A.G.M., Maresca J.A., Bryant D.A.: Bacteriochlorophyll biosynthesis in green bacteria. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.):

Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 201-221. Springer, Dordrecht 2006.

Frigaard N.U., Voigt G.D., Bryant D.A.: *Chlorobium tepidum* mutant lacking bacteriochlorophyll *c* made by inactivation of the *bchK* gene, encoding bacteriochlorophyll *c* synthase. – J. Bacteriol. **184**: 3368-3376, 2002.

Gan F., Zhang S., Rockwell N.C. *et al.*: Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light. – Science **345**: 1312-1317, 2014.

Garcia-Gil L.J., Gich F.B., Fuentes-Garcia X.: A comparative study of *bchG* from green photosynthetic bacteria. – Arch. Microbiol. **179**: 108-115, 2003.

Garg H., Loughlin P.C., Willows R.D., Chen M.: The C2^1-formyl group in chlorophyll *f* originates from molecular oxygen. – J. Biol. Chem. **292**: 19279-19289, 2017.

Glaeser J., Bañeras L., Rütters H., Overmann J.: Novel bacteriochlorophyll *e* structures and species-specific variability of pigment composition in green sulfur bacteria. – Arch. Microbiol. **177**: 475-485, 2002.

Gloe A., Pfennig N., Brockmann Jr. H., Trowitzsch W.: A new bacteriochlorophyll from brown-colored *Chlorobiaceae*. – Arch. Microbiol. **102**: 103-109, 1975.

Granick S.: The structural and functional relationships between heme and chlorophyll. – Harvey Lect. **44**: 220-245, 1948.

Green B., Anderson J., Parson W.W.: Photosynthetic membranes and their light-harvesting antennas. – In: Green B., Parson W. (ed.): Light-Harvesting Antennas in Photosynthesis. Pp. 1-28. Kluwer Academic Publishers, Dordrecht 2003.

Harada J., Mizoguchi T., Tsukatani Y. *et al.*: Chlorophyllide *a* oxidoreductase works as one of the divinyl reductases specifically involved in bacteriochlorophyll *a* biosynthesis. – J. Biol. Chem. **289**: 12716-12726, 2014.

Harada J., Saga Y., Yaeda Y. *et al.*: In vitro activity of C-20 methyltransferase, BchU, involved in bacteriochlorophyll *c* biosynthetic pathway in green sulfur bacteria. – FEBS Lett. **579**: 1983-1987, 2005.

Harada J., Teramura M., Mizoguchi T. *et al.*: Stereochemical conversion of C3-vinyl group to 1-hydroxyethyl group in bacteriochlorophyll *c* by the hydratases BchF and BchV: adaptation of green sulfur bacteria to limited-light environments. – Mol. Microbiol. **98**: 1184-1198, 2015.

Hastings G., Durrant J.R., Barber J. *et al.*: Observation of pheophytin reduction in photosystem two reaction centers using femtosecond transient absorption spectroscopy. – Biochemistry **31**: 7638-7647, 1992.

Helfrich M., Bommer B., Oster U. *et al.*: Chlorophylls of the *c* family: Absolute configuration and inhibition of NADPH:protochlorophyllide oxidoreductase. – BBA-Bioenergetics **1605**: 97-103, 2003.

Heyes D.J., Heathcote P., Rigby S.E.J. *et al.*: The first catalytic step of the light-driven enzyme protochlorophyllide oxidoreductase proceeds *via* a charge transfer complex. – J. Biol. Chem. **281**: 26847-26853, 2006.

Ho M.Y., Shen G., Canniffe D.P. *et al.*: Light-dependent chlorophyll *f* synthase is a highly divergent paralog of PsbA of photosystem II. – Science **353**: aaf9178, 2016.

Huster M.S., Smith K.M.: Biosynthetic studies of substituent homologation in bacteriochlorophylls *c* and *d*. – Biochemistry **29**: 4348-4355, 1990.

Ishikita H., Saenger W., Biesiadka J. *et al.*: How photosynthetic reaction centers control oxidation power in chlorophyll pairs P680, P700, and P870. – P. Natl. Acad. Sci. USA **103**: 9855-9860, 2006.

Klimov V.V.: Discovery of pheophytin function in the photosynthetic energy conversion as the primary electron acceptor

of Photosystem II. – Photosynth. Res. **76**: 247-253, 2003.

Kobayashi M., Akiyama M., Kise H., Watanabe T.: Unusual tetrapyrrole pigments of photosynthetic antenna and reaction centers: specially-tailored chlorophylls. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.): Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 56-63. Springer, Dordrecht 2006.

Kühl M., Chen M., Larkum A.W.D.: Algae and cyanobacteria in extreme environments. – In: Seckbach J. (ed.): Cellular Origin, Life in Extreme Habitats and Astrobiology. Pp. 101-123. Springer, Dordrecht 2007.

Lange C., Kiesel S., Peters S. *et al.*: Broadened substrate specificity of 3-hydroxyethyl bacteriochlorophyllide *a* dehydrogenase (BchC) indicates a new route for the biosynthesis of bacteriochlorophyll *a*. – J. Biol. Chem. **290**: 19697-19709, 2015.

Larkum A.W.D., Kühl M.: Chlorophyll *d*: the puzzle resolved. – Trends Plant Sci. **10**: 355-357, 2005.

Larkum A.W.D., Ritchie R.J., Raven J.A.: Living off the Sun: chlorophylls, bacteriochlorophylls and rhodopsins. – Photosynthetica **56**: 11-43, 2018.

Li B., Bridwell-Rabb J.: Aerobic enzymes and their radical SAM enzyme counterparts in tetrapyrrole pathways. – Biochemistry **58**: 85-93, 2019.

Liu Z.F., Bryant D.A.: Identification of a gene essential for the first committed step in the biosynthesis of bacteriochlorophyll *c*. – J. Biol. Chem. **286**: 22393-22402, 2011.

Manning W.M., Strain H.H.: Chlorophyll *d*, a green pigment of red algae. – J. Biol. Chem. **151**: 1-19, 1943.

Maresca J.A., Chew A.G.M., Ponsatí M.R. *et al.*: The *bchU* gene of *Chlorobium tepidum* encodes the bacteriochlorophyll C-20 methyltransferase. – J. Bacteriol. **186**: 2558-2566, 2004.

Melkozernov A.N., Blankenship R.E.: Photosynthetic functions of chlorophylls. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.): Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 397-410. Springer, Dordrecht 2006.

Miyashita H., Adachi K., Kurano N. *et al.*: Pigment composition of a novel oxygenic photosynthetic prokaryote containing chlorophyll *d* as the major chlorophyll. – Plant Cell Physiol. **38**: 274-281, 1997.

Moss G.P.: Nomenclature of tetrapyrroles. IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Recommendations 1986. – Eur. J. Biochem. **178**: 277-328, 1988.

Niedzwiedzki D.M., Blankenship R.E.: Singlet and triplet excited state properties of natural chlorophylls and bacteriochlorophylls. – Photosynth. Res. **106**: 227-238, 2010.

Nomata J., Mizoguchi T., Tamiaki H., Fujita Y.: A second nitrogenase-like enzyme for bacteriochlorophyll biosynthesis: reconstitution of chlorophyllide *a* reductase with purified X-protein (BchX) and YZ-protein (BchY-BchZ) from *Rhodobacter capsulatus*. – J. Biol. Chem. **281**: 15021-15028, 2006.

Oh J.I., Eraso J.M., Kaplan S.: Interacting regulatory circuits involved in orderly control of photosynthesis gene expression in *Rhodobacter sphaeroides* 2.4.1. – J. Bacteriol. **182**: 3081-3087, 2000.

Pinta V., Picaud M., Reiss-Husson F., Astier C.: *Rubrivivax gelatinosus acsF* (previously *orf358*) codes for a conserved, putative binuclear-iron-cluster-containing protein involved in aerobic oxidative cyclization of Mg-protoporphyrin IX monomethylester. – J. Bacteriol. **184**: 746-753, 2002.

Porra R.J., Schäfer W., Gad'on N. *et al.*: Origin of the two carbonyl oxygens of bacteriochlorophyll *a*: Demonstration of two different pathways for the formation of ring E in *Rhodobacter*

sphaeroides and *Roseobacter denitrificans*, and a common hydratase mechanism for 3-acetyl group formation. – Eur. J. Biochem. **239**: 85-92, 1996.

Porra R.J.: Recent progress in porphyrin and chlorophyll biosynthesis. – Photochem. Photobiol. **65**: 492-516, 1997.

Raven P.H., Evert R.F., Eichhorn S.E.: Photosynthesis, light, and life. – In: Biology of Plants (7th Edition). Pp. 119-127. W.H. Freeman and Company Publishers, New York 2005.

Reid J.D., Hunter C.N.: Magnesium-dependent ATPase activity and cooperativity of magnesium chelatase from *Synechocystis* sp. PCC6803. – J. Biol. Chem. **279**: 26893-26899, 2004.

Saunders A.H., Golbeck J.H., Bryant D.A.: Characterization of BciB: A ferredoxin-dependent 8-vinyl-protochlorophyllide reductase from the green sulfur bacterium *Chloroherpeton thalassium*. – Biochemistry **52**: 8442-8451, 2013.

Scheer H.: An overview of chlorophylls and bacteriochlorophylls: Biochemistry, biophysics, functions and applications. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.): Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 1-19. Springer, Dordrecht 2006.

Schliep M., Crossett B., Willows R.D., Chen M.: ^{18}O labeling of chlorophyll *d* in *Acaryochloris marina* reveals that chlorophyll *a* and molecular oxygen are precursors. – J. Biol. Chem. **285**: 28450-28456, 2010.

Suzuki J.Y., Bauer C.E.: A prokaryotic origin for light-dependent chlorophyll biosynthesis of plants. – P. Natl. Acad. Sci. USA **92**: 3749-3753, 1995.

Suzuki J.Y., Bollivar D.W., Bauer C.E.: Genetic analysis of chlorophyll biosynthesis. – Annu. Rev. Genet. **31**: 61-89, 1997.

Tamiaki H., Komada J., Kunieda M. *et al.*: *In vitro* synthesis and characterization of bacteriochlorophyll-*f* and its absence in bacteriochlorophyll-*e* producing organisms. – Photosynth. Res. **107**: 133-138, 2011.

Tamiaki H., Teramura M., Tsukatani Y.: Reduction processes in biosynthesis of chlorophyll molecules: chemical implication of enzymatically regio- and stereoselective hydrogenations in the late stages of their biosynthetic pathway. – B. Chem. Soc. Jpn. **89**: 161-173, 2016.

Tanaka A., Ito H., Tanaka R. *et al.*: Chlorophyll *a* oxygenase (CAO) is involved in chlorophyll *b* formation from chlorophyll *a*. – P. Natl. Acad. Sci. USA **95**: 12719-12723, 1998.

Tanaka R., Tanaka A.: Chlorophyll cycle regulates the construction and destruction of the light-harvesting complexes. – BBA-Bioenergetics **1807**: 968-976, 2011.

Taniguchi M., Lindsey J.S.: Synthetic chlorins, possible surrogates for chlorophylls, prepared by derivatization of porphyrins. – Chem. Rev. **117**: 344-535, 2017.

Teramura M., Harada J., Mizoguchi T. *et al.*: *In vitro* assays of BciC showing C13^2-demethoxycarbonylase activity requisite for biosynthesis of chlorosomal chlorophyll pigments. – Plant Cell Physiol. **57**: 1048-1057, 2016a.

Teramura M., Harada J., Tamiaki H.: *In vitro* stereospecific hydration activities of the 3-vinyl group of chlorophyll

derivatives by BchF and BchV enzymes involved in bacteriochlorophyll *c* biosynthesis of green sulfur bacteria. – Photosynth. Res. **130**: 33-45, 2016b.

Thweatt J.L., Ferlez B.H., Golbeck J.H., Bryant D.A.: BciD is a radical S-adenosyl-l-methionine (SAM) enzyme that completes bacteriochlorophyllide *e* biosynthesis by oxidizing a methyl group into a formyl group at C-7. – J. Biol. Chem. **292**: 1361-1373, 2017.

Tsuchiya T., Mizoguchi T., Akimoto S. *et al.*: Metabolic engineering of the Chl *d*-dominated cyanobacterium *Acaryochloris marina*: Production of a novel Chl species by the introduction of the chlorophyllide *a* oxygenase gene. – Plant Cell Physiol. **53**: 518-527, 2012.

Tsukatani Y., Harada J., Nomata J. *et al.*: *Rhodobacter sphaeroides* mutants overexpressing chlorophyllide *a* oxidoreductase of *Blastochloris viridis* elucidate functions of enzymes in late bacteriochlorophyll biosynthetic pathways. – Sci. Rep. **5**: 9741, 2015.

Tsukatani Y., Yamamoto H., Harada J. *et al.*: An unexpectedly branched biosynthetic pathway for bacteriochlorophyll *b* capable of absorbing near-infrared light. – Sci Rep. **3**: 1217, 2013a.

Tsukatani Y., Yamamoto H., Mizoguchi T. *et al.*: Completion of biosynthetic pathways for bacteriochlorophyll *g* in *Heliobacterium modesticaldum*: The C8-ethylidene group formation. – BBA-Bioenergetics **1827**: 1200-1204, 2013b.

Van Gorkom H.J.: Identification of the reduced primary electron acceptor of Photosystem II as a bound semiquinone anion. – BBA-Bioenergetics **347**: 439-442, 1974.

Vogl K., Tank M., Orf G.S. *et al.*: Bacteriochlorophyll *f*: properties of chlorosomes containing the "forbidden chlorophyll". – Front. Microbiol. **3**: 298, 2012.

Walker C.J., Willows R.D.: Mechanism and regulation of Mg-chelatase. – Biochem. J. **327**: 321-333, 1997.

Wang P.R., Gao J.X., Wan C.M. *et al.*: Divinyl chlorophyll(ide) *a* can be converted to monovinyl chlorophyll(ide) *a* by a divinyl reductase in rice. – Plant Physiol. **153**: 994-1003, 2010.

Willows R.D.: Biosynthesis of chlorophylls from protoporphyrin IX. – Nat. Prod. Rep. **20**: 327-341, 2003.

Willows R.D., Li Y., Scheer H., Chen M.: Structure of chlorophyll *f*. – Org. Lett. **15**: 1588-1590, 2013.

Xu M., Kinoshita Y., Matsubara S., Tamiaki H.: Synthesis of chlorophyll-*c* derivatives by modifying natural chlorophyll-*a*. – Photosynth. Res. **127**: 335-345, 2016.

Yamazaki S., Nomata J., Fujita Y.: Differential operation of dual protochlorophyllide reductases for chlorophyll biosynthesis in response to environmental oxygen levels in the cyanobacterium *Leptolyngbya boryana*. – Plant Physiol. **142**: 911-922, 2006.

Zapata M., Garrido J.L., Jeffrey S.W.: Chlorophyll *c* pigments: Current status. – In: Grimm B., Porra R.J., Rüdiger W., Scheer H. (ed.): Chlorophylls and Bacteriochlorophylls. Advances in Photosynthesis and Respiration. Pp. 40-50. Springer, Dordrecht 2006.