Chlorophyll Modifications and Their Spectral Extension in Oxygenic Photosynthesis

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redshifted chlorophylls, chlorophyll biosynthesis, formyl group substitution, spectral extension, evolution of chlorophylls, light and oxygen

Abstract

Chlorophylls are magnesium-tetrapyrrole molecules that play essential roles in photosynthesis. All chlorophylls have similar five-membered ring structures, with variations in the side chains and/or reduction states. Formyl group substitutions on the side chains of chlorophyll *a* result in the different absorption properties of chlorophyll *b*, chlorophyll *d*, and chlorophyll *f*. These formyl substitution derivatives exhibit different spectral shifts according to the formyl substitution position. Not only does the presence of various types of chlorophylls allow the photosynthetic organism to harvest sunlight at different wavelengths to enhance light energy input, but the pigment composition of oxygenic photosynthetic organisms also reflects the spectral properties on the surface of the Earth. Two major environmental influencing factors are light and oxygen levels, which may play central roles in the regulatory pathways leading to the different chlorophylls. I review the biochemical processes of chlorophyll biosynthesis and their regulatory mechanisms.

Contents

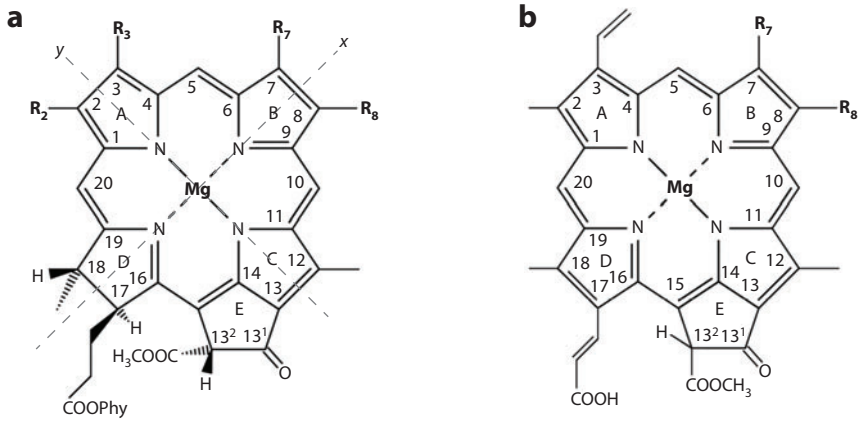
OVERVIEW OF THE STRUCTURE AND SPECTRAL PROPERTIES OF CHLOROPHYLLS

Chlorophylls are magnesium (Mg)-tetrapyrrole molecules that play essential roles in photosynthesis. Their functions include harvesting light energy, transferring excitation energy to reaction centers, and driving charge separation reactions in reaction centers. These molecules contain similar five-membered ring structures, with variations in the side chains and/or reduction states (**Figure 1**). Such alterations in the rings or side chains in various types of chlorophylls result in different absorption properties (**Figure 2*a***). The presence of various types of chlorophylls allows photosynthetic organisms to harvest sunlight at different wavelengths (**Figure 2*b***) and to enhance the input light energy. The pigment composition of oxygenic photosynthetic organisms reflects the spectral properties on the surface of the Earth (1).

The first chlorophyll molecule was isolated and reported by French chemists in 1817. Five chlorophylls from natural photosynthetic organisms have been characterized to date: chlorophyll *a* (Chl *a*, or 8-vinyl Chl *a*), Chl *b* (8-vinyl Chl *b*), Chl *c*, Chl *d*, and Chl *f*. Chl *a*, Chl *b*, and Chl *c* were identified in the nineteenth century, and Chl *d* was reported in 1943, more than 70 years later (5). The fifth chlorophyll, Chl *f*—the most redshifted—was isolated from stromatolites and identified in 2010 (6). Chl *g* was proposed to be a 7-formyl-Chl d_{p672}, an artificial chlorophyll species generated by a molecular-engineered *Prochlorothrix hollandica* chlorophyllide *a* oxygenase (*phCAO*) *Acaryochloris* mutant line (7).

Chl *a* is the most abundant of all. It is present in the reaction center and light-harvesting complexes (LHCs) of almost all oxygenic photosynthetic organisms, including higher plants, algae, and cyanobacteria (8). Chl *b* is the second-most-abundant chlorophyll in oxygenic photosynthetic organisms. It functions only as the accessory chlorophyll in the light-harvesting system but cannot act as the so-called special pair in the reaction center (9). To date, Chl *c* has not been found in plants, but it is widely distributed in algae such as diatoms (10). A Chl *c*–like pigment, 8-vinyl protochlorophyllide (MgDVP), has been found in some cyanobacteria, where it acts as a light-harvesting pigment (11). Chl *c* has fully unsaturated porphyrin (**Figure 1*b***) that is different from that in other chlorophylls; this topic is beyond the scope of this review. Zapata et al. (12) provide a full review of Chl *c* and its function.

The discovery of Chl *d* in *Acaryochloris marina* (13) confounded the traditional thinking



Chlorin type

Chlorophylls	R_2	R_3	R_7	R_8	Q_y (nm)
Chl *a*	CH_3	$CH{=}CH_2$	CH_3	$CH_2{-}CH_3$	665*
8-vinyl Chl *a* (Chl a_2)	CH_3	$CH{=}CH_2$	CH_3	$CH{=}CH_2$	666#
Chl *b*	CH_3	$CH{=}CH_2$	CHO	$CH_2{-}CH_3$	652*
8-vinyl Chl *b* (Chl b_2)	CH_3	$CH{=}CH_2$	CHO	$CH{=}CH_2$	658#
Chl *d*	CH_3	CHO	CH_3	$CH_2{-}CH_3$	696*
Chl *f*	CHO	$CH{=}CH_2$	CH_3	$CH_2{-}CH_3$	707*

Porphyrin type

Chl *c*	R_7	R_8
Chl *c*1	CH_3	$CH_2{-}CH_3$
Chl *c*2	CH_3	$CH{=}CH_2$
Chl *c*3	$COOCH_3$	$CH{=}CH_2$

Figure 1

Chemical structure of chlorophylls (Chls). (*a*) Chemical structure of Chls, which have chlorin-type macrocycles. (*b*) Chemical structure of the Chl *c* family, whose members are of the porphyrin type. The numbering system accords with IUPAC/IUBMB (International Union of Pure and Applied Chemistry/International Union of Biochemistry and Molecular Biology). Chlorin-type Chls are esterified with the phytyl chain (Phy) of $C_{20}H_{39}$. The *y* axis is closest to the C3 and C13 positions (2). Chl *b*, Chl *d*, and Chl *f* have formyl group substitutions at the C7, C3, and C2 positions, respectively. The structure differences are colored red. Asterisks mean that the Q_y (nm) of Chl *a*, Chl *b*, Chl *d*, and Chl *f* was recorded in 100% methanol (3). Pound signs mean that the Q_y (nm) of 8-vinyl Chl *a* and 8-vinyl Chl *b* was recorded in a mixture of methanol and acetone (4).

about the essential functions of Chl *a* in photosynthetic organisms. Chl *d* is the only chlorophyll found to date that can replace all functions of Chl *a* involving light harvesting (14–17) and charge separation in reaction centers (18–20). Chl *d*–containing cyanobacteria are widely distributed in IR-enriched ecological niches (21–25). A Chl *f*–containing cyanobacterium was recently purified and named *Halomicronema hongdechloris*, according to 16S ribosomal DNA–based phylogenetic analysis and morphological properties (26). Chl *f* is a minor chlorophyll and Chl *a* the major chlorophyll in *H. hongdechloris*. A freshwater cyanobacterium (strain KC1) containing Chl *f* was isolated from Lake Biwa, Japan. This organism is a unicellular cyanobacterium and is not closely related to *H. hongdechloris*, according to 16S ribosomal DNA sequence classification (27). The functions of Chl *f* are still unknown, but this chlorophyll is responsive to light (26, 27). Two other chlorophyll derivatives, 8-vinyl Chl *a* and 8-vinyl Chl *b*, are the major pigments in the marine picoplankton genus *Prochlorococcus* (28), the smallest known cyanobacterium, with a tiny cell diameter of 0.5 to 0.7 μm. *Prochlorococcus* cells are the dominant phytoplankton communities in most tropical and temperate open-ocean ecosystems (29), where nutrient concentrations and biomass are relatively low (30, 31).

All chlorophylls exhibit two major absorption bands (**Figure 2*a***), leaving a considerably wide gap in the absorption spectrum that is known as the green window. The green window gives chlorophylls their well-known green color. Isolated chlorophyll *a* in methanol has

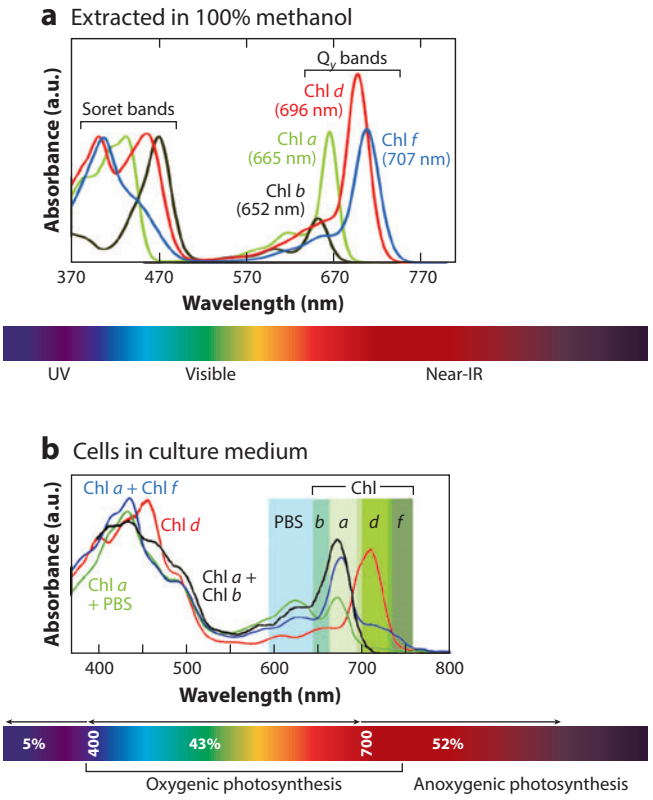


Figure 2

Absorption spectra of chlorin-type chlorophylls (Chl *a*, Chl *b*, Chl *d*, and Chl *f*). Soret-band maxima are arbitrarily scaled to a common height for comparison. (*a*) Absorption spectra of isolated Chls in 100% methanol. (*b*) In vivo absorption spectra of oxygenic photosynthetic bacteria containing different photopigments. The covering absorption regions of different photopigments are labeled. The colored bars represent the absorption regions of different photopigments. Abbreviation: PBS, phycobilisomes.

a maximal red absorption (Q_y) of 665 nm and a blue absorption of 436 nm (the Soret band). However, in phototrophs, the protein environment changes the spectral properties; for instance, Chl *a* has a maximal Q_y absorption of ~680 nm in photosystem II (PSII) and a maximal Q_y absorption of ~700 nm in PSI.

Chl *a* is used as a reference compound in structural analyses of other chlorophylls and related photopigments. The basic structure of Chl *a* is defined by total synthesis of the tetrapyrrole moiety (32) and the C-20 phytol (33). It has a 17,18-dihydroporphyrin ring, coordinated to a central Mg atom, that is designated chlorin as opposed to bacteriochlorin (7,8,17,18-tetrahydroporphyrin) (**Figure 1*a***). The derivatives of chlorophyll, excluding the Chl *c* group (**Figure 1*b***), have the same chlorin structure as Chl *a*. Chl *b*, Chl *d*, and Chl *f* are formyl substitution derivatives of Chl *a* (**Figure 1*a***). These derivatives demonstrate different spectral shifts according to the formyl substitution position. The formyl group located at the C7 position in Chl *b* causes a blueshift to a Q_y maximum of 652 nm but, at the C3 position in Chl *d* and at the C2 position in Chl *f*, causes redshifts of Q_y to 696 nm and 707 nm, respectively (**Figure 2**). 8-Vinyl Chl *a* and 8-vinyl Chl *b* are derivatives of Chl *a* and Chl *b*, respectively, with an additional vinyl group at the C8 position, whereas all other chlorophylls have a reduced ethyl group at the C8 position (**Figure 1*a***) (4).

OVERVIEW OF CHLOROPHYLL BIOSYNTHESIS

The biosynthesis pathway of chlorophyll is well established in higher plants. However, some details of its compartmentalization and regulation remain obscure. The newly discovered chlorophylls add additional uncertainty to our understanding of the biosynthesis pathways and their regulation mechanisms.

The tetrapyrrole biosynthesis pathway (e.g., that of chlorophylls, hemes, and cobalamin) is shared between the different tetrapyrroles up to a certain point (steps 1–3 in **Figure 3**). The steps from glutamate (Glu) to protoporphyrin IX (P_{IX}) are common to the biosynthesis pathways of chlorophylls and hemes. The two pathways branch at the point where two different metals are inserted (steps 4 and 5 in **Figure 3**). The Mg branch leads to the end product of chlorophylls, which starts with the insertion of Mg^{2+} into P_{IX}. The iron (Fe) branch produces heme, into which Fe is inserted by Fe chelatase. There are six known steps to synthesis of Chl *a* after the biosynthesis pathway branches away from heme biosynthesis: Mg insertion into P_{IX} (step 4); methylation at the C13 position (step 6); monomethyl ester cyclic oxygenase

forming the fifth ring, ring E (MgDVP) (step 7); 8-vinyl group reduction generating protochlorophyllide (Pchlide) *a* (step 8); the reduction at ring D of Pchlide forming chlorophyllide (Chlide) *a* (step 9); and esterification with a phytol-pyrophosphate chain, resulting in the formation of Chl *a* (step 10) (**Figure 3**). The common ancestry of chlorophyll biosynthesis pathways has been convincingly demonstrated by the presence of extensive sequence similarity among enzymes that catalyze similar steps in the known biosynthesis pathways of Chl *a* (34).

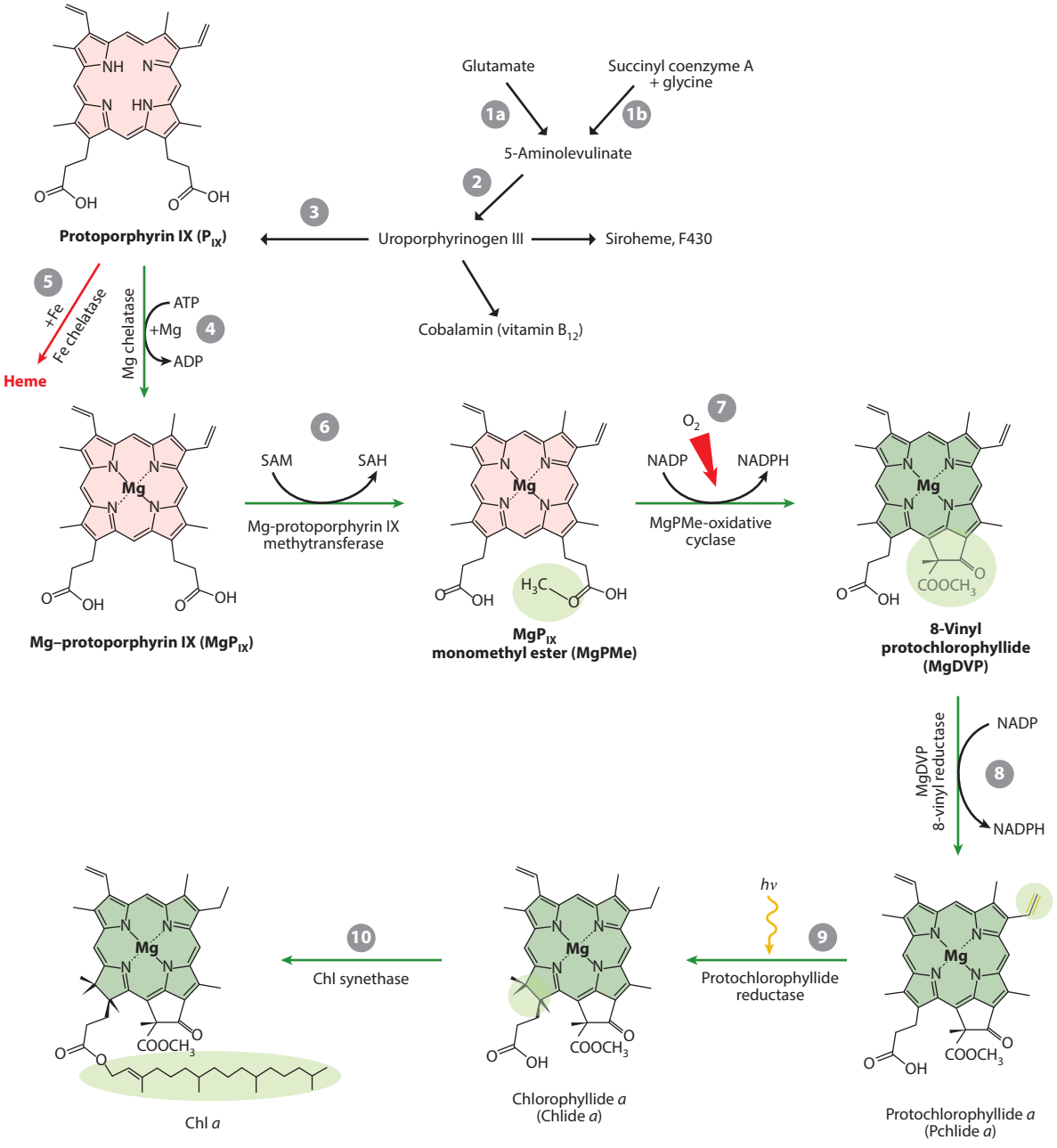
Step 4: Mg Insertion into Protoporphyrin IX

Mg chelatase catalyzes the branch point between chlorophylls and heme synthesis, so it is reasonable to expect that Mg chelatase is a key regulator of chlorophyll biosynthesis (35). The balance of the activity of the branches in the pathway reflects the cellular demand for each end product. Fe chelatase inserts Fe^{2+} into P_{IX} in a reaction that does not require input of energy and is inhibited by the presence of ATP. In contrast, the insertion of Mg^{2+} by Mg chelatase requires the input of a significant amount of energy: the hydrolysis of ~15 ATP molecules per metal insertion (36, 37). Therefore, the ratio of ATP to ADP plays an important role in the allocation of P_{IX} between the heme and chlorophyll biosynthesis branches (38). Mg chelatase belongs to the class of AAA^+-type chelatases and is composed of three subunits: H, I, and D (**Figure 4**). These subunits are conserved from cyanobacteria to higher plants (ChlH, ChlI, and ChlD) and are commonly referred to as BchH, BchI, and BchD in bacteriochlorophyll biosynthesis (39). The ChlH subunit, with a mass of ~140 kDa in higher plants, is the porphyrin-binding subunit of Mg chelatase (40, 41). The ChlI or BchI subunit is a member of the AAA^+ superfamily of ATPase, and it binds Mg^{2+} and ATP. However, the ChlD or BchD subunit appears to be an inactive AAA^+ ATPase, although there is structural similarity between the D and I subunits in their N-terminal halves (42, 43). The extended C-terminal portion of the BchD subunit contains an integrin I domain that plays a central role in transmitting conformational changes of complexes of BchI and BchD in *Rhodobacter capsulatus* (44). At high concentrations of Mg^{2+} and in the presence of ATP, the ChlI subunit forms a ring structure (45, 46). The function of ChlD may be to provide a stable platform for ChlI subunits and form a ChlI/ChlD polymeric complex. ATP binding, but not ATP hydrolysis, is important for activation of the ChlI/ChlD complex (42), and the ChlI/ChlD complex subsequently interacts with the ChlH subunit and drives the ATP-dependent insertion of Mg^{2+} into P_{IX} (**Figure 4**) (47–49).

The three subunits of Mg chelatase are weakly associated with one another, and the three-subunit Mg chelatase complex is relatively unstable. The assembly and disassembly kinetics of Mg chelatase may reflect its multiplicity of regulatory mechanisms. The free Mg^{2+} concentration is an important regulatory element of the activity of Mg chelatase (36, 50). The translocation of the ChlH subunit from the stroma to the chloroplast envelope membrane has been observed in response to an increased concentration of Mg^{2+} (51), which may be the process by which the substrate P_{IX} is transferred to the ChlH subunit (52). In the presence of a high concentration of Mg^{2+}, MgP_{IX} is enclosed in the cagelike ChlH protein and chaperoned to the active site of MgP_{IX} methyltransferase (ChlM) (**Figure 4**), the next reaction in the pathway—the methylation of MgP_{IX} (step 6 in **Figure 3**) (41).

The redox state plays an important function in posttranslational regulation of Mg chelatase in higher plants (53). The ChlI unit functions as an active ATPase and is the target of regulation by thioredoxin (54–56). Thioredoxins are small proteins that mediate the dithiol-disulfide exchange of Cys residues. Silencing the expression of thioredoxins in pea plants gives rise to the oxidized form of ChlI, which loses its ATPase activity, thereby producing an inactive Mg chelatase (56). Higher plants and

cyanobacteria normally possess only one copy of ChlI, but some organisms, such as *Arabidopsis thaliana* (57), have two isoforms of ChlI. ChlI2 in *Arabidopsis* is involved in assembling the oligomeric ChlI ring structure and is necessary for chlorophyll biosynthesis (58). Changes in the accessible oxygen concentration and in the light environment also affect the redox state of Mg chelatase, switching the biosynthesis pathway to heme or to chlorophyll. P_{IX} and MgP_{IX} can be excited by light and will form triplet excited states if they accumulate in vivo. The excited P_{IX} and MgP_{IX} immediately interact with oxygen and yield reactive oxygen



species (ROS), which inactivate Mg chelatase (59) and harm the cells.

The genomes uncoupled 4 (GUN4) protein activates Mg chelatase (60), and its C-terminal residues play an essential role in activating the ChlH subunit (61). In cyanobacteria and higher plants, GUN4 binds to the ChlH subunit and forms a supercomplex, binding with P_{IX} and MgP_{IX} (**Figure 4**), which may also be involved in heme biosynthesis by controlling the flow of substrate into the heme or chlorophyll biosynthesis branch (62). This supercomplex helps deliver porphyrins (P_{IX} and MgP_{IX}) into chlorophyll biosynthesis enzymatic sites and promotes the interaction between ChlH and thylakoid membranes (63–65). The excess MgP_{IX} in the chloroplast binds to GUN4 as part of the posttranslational regulation of tetrapyrrole metabolism because GUN4/porphyrin complexes may become an easy target for degradation (66).

Step 6: Methylation at the C13 Position of Mg–Protoporphyrin IX

S-Adenosyl-L-methionine (SAM)/ChlM is the enzyme that catalyzes the transfer of a methyl group to the 13-propionate side chain of MgP_{IX} and produces a MgP_{IX} monomethyl ester (MgPMe) (step 6 in **Figure 3**). Chlorophyll formation depends entirely on the ChlM protein, and the *chlM*-null mutant of *Arabidopsis* becomes deficient in newly synthesized chlorophyll after a few days. The methyl units come from SAM, a universal methyl donor. Impairing the transport of SAM into chloroplasts or decreasing the methylation index affects the activity of ChlM and plastid biogenesis (67, 68).

Mg chelation is coupled to this methyltransferase reaction in purple photosynthetic bacteria (69). The formation of a tightly bound ChlH/ChlM/P_{IX} complex channels the substrate (P_{IX}) directly to the enzymatic active site and enhances the activity of the methyltransferase (70–72); therefore, ChlM (or BchM) and the interaction between the H subunit of Mg chelatase and ChlM (or BchM) play an important role in the allocation of P_{IX} between the heme and chlorophyll biosynthesis branches. The inactivation of ChlM inhibits the activity of Mg chelatase and stimulates the increased activity of Fe chelatase. However, the enhanced activation of ChlM leads to opposite profiles: increased activity of Mg chelatase and reduced activity of Fe chelatase (73, 74). Chlorophyll is essential for the maturation and correct folding of chlorophyll-binding proteins and their

Figure 3

Chlorophyll *a* (Chl *a*) biosynthesis pathway. Step 1*a*: There are three enzymatic steps for synthesizing aminolevulinate from glutamate, which is the main pathway in plants, cyanobacteria, and Archaea. Step 1*b*: An alternative pathway for synthesizing aminolevulinate is found in α-proteobacteria, animals, and fungi. Step 2: There are three enzymatic steps for synthesizing uroporphyrinogen III from aminolevulinate: porphobilinogen synthase, hydroxymethylbilane synthase, and uroporphyrinogen III synthase. Step 3: Three enzymatic steps lead to the last common intermediate product, protoporphyrin IX (P_{IX}). The three enzymes are uroporphyrinogen decarboxylase, coproporphyrinogen III oxidase, and protoporphyrinogen oxidase. Step 4: Magnesium (Mg) is inserted into P_{IX} by Mg chelatase (composed of H, I, and D subunits). This process requires Mg^{2+} and ATP, giving rise to MgP_{IX}. Step 5: Ferrochelatase leads to the end product of heme. Step 6: Methylation occurs at the C13 position by MgP_{IX} methyltransferase (ChlM), which transfers a methyl group from *S*-adenosyl-L-methionine (SAM) to the carboxyl group of MgP_{IX} and produces MgP_{IX} monomethyl ester (MgPMe). SAH represents *S*-adenosyl-L-homocysteine. Step 7: The formation of the isocyclic ring (ring E) is catalyzed by an oxidative cyclase enzyme (AcsF or BchE). Two mechanisms of cyclization have been reported. In the first, the oxo group oxygen is derived from molecular oxygen by an oxygenase (AcsF). In the second, the oxo group oxygen is derived from water under anaerobic conditions by a hydratase (BchE). Oxygen is one of the important regulatory elements. The red arrow represents oxygen input. Step 8: The 8-vinyl group is reduced to an 8-ethyl group by an 8-vinyl reductase and produces protochlorophyllide *a* (Pchlide *a*). The green ovals represent the changed enzymatic reaction positions. Step 9: The ring D reduction by Pchlide reductases, producing chlorophyllide *a* (Chlide *a*). There are two types of Pchlide reductases: the light-dependent reaction (LPOR) and the light-independent reaction (DPOR). Light is an important regulatory element. Step 10: The last step in the synthesis of Chl *a* is the esterification of Chlide *a* with a phytol chain (Phy), which is catalyzed by Chl synthase (ChlG).

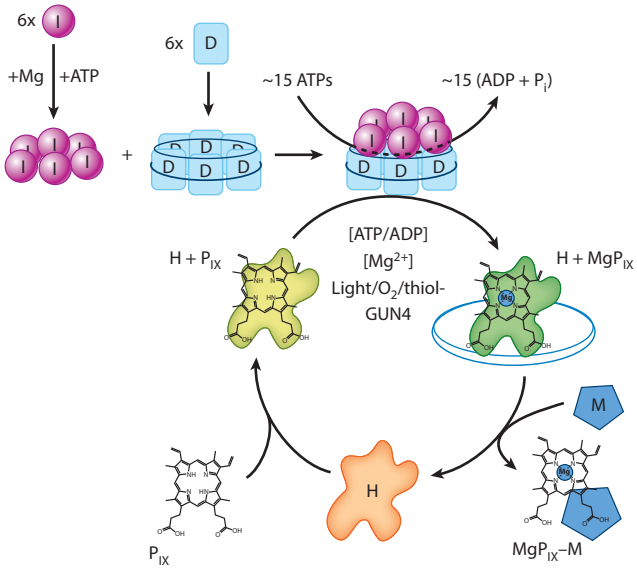


Figure 4

Functional model of magnesium (Mg) chelatase. Mg chelatase is composed of three subunits: H, I, and D. The formation of the I/D complex requires ATP and provides a Mg binding site. The I/D complexes form a polymeric ring and bind with the H subunit. The H subunit provides a binding site for protoporphyrin IX (P_{IX}) and MgP_{IX}. The product of MgP_{IX} is directly delivered to the next step reaction by forming complexes of H/MgP_{IX}/M. No free P_{IX} and MgP_{IX} is found in the system. D (*light blue rectangles*) represents ChlD subunits; H (*irregular shapes*) represents ChlH subunits; I (*purple circles*) represents ChlI subunits; M (*blue pentagons*) represents ChlM (MgP_{IX} methyltransferase) protein. The blue oval ring represents the polymeric I/D complex. Mg chelatase is tightly regulated by many factors, including the ratio of ATP to ADP, the concentration of Mg^{2+}, and environmental elements [light, oxygen, and cell redox states (thiol)]. The genomes uncoupled 4 protein (GUN4) involves Mg chelatase reactions by recruiting P_{IX} or removing the product MgP_{IX} from ChlH.

insertion into thylakoids. The inactivation of ChlM inhibits the assembly of chlorophyll-binding proteins in thylakoids due to the inactivity of chlorophyll biosynthesis (73).

Step 7: Mg–Protoporphyrin IX Monomethyl Ester Cyclase

MgPMe cyclase catalyzes the incorporation of atomic oxygen into MgPMe to form MgDVP (**Figure 3**). This oxidative cyclization creates ring E, the distinctive isocyclic ring of all chlorophylls. This ring structure is the unique characteristic of chlorophylls compared with all other tetrapyrroles. The two unrelated cyclization mechanisms based on the origin of the oxygen atom are the aerobic cyclase (AcsF) and the anaerobic cyclase (BchE) (75, 76). Most oxygenic photosynthetic organisms use aerobic cyclases, which carry out the MgPMe oxidative cyclic reaction in an oxygen-dependent manner. The anaerobic cyclase is a radical SAM enzyme and is widely distributed in anaerobic anoxygenic photosynthetic organisms, such as green sulfur bacteria. The lack of sequence similarities between AcsF and BchE suggests that they arose from different ancestors (75). However, recent genomic sequence analyses reveal that some photosynthetic organisms contain both AcsF and BchE (**Table 1**). For example, two *acsF* genes (*sll1214* and *sll1874*) and three homologs of the *bchE* gene (*slr0905*, *sll1242*, and *slr0309*) exist in the *Synechocystis* PCC6803 genome. The coexistence of aerobic and anaerobic enzymes for one reaction may be a footprint of the evolutionary transition from anaerobic to aerobic metabolism (76). However, the three BchE candidates have no direct effect on chlorophyll biosynthesis (77), whereas two genes encoding AcsF in *Synechocystis* PCC6803 function as cyclase and their activities respond to different oxygen concentration levels (77). One of these genes (*sll1214*) is expressed under normal oxygen concentrations, and the other (*sll1874*) is upregulated under microaerobic conditions (78). The function of the three BchE homologs in *Synechocystis* PCC6803 remains uncertain. Almost all unicellular photosynthetic organisms, including algae and cyanobacteria, contain two or more copies of cyclase-encoding genes. Those genes apparently are responsive to varying environmental conditions, such as oxygen levels and nutrient stress.

The formation of the isocyclic ring includes a series of reactions that may be catalyzed by a multisubunit enzymatic complex, which has both membrane-bound and soluble components (79, 80). However, this multisubunit complex is still uncharacterized. A *Ycf54* mutant of *Synechocystis* PCC6803 accumulates MgPME, the cyclase substrate, which suggests that the Ycf54 (hypothetical chloroplast open

Table 1 Examples of anaerobic pathways mixed with aerobic pathways[a]

		Species	AcsF[b]	BchE[b]
Aerobes	Higher plants	*Arabidopsis thaliana*	+	−
	Cyanobacteria	*Prochlorococcus*	+	−
		Synechococcus sp.	+	−
		Anabaena PCC7120	+	+
		Gloeobacter PCC7421	+	+
		Thermosynechococcus BP-1	+	−
		Synechocystis PCC6803	+	+
		Acaryochloris marina	+	−
Facultative organisms		*Roseobacter*	+	−
		Rhodobacter	+	+
Anaerobes		*Heliobacterium modesticaldum*	−	+
		Chloroflexus aurantiacus	+	+
		Chlorobium tepidum	−	+

[a]Data are from searches of the Kyoto Encyclopedia of Genes and Genomes and National Center for Biotechnology Information databases (November 2012).
[b]Abbreviations: AcsF, Mg–protoporphyrin IX monomethyl ester aerobic cyclase; BchE, Mg–protoporphyrin IX monomethyl ester anaerobic cyclase.

reading frame 54) protein is essential for cyclase function and may be a subunit of the cyclase complex (81).

Steps 8 and 9: The 8-Vinyl Group Reduction and the Ring D Reduction Forming Chlorophyllide

The 8-vinyl reductase step is the next step that leads to Pchlide (step 8 in **Figure 3**). It is the earliest step in chlorophyll biosynthesis that is responsible for the chemical diversity in this class of molecules. Almost all chlorophylls have an ethyl group at the C8 position; exceptions are chlorophylls found in some marine picophytoplankton species. Marine *Prochlorococcus* species lack the reductase for the vinyl group at the C8 position and thus produce 8-vinyl Chl *a* and 8-vinyl Chl *b* (**Figure 1*a***) (4, 82). *Prochlorococcus* species are distributed widely in the open ocean; deeper in the water column, blue light is the major component of light irradiance. 8-Vinyl Chl *a* and 8-vinyl Chl *b* (also named Chl a_2 and Chl b_2, respectively) have enhanced blue-light absorption because of shifted maximal absorption peaks. In plants, the reductase for transforming the vinyl group to an ethyl group at the C8 position of chlorophylls has broad substrate specificity and is responsible for the multibranched chlorophyll biosynthesis pathways (83). The activities of 8-vinyl reductase have been detected at five levels of MgDVP, MgPMe, Pchlide *a*, Chlide *a*, and 8-vinyl Chl *a* (83, 84). However, the 8-vinyl reductases from different species show diverse and differing substrate preferences. There are more than five different 8-vinyl reductases with different reductive activities on the same or on different substrates (83). Both 8-ethyl and 8-vinyl chlorophyll precursors accumulate in higher plants, and the ratio between the two forms changes depending on growth conditions. The catalyzing activity of 8-vinyl reductase with various intermediate molecules of chlorophyll biosynthesis confirms the importance of the reduced 8-vinyl side chain, especially for organisms living under low-light and growth-limiting light conditions (85). The mosaic distribution of 8-vinyl reductases and various proposed types of vinyl reductases reflect the limits of our current knowledge about vinyl reductase in the chlorophyll biosynthesis

pathway. Further investigation will be required to learn more about this reaction step (86, 87).

Interestingly, the next step (step 9 in **Figure 3**), catalyzed by Pchlide oxidoreductase (POR) in cyanobacteria, shows equal activity in vitro with either monovinyl (3-vinyl) Pchlide or divinyl (3,8-divinyl) MgDVP. In other words, POR can use both Pchlides as its substrate (88).

Pchlide reduction at ring D produces a Chlide, the direct precursor of Chl *a*. This step plays an important role in regulating plant development and the assembly of the photosynthetic apparatus. Two enzymes—light-independent POR (DPOR, where D stands for dark) and light-dependent POR (LPOR)—catalyze the reduction of the carbon double bond between C17 and C18 of Pchlide to the carbon single bond between C17 and C18 of Chlide (step 9 in **Figure 3**), depending on whether the enzymatic reduction requires light (55). These two enzymes have entirely unrelated mechanisms for performing the same stereospecific reduction of the double bond of the fourth ring, supporting the idea that they are two evolutionarily distinct enzymes. LPOR is ubiquitous among cyanobacteria and all eukaryotic photosynthetic organisms, and it has one nuclear-encoded subunit that is translocated to the chloroplast posttranslationally in plants. In contrast, DPOR is a protein complex composed of three subunits: L, B, and N. The three subunits are conserved from cyanobacteria to higher plants (ChlL, ChlB, and ChlN) and are commonly referred to as BchL, BchB, and BchN in bacteriochlorophyll biosynthesis. DPOR is not universally distributed in eukaryotic photosynthetic organisms; however, it is ubiquitously distributed among prokaryotic phototrophs, including oxygenic photosynthetic prokaryotic organisms (cyanobacteria) and anoxygenic photosynthetic prokaryotic organisms (such as purple bacteria and green sulfur bacteria). The DPOR-encoding genes are absent in the plastid genomes of the red alga *Cyanidioschyzon merolae* and angiosperms; they are also absent in the plastid genomes of diatoms (89, 90), haptophytes (91), cryptophytes (92), and *Chromera velia* (93). However, DPOR has been characterized in several types of plastid-containing algae and lower plants, such as green algae (94, 95), mosses (96), glaucophytes (97), and red algae (98). The presence of pseudogenes encoding DPOR subunits in the nuclear genome of *Chroomonas mesostigmatica* suggests that they may have recently been transferred from the plastid genome (99).

A sequence comparison revealed that DPOR is a nitrogenase-like enzyme, and the crystal structure of DPOR confirmed the similarity between nitrogenase and DPOR. There are two functional parts of a DPOR protein complex, a dimeric L subunit component and a heterotetrameric B and N subunit component, which are structurally related to Fe nitrogenase and MoFe nitrogenase, respectively (100, 101). Each B/N heterodimer binds one Pchlide and one 4Fe–4S cluster, and a *trans*-specific reduction mechanism has been proposed to reduce the double carbon bond between C17 and C18 in Pchlide to form Chlide (100). In cyanobacteria, there are two phylogenetically different forms of DPOR. One is closely related to the plant and algal sequences and is rooted by *Chloroflexi* and a diverse group of proteobacteria. The second cluster of cyanobacterial DPOR sequences consists mainly of various *Prochlorococcus* and marine *Synechococcus* species and is closely related to proteobacterial sequences (99, 102).

The catalytic reaction can be initiated on a preassembled enzyme/substrate/NADPH complex (a LPOR/Pchlide/NADPH complex) by use of the light absorbed by Pchlide. The reduction mechanism in LPOR is different from that in DPOR, which includes four light-independent reaction steps after the Pchlide-binding complex is triggered by light (103). The unique characteristic of LPOR is that it is directly driven by light, although there are a wide variety of gene family organizations and regulatory mechanisms (104). The reduction of Pchlide *a* into Chlide *a* (step 9 in **Figure 3**) is caused by a sequential two-photon-initiated mechanism: The absorption of the first photon creates the active conformation of enzyme

complexes, and catalysis occurs upon absorption of the second photon (105). A proton is donated to the C18 position, and a hydride is transferred from NADPH to the C17 position (103); the catalytic-site residues (Tyr) are highly conserved and essential for LPOR's catalytic function (106).

The kinetics of assembling and disassembling this enzyme/substrate/NADPH complex, such as the binding of substrate and NADPH, reflects the regulatory processes. LPOR is present at high levels as a ternary complex with Pchlide and NADPH, forming prolamellar bodies in etioplasts of dark-grown seedlings (104). Most angiosperms have only LPOR, but some have multiple isoforms of LPOR. These multiple isoforms operate at different growth stages by light-dependent and development-dependent regulatory mechanisms. *Arabidopsis* contains three isoforms of LPOR: LPORA, LPORB, and LPORC. LPORA and LPORB accumulate extensively in etioplasts as supercomplexes of LPOR/Pchlide/NADPH; however, the expression levels of LPORA and LPORB differ in response to light. LPORA expression is strongly downregulated by light, and LPORB expression is less light sensitive (107). LPORC in *Arabidopsis* is the dominant form in mature green tissues (108). In barley, LPORA and LPORB are found in large light-harvesting LPOR/Pchlide complexes in the prolamellar bodies of etioplasts (107). LPORA and LPORB specifically bind to Pchlide *b* and Pchlide *a*, respectively. The light-harvesting LPOR/Pchlide complexes also function as light traps (i.e., they remove excess light) and photoprotectors (i.e., they avoid Pchlide-derived singlet-oxygen generation) (109, 110). In cucumber and pea, only one LPOR gene is present, and its expression level is positively regulated by light (111) and remains unchanged during greening and development (112).

Step 10: Chlorophyll Synthase

In the last step of chlorophyll biosynthesis, chlorophyll synthase (ChlG) esterifies Chlide *a* (or Chlide *b*) with the phytol-pyrophosphate chain and produces Chl *a* (or Chl *b*). This hydrophobic side chain allows chlorophyll to be stabilized with photosynthetic proteins localized in the thylakoid membranes. Using sense and antisense ChlG RNA in *Arabidopsis*, Shalygo et al. (113) showed that ChlG is involved in the feedback control of the entire chlorophyll biosynthesis pathway. However, overexpression of ChlG in plants seems not to produce any negative feedback from the excessive chlorophyll-synthesizing activities. Chlorophyll synthase also accepts geranylgeranyl diphosphate as a substrate. The hydrogenation of geranylgeraniol to phytol occurs before or after it is incorporated into the pigment, allowing some flexibility in the pathway. The esterified chlorophylls are specifically required for chlorophyll-binding protein complexes, such as the plastid-encoded Chl *a*–binding proteins P700, D1, D2, CP43, and CP47.

THE MODIFICATIONS THAT LEAD TO OTHER TYPES OF CHLOROPHYLLS

All chlorophylls have similar structures. The differences among them are introduced at the end of the biosynthesis pathways (**Figure 5**). In general, Chlide *a* (or Chl *a*) is the precursor of all other types of chlorophylls—Chl *b*, Chl *d*, and Chl *f* (114–116). The biosynthesis pathway leading to Chl *c* as an end product is uncertain. Chl *c* may be an evolutionary precursor of chlorophylls (117). However, using isotopes of nitrogen and carbon as biomarkers for geochemical analysis, Kashiyama et al. (118) proposed that Chl *c*–containing algae use mainly nitrogen that has been fixed by cyanobacteria. This finding suggests that Chl *c* is derived from Chl *a* or Chlide *a*.

Chl *b* and Chl *d* are synthesized from Chl *a* (or Chlide *a*), as shown by ^{18}O-labeling experiments (**Figure 5**) (115, 119, 120). Chl *b* biosynthesis includes transformation of the 7-methyl group to the 7-formyl group, and the formyl oxygen originates from molecular oxygen (121). How Chl *f* is biosynthesized is uncertain; it may use the same mechanism as Chl *b* biosynthesis

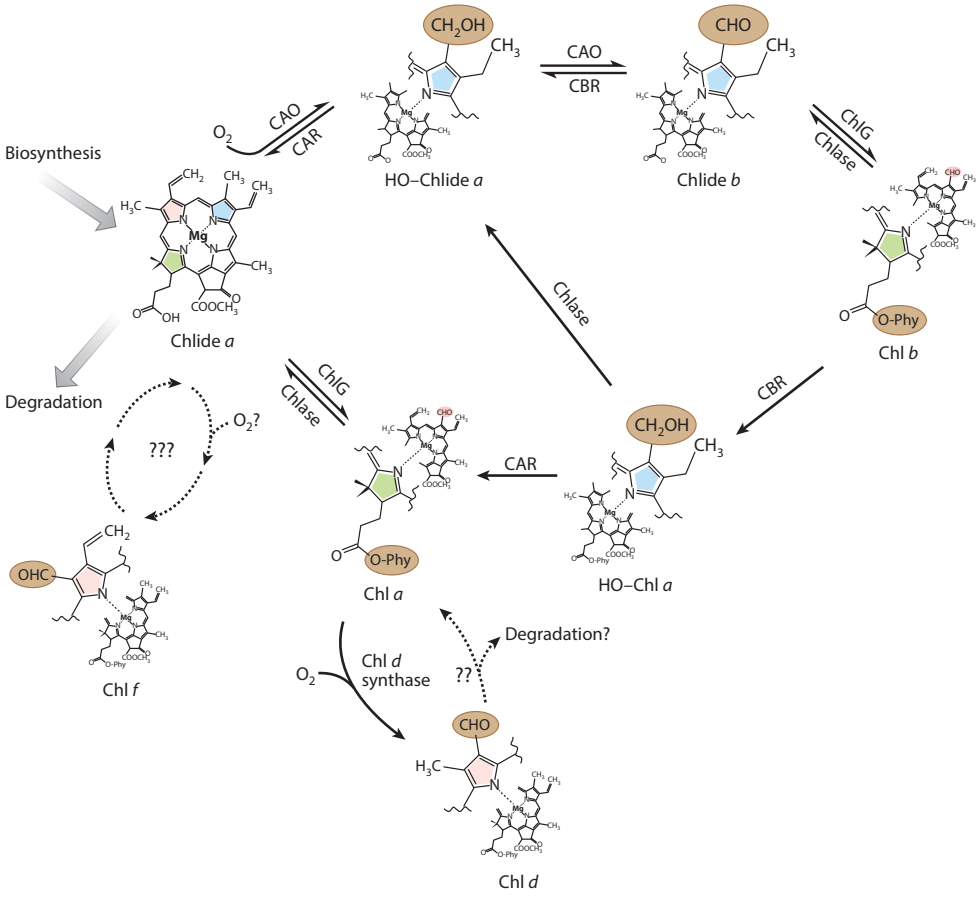


Figure 5

Chlorophyll (Chl) cycle and formyl formation. Chlorophyllide *a* (Chlide *a*) is the central intermediate for biosynthesis, degradation, and the reversible conversion between Chl *a* and Chl *b*. Chl *d* is synthesized via Chl *a*, not Chlide *a*; however, there are unknown pathways for its degradation, and whether degradation occurs via Chl *a* is unknown. The biosynthesis and degradation mechanisms for Chl *f*, and whether the oxygen in the formyl group in Chl *f* comes from molecular oxygen, are unknown. The changed sites are enlarged and colored as follows: Green represents ring D, blue represents ring B, and pink represents ring A. Abbreviations: Chlase, chlorophyllase; CAO, Chlide *a* oxygenase; CAR, 7′-hydroxy Chlide *a* reductase; CBR, Chlide *b* reductase; ChlG, Chl synthase.

because it also includes the transformation of a methyl group to a formyl group, but at the C2 position (6, 116). The mechanism of Chl *d* biosynthesis differs from that of Chl *b* and Chl *f* because Chl *d* requires a transformation from a vinyl group to a formyl group. Chl *d* synthase has been proposed to be a P450-type enzyme because bubbling carbon monoxide–enriched air inhibits Chl *d* biosynthesis and this reaction can be reversed by oxygen-enriched air (122).

Chlorophyll *b* and the Chlorophyll Cycle

Chl *b* is biosynthesized from Chlide *a* via the CAO enzyme, which is a Rieske FeS center–containing, non-heme-Fe monooxygenase that uses molecular oxygen and NADPH to perform two successive hydroxylations at the C7 position of Chlide *a* (**Figure 5**) (121). All CAO genes share high sequence similarity and occur widely in higher plants, prochlorophytes

(cyanobacteria containing Chl *b*), and green algae, suggesting that the *CAO* gene had a common ancestor in prokaryotes that was passed on to chloroplasts (123). Higher plant *CAO* expressed in bacteria can actively convert Chlide *a*, but not Pchlide *a*, to Chlide *b* in vitro (124). The formation of 7-formyl Chl d_{p672} in a *phCAO Acaryochloris* mutant line implicates the spatial specificity of CAO catalytic activity; in other words, CAO converts the methyl group to formyl group only at the C7 position (7).

Chl *b* is essential for the assembly and function of most LHCs. The gene encoding CAO is located in the nucleus, and the mature CAO protein is located in thylakoid membranes. The Chlide *b*–binding LHC precursors are imported into chloroplasts via translocon supercomplexes, which are located at the inner chloroplast envelopes and interact with CAO (125). Chl *b* is bound only in LHC systems in higher plants and is coordinately regulated, together with *Lhcb* gene expression, by irradiance (126). A large, hydrophilic NH_2-terminal domain at the N-terminal end of CAO recognizes and provides the binding site for the Chlide *b*–binding LHC systems. The *Arabidopsis PhCAO* mutant is generated by transforming and expressing a *CAO* gene from *Prochlorothrix hollandica*, a Chl *b*–containing cyanobacterium. *Prochlorothrix* species contain six-helix transmembrane chlorophyll-binding protein complexes as LHCs, which are homologous to the PSII core antenna system (CP43 and CP47) but are not related to the LHC systems in higher plants (127, 128). Presumably, expressed PhCAO protein interacts with Chl *b* that is bound in the PSII core antenna, homologous to the six-helix transmembrane chlorophyll-binding protein complex antenna system in *Prochlorothrix*, as well as the LHC systems in the *Arabidopsis PhCAO* mutant line (129). In contrast, expression of an *Arabidopsis CAO* (*AtCAO*) gene in *Synechocystis* PCC6803 requires the simultaneous overexpression of *Lhcb* (130). These distinctive roles of CAO suggest that it has an important function in assembling the chlorophyll-binding protein complexes in higher plants.

Prochlorococcus species are the only photosynthetic organisms that contain 8-vinyl Chl *a* and 8-vinyl Chl *b*. Investigators had assumed that 8-vinyl Chl *b* is synthesized from 8-vinyl Chl *a* by a homologous *CAO* gene. However, the complete genomic DNA sequences of several species of *Prochlorococcus* reveal that there is no homologous *CAO* gene in this organism (31, 131). The gene named *Pro0890* in *Prochlorococcus* SS120 may be the most plausible candidate for an enzyme (CAO) needed for the biosynthesis of 8-vinyl Chlide *b* and, therefore, 8-vinyl Chl *b*. Conversely, phylogenetic analyses indicate that this gene, *Pro0890*, is only distantly related to *CAO* genes found in higher plants and green algae. Another notable phenomenon is that low light–adapted *Prochlorococcus* strains, SS120 and NATL1, can synthesize both 8-vinyl Chl *b* and Chl *b* when grown under high-light conditions, and Chl *b* is assumed to be derived from 8-vinyl Chl *b*. Surprisingly, no 8-vinyl Chl *b* has been detected in *Prochlorococcus* MED4, a high light–adapted strain. Therefore, how 8-vinyl Chl *b* is biosynthesized remains an open question, and answering it may provide a clue to how these organisms grow over such a wide range of light environments and constitute up to half of global marine primary production (31). Using advanced bioinformatics tools combined with molecular genetics techniques, investigators identified the Chl *b* synthase gene (*PMM0808*) in *Prochlorococcus* MIT9313 and confirmed that it is distantly related to the *CAO* genes (132).

Chl *b* can be reversibly converted back to Chl *a* through a chlorophyll cycle pathway (**Figure 5**), suggesting that Chl *b* is a late-evolved pigment (123, 133). The interconversion between Chl *a* and Chl *b* may provide higher plants with the ability to optimize their adaptation to varying light conditions. The regulation of the chlorophyll cycle plays an essential role in controlling the ratio of Chl *a* to Chl *b* because each photosynthetic protein requires chlorophylls in a strictly fixed stoichiometry for optimal energy transfer (55). However, overexpression of *CAO* messenger RNA in plants has only a minor effect on the ratio of Chl *a* to

Chl *b*, although *CAO* messenger RNA upregulation affects the amount of LHCII (134–136). In the absence of Chl *b* or a decreased amount of Chl *b* under high light, LHC protein content decreases due to the degradation of unbound LHC apoproteins by proteases (137, 138).

Chlorophyll *d*

In 1943, Chl *d* was identified as a minor photopigment in 18 red macroalgae pigment extractions (5). However, it was considered an artifactual by-product for more than 50 years until the 1996 discovery of a novel cyanobacterium, *Acaryochloris marina*, which contains mainly Chl *d* (>95% of total chlorophylls) (13). This discovery introduced a new episode in the story of Chl *d*. Murakami et al. (139) confirmed the existence of a Chl *d*–containing cyanobacterium living on the surface of red algae; this finding is almost certainly why Chl *d* was reported as a minor pigment in the red algae pigment extractions in 1943 (5). *A. marina* is a monophyletic clade but is widely distributed in ecological niches in which far-red light is enriched (24, 25). Chl *d* biosynthesis has not been observed in any other photosynthetic organism (140).

The protein sequences of the photosynthetic reaction centers PsaA/PsaB and PsbA/PsbD are highly similar to the analogous proteins of Chl *a*–containing photosynthetic organisms (141). In vitro reconstitution experiments have also confirmed that no specific ligands are required to bind Chl *d* instead of Chl *a* (142). Interestingly, Chl *a* in *Acaryochloris* is always a minor photopigment: It makes up less than 5% of total chlorophyll, whatever the culture conditions, except in one carbon monoxide–inducing experiment in which the ratio of Chl *a* to Chl *d* in *Acaryochloris* cells increased to ~17% (122). The only difference in structure between Chl *a* and Chl *d* is at the C3 position, where the vinyl group in Chl *a* is replaced by a formyl group in Chl *d* (**Figure 1*a***). Oxygenic photosynthesis uses Chl *d* to extend the photosynthetic spectral absorption region from 400–700 nm to 390–750 nm, with ~14% increased light-energy input (3).

The complete genome sequence of *Acaryochloris* was published in 2008 (141). However, no obvious putative genes were assigned as Chl *d* synthase genes due to the limited knowledge about Chl *d* biosynthesis. On the basis of annotation and sequence comparison, all the genes encoding enzymes in the Chl *a* biosynthesis pathway were identified. An ^{18}O-labeling experiment confirmed that Chl *d* synthesis is an oxygenation reaction and that Chl *a* is the precursor to Chl *d* (**Figure 5**) (115).

Chlorophyll *f*

Chl *f* is the most recently discovered chlorophyll (6). The function of Chl *f* in the cyanobacterium that contains it is uncertain but intriguing. A central question about the evolution of redshifted chlorophylls is how far this redshift can be extended while still meeting the energy requirements for driving photosynthetic reactions. Can Chl *f* play a role in the reaction center if it is still able to provide a high-enough redox potential for the oxygen evolution reaction in PSII? Also, we do not know whether an uphill energy-transferring mechanism is involved if Chl *f* is located in LHC systems and the captured energy has to be transferred to Chl *a* in the reaction centers. Chen et al. (26) observed a reversible change between Chl *a* and Chl *f* in response to the quantity of light in the newly characterized Chl *f*–containing cyanobacterium *H. hongdechloris*. Chl *f* cannot be detected if *H. hongdechloris* is white-light adapted for more than 2 weeks. No biochemical information about Chl *f* biosynthesis is available.

PERSPECTIVES ON THE REGULATION AND ADAPTATION OF VARIOUS CHLOROPHYLLS

Chlorophyll biosynthesis is expected to be tightly regulated at various levels by environmental and endogenous factors. Biosynthesis is determined primarily by two environmental

elements, light and oxygen. Because chlorophylls are molecules that stand between photosynthetic reactions on Earth and the light energy delivered by the Sun, the activities and amounts of chlorophyll in photosynthetic systems are highly regulated by and adapted to the environment.

Light

Light is an important environmental driver of photosynthesis and is also the most influential factor controlling the chlorophyll biosynthesis pathways. Light is directly involved in chlorophyll biosynthesis by driving the LPOR enzyme, which forms an inactive enzyme/substrate/NADPH complex in the dark. The addition of light completes the enzymatic transformation. Using ultrafast pump-probe absorption difference spectroscopy, Sytina et al. (105, 143) revealed that the activation of LPOR is initiated by the absorption of light by the bound Pchlide *a* in this complex. A short flash of light is enough to initiate the activities of the inactive LPOR/Pchlide/NADPH complex.

Light is an important environmental signal that triggers the photosynthesis reaction and regulates chlorophyll biosynthesis through redox and feedback mechanisms (144). Chlorophyll synthesis is restricted to photosynthesizing tissues, and in angiosperms it is strictly light dependent. Excited chlorophyll and its biosynthetic intermediate molecules are dangerous if present as free molecules in the cells because they produce sufficient ROS to kill cells. Remarkably, chlorophylls do not occur as free pigment molecules, so the negative effects of ROS generated by excited chlorophylls are avoided; instead, they are always bound to proteins with carotenoids. A protein bound to an intermediate product of chlorophyll biosynthesis is a direct target for proteases (145).

In most photosynthetic organisms, protective carotenoid systems quench the triplet state of chlorophylls. The formation of a LPOR/Pchlide/NADPH complex also protects the free Pchlides from the cells (109, 110). Photosynthetic organisms acclimate to the level of irradiance (photoacclimation) by adjusting the size of the antennae and the production of photopigments with various absorption properties; for example, they produce redshifted chlorophyll to extend the absorption range and capture a broader range of wavelengths in the reaction centers (**Figure 2*b***). *Prochlorococcus* takes advantage of 8-vinyl Chl *a* and 8-vinyl Chl *b* to adapt to open-oceanic environments, capturing more blue light (1). Chl *f* is the only chlorophyll reported to be acclimated directly under IR light (26). The regulatory mechanism for this photoswitchable conversion between Chl *a* and Chl *f* is unknown.

Additionally, the chlorophyll biosynthesis pathway is tightly controlled by feedback and feed-forward mechanisms. The total chlorophyll content is decreased under high-light conditions and is increased with more layers of thylakoid membranes under low-light conditions.

Oxygen

The oxygen generated by oxygenic photosynthetic reactions powered the evolution of life on Earth. Accumulation of molecular oxygen in the atmosphere fundamentally changed the redox balance on Earth and permitted the development of both aerobic metabolism and advanced life forms (146). Since then, oxygen concentration has played an important role in the regulation of photosynthesis and chlorophyll biosynthesis (147–149). By increasing the oxygen concentration in the atmosphere, ancient life forms, living in anaerobic environments or in limited accessible-oxygen-molecule concentrations, had to adapt and evolve new strategies to deal with the increased oxygen concentration, including ROS generated by excited porphyrin molecules in the cells. The yin-and-yang balance of chlorophyll biosynthesis has been central to the development of the biosphere.

The coexistence of oxygen-dependent and oxygen-independent enzymes may represent either the evolutionary transition from anaerobic to aerobic metabolic reactions (76) or an adaptation strategy for regulating reactions

Table 2 Calculated heats of formation (ΔH) of chlorophyll (Chl) Mg-peptide ligands in different environments, with different dielectric constants (ε)[a]

Species	ΔH (kcal/mol)[b]						Relative stability[c]
	$\varepsilon = 1$	$\varepsilon = 2.2$	$\varepsilon = 4.3$	$\varepsilon = 6.9$	$\varepsilon = 12.3$	$\varepsilon = 78.4$	$\varepsilon = 2.2$
Chl *a*–binding peptide	−892	−1,001	−1,065	−1,093	−1,001	−1,047	1
Chl *d*–binding peptide	−806	−913	−977	−1,005	−1,027	−949	2
Chl *a*–binding peptide	−706	−824	−891	−924	−944	−870	3
Chl *f*–binding peptide	+732	+322	+88	−18	−100	−198	4
Chl *b*–binding peptide	+770	+379	+253	+115	+1	−133	5

[a]Data are from the PM5 method (see Reference 151 for more details).
[b]Values of ε are as follows: gas phase, 1.0; benzene, 2.2; ether, 4.3; aniline, 6.9; pyridine, 12.3; water, 78.4.
[c]The greater the negative value is, the more thermodynamically stable is the species. The positive value of the Chl *f*–binding peptide is similar to that of the Chl *b*–binding peptide at $\varepsilon < 5.0$.

under continuously changing microenvironmental conditions. The formation of the isocyclic ring is a decisive step in chlorophyll biosynthesis (step 7 in **Figure 3**). The tightly regulated step of the fifth-ring cyclization requires oxygen and an NADPH-regenerating system (35). Multiple copies of cyclase-encoding genes in oxygenic photosynthetic unicellular organisms represent one of the strategies that have evolved to deal with varying environmental conditions because they are more easily affected by environments (**Table 1**).

Oxygen atoms are the most electronegative atoms commonly found in biological molecules, and formyl groups exert significant effects on chlorophyll molecules. Formyl substitution in Chl *b*, Chl *d*, and Chl *f* occurs during the late stage of chlorophyll biosynthesis; it seems to be a common alteration strategy to fine-tune the absorbance properties of chlorophylls so that oxygenic photosynthetic organisms can take advantage of extending absorbance wavelength ranges. The formyl substitution at the C7 position draws electron density toward the periphery of Chl *b*; that is, the electrons are drawn away from the core of the molecule along the molecular *x* (Q_x) axis, causing weak Q_y absorbance in Chl *b* (**Figure 1*a***) (150). In contrast, the formyl substitution at the C3 position in Chl *d* exerts an electron-withdrawing effect along the Q_y axis, which causes the Q_y absorption maximum to be redshifted by more than 30 nm (**Figure 2*a***). The formyl substitution at the C2 position in Chl *f* further redshifts the Q_y absorbance maximum due to the electron-withdrawing effects of the combination of formyl at the C2 position and vinyl at the C3 position. However, a computer model of the heat of formation (ΔH) of Chl *f*, based on the model system reported by Chen & Cai (151), indicates that the Chl *f*–binding peptide system has a positive ΔH, similar to that of the Chl *b*–binding peptide system (**Table 2**). These results suggest that Chl *f*, along with Chl *b*, may preferentially bind ligands containing an oxygen atom, such as water. In contrast, Chl *a* and Chl *d* have a broad range of ligands from the imidazole group of histidine to water (2).

Our current knowledge about the formyl substitutions in Chl *b* and Chl *d* is very limited, but both formyl formations have one factor in common. Molecular oxygen is required, although the oxygen level seems not to be a limiting factor for the biosynthesis of Chl *b* and Chl *d*, because photosynthesis continuously provides nearby oxygen.

Two unrelated PORs, LPOR and DPOR, coexist in cyanobacteria. Dual regulatory mechanisms by light and oxygen level have been observed (152). LPOR is essential for the growth of cyanobacteria under aerobic, high-light conditions (153), whereas DPOR reaches its maximal activity to complement the loss of LPOR activities under anaerobic conditions.

Adaptation

Reconversion of Chl *b* into Chl *a* is required for adaptation to varying light intensities as well as for the transfer of chlorophyll into the catabolic degradation pathway (**Figure 5**) (2). The ratio of Chl *a* to Chl *b* is also important for the regulation of antenna size in eukaryotic photosynthetic organisms if they use Chl *b* as an accessory photopigment. The introduction of the cyanobacterial *CAO* gene into higher plants would cause photodamage under high-light conditions and would not be useful in increasing photosynthetic efficiency (130). However, Biswal et al. (154) argue that controlled upregulation of Chl *b* biosynthesis can increase the antenna size and electron-transport rate by 40% to 80% and, hence, enhance the biomass of photosynthesis.

Chl *b* shows strong absorption intensity in the blue region with a moderate Q_y absorption intensity, which matches the light conditions on land. The redshifted chlorophylls, Chl *d* and Chl *f*, are found only in unique filtered-light environments, where the visible light is removed by the absorption of overlying organisms using Chl *a* and Chl *b* but near-IR light intensity is high. The occurrence of redshifted chlorophylls may have arisen from selective pressure from the light. The incorporation of oxygen atoms from molecular oxygen strongly suggests that formyl substitution in the later steps of chlorophyll biosynthesis evolved recently to allow the organism to adapt to different light environments. New genes related to these new enzymatic reactions may be recruited through lateral gene transfer.

SUMMARY POINTS

1. The chlorophyll biosynthesis branch is composed of six steps after P_{IX}, two of which are catalyzed by two sets of unrelated enzymes: (*a*) AcsF and BchE and (*b*) LPOR and DPOR. These two steps are tightly regulated by oxygen levels and light, respectively.
2. During the past few decades, the genes of the biosynthesis enzymes have been cloned and their expression patterns in response to light have been studied. However, the regulatory mechanisms driven by oxygen level remain uncertain.
3. Chlorophyll biosynthesis is tightly regulated at various levels. Investigators have proposed several regulatory models of how the regulation of gene expression, protein environment, and responses to environmental changes can affect the flow of tetrapyrrole biosynthesis.
4. Higher atmospheric oxygen concentration provided positive selective pressure for modification of chlorophylls by formyl substitution, which strongly suggests that formyl substitution in the later steps of chlorophyll biosynthesis evolved relatively recently to adapt organisms to varied light environments.
5. The ability to extend the photosynthetically active spectral region by use of redshifted chlorophylls offers potential for biotechnological applications to enhance photosynthetic efficiency.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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