Chapter 3

Chlorophyll Biosynthesis in Higher Plants

Baishnab C. Tripathy*
School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

Gopal K. Pattanayak
Department of Molecular Genetics and Cell Biology, University of Chicago, 1103 East 57th Street, EBC 409, Chicago, IL 60637, USA

Summary

Chlorophyll (Chl) is essential for light harvesting and energy transduction in photosynthesis. The Chl biosynthesis pathway in higher plants is complex and is mediated by more than 17 enzymes. The formation of Chl can be subdivided into four parts: (1) synthesis of 5-aminolevulinic acid (ALA), the precursor

*Author for correspondence, e-mail: bctripathy@mail.jnu.ac.in

J.J. Eaton-Rye, B.C. Tripathy and T.D. Sharkey (eds.), Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation, Advances in Photosynthesis and Respiration 34, pp. 63–94,
DOI 10.1007/978-94-007-1579-0_3,

of Chl and heme; (2) formation of a pyrrole ring porphobilinogen from the condensation reaction of two molecules of ALA and assembly of four pyrroles leading to the synthesis of the first closed tetrapyrrole having inversion of ring D, i.e., uroporphyrinogen III; (3) synthesis of protoporphyrin IX via several decarboxylation and oxygenation reactions, and (4) insertion of Mg to the protoporphyrin IX (PPIX) moiety steering it to the Mg-branch of tetrapyrrole synthesis leading to the formation of Chl. In higher plants, tetrapyrrole synthesis occurs in plastids, where it is initiated by the reduction of the glutamyl moiety of glutamyl-tRNA to glutamate-1-semialdehyde. The first branch point in the pathway is the methylation of uroporphyrinogen III that directs it toward the synthesis of siroheme, an essential component of nitrite reductase and sulfite reductase, whereas decarboxylation steers it towards PPIX synthesis. A second branch point of the tetrapyrrole biosynthesis pathway is Fe insertion to PPIX leading to the synthesis of hemes. Mg-insertion to the PPIX moiety leads to the synthesis of Mg-protoporphyrins and chlorins. During the day when the ATP levels are high, the magnesium branch of the pathway is favoured, as Mg-chelatase needs ATP for the Mg-PPIX synthesis. In this chapter, we discuss the mechanism of Chl biosynthesis; heterogeneity of the monovinyl and divinyl protochlorophyllide pool; regulation of Chl biosynthesis; the intraplastidic Chl biosynthesis route, and the evolution of Chl biosynthesis.

Abbreviations: ALA – 5-aminolevulinic acid; ALAD – Aminolevulinic dehydratase; CAO – Chlorophyllide *a* oxygenase; CBR – Chlorophyll *b* reductase; Chl – Chlorophyll; Chlide – Chlorophyllide; CHLG – Chlorophyll synthase; CHLP – geranyl-geranyl reductase; CPOX – Coproporphyrinogen oxidase; DV-Pchlide – Divinyl protochlorophyllide; DVR – Divinyl reductase; FLU – Negative regulator of the chlorophyll biosynthesis pathway; FD – Feredoxin; GA – Gibbralic acid; GGPP – Geranyl geranyl pyrophosphate; GluRS – Glutamyl-tRNA synthetase; GluTR – Glutamyl-tRNA reductase; GSA – Glutamate 1-semialdehyde; GSA-AT – Glutamate 1-semialdehyde aminotransferase; GUN – Genome uncoupled; LHCII – Light-harvesting complex II; Lin2 – Lesion initiation 2; lip1 – Light-independent photomorphogenesis 1; MgCh – Magnesium chelatase; MPE – Mg-protoporphyrin IX monomethylester; MPEC – Mg-protoporphyrin IX monomethylester cyclase; MTF – Mg-protoporphyrin IX methyltransferase; MV-Pchlide – Monovinyl protochlorophyllide; PBG – Porphobilinogen; PBGD – Porphobilinogen deaminase; PC – Plastocyanin; Pchlide – Protochlorophyllide; PhPP – Phytyl diphosphate; PLBs – Prolamellar bodies; POR – Protochlorophyllide oxidoreductase; PPIX – Protoporphyrin IX; PPOX – Protoporphyrinogen oxidase; PQ – Plastoquinone; Protogen IX – Protoporphyrinogen IX; PS II – Photosystem II; SAM – *S*-adenosyl- methionine; SDR – Short chain dehydrogenases/reductases; UROD – Uroporphyrinogen III decarboxylase; Urogen III – Uroporphyrinogen III; UROS – Uroporphyrinogen III synthase

I. Introduction

Chlorophyll (Chl), the most abundant pigment, ubiquitously distributed in all plant species, is essential for light harvesting and energy transduction in photosynthesis. The major site of Chl biosynthesis in higher plants is the plastid. All enzymes of the Chl biosynthetic pathway are nuclear encoded and post-translationally imported into chloroplasts. Chl synthesis is synchronized with the formation of other pigments such as carotenoids and with pigment-binding proteins; Chl synthesis also incorporates coordination between chloroplast and nucleus. In higher plants, most of the genes responsible for the Chl biosynthesis pathway are identified and the enzymatic steps of the pathway are characterized (Fig. 3.1). For a background on plastids, on Chls, on carotenoids, and on Chl biosynthesis in chloroplasts, the readers are referred to books edited by Wise and Hoober (2006), Grimm et al. (2006), Frank et al. (1999) and Rebeiz et al. (2010), respectively.

Throughout this chapter nuclear genes will be designated in uppercase italics and their corresponding protein as uppercase normal font; mutant plants will be in lowercase italics. In some cases a lowercase letter may form part of the accepted abbreviation for a nuclear-encoded enzyme (e.g.,

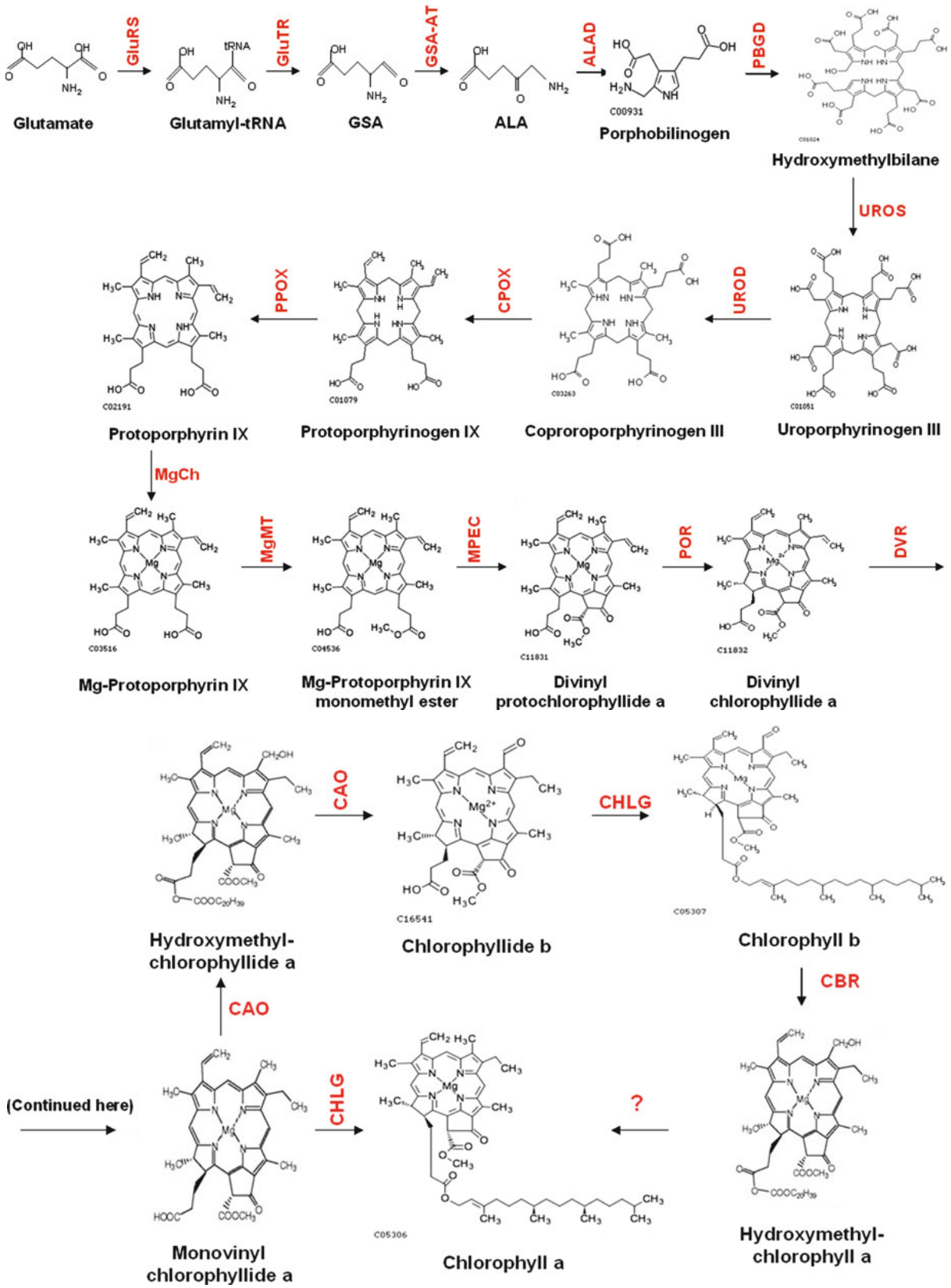

Fig. 3.1. Chlorophyll biosynthesis pathway in higher plants. All abbreviations in this figure are defined in the *Abbreviations* list for this chapter.

Glutamyl-tRNA synthetase is abbreviated GluTR and its corresponding gene is *GluTR*). In these cases the lower case letter has been retained and the reader should consult the *Abbreviations* list to clarify individual examples as required.

II. Enzymes Involved in Chlorophyll Biosynthesis

A. Glutamyl-tRNA Synthetase

Glutamyl-tRNA synthetase (GluRS), also known as aminoacyl-tRNA synthetase, ligates glutamate to $tRNA^{GLU}$ (Huang et al., 1984; Kannagara et al., 1984, 1994) (Fig. 3.1). GluRS belongs to the class I aminoacyl-tRNA synthetases. Aminoacyl-tRNA synthetases generally catalyze the formation of an aminoacyl-AMP from an amino acid and ATP, prior to the aminoacyl transfer to tRNA. However, GluRS avoids the aminoacyl-AMP formation in the absence of tRNA.

In eukaryotic cells two different kinds of GluRS are present, one inside the chloroplast and the other in the cytosol. Both enzymes are encoded by nuclear DNA, and one of the enzymes is imported into the chloroplast where it ligates glutamate to $tRNA^{GLU}$ (Schon et al., 1988). In barley, $tRNA^{GLU}$ is encoded by chloroplast DNA. It contains the UUC glutamate anticodon and is involved in aminolevulinic acid (ALA) synthesis (Schon et al., 1986).

GluRS has been purified from barley (Bruyant and Kannagara, 1987), wheat (Ratinaud et al., 1983), tobacco (Kim et al., 2005) and *Arabidopsis thaliana* (Day et al., 1998). Virus-induced gene silencing of *GluRS* in tobacco results in a severe leaf-yellowing phenotype (Kim et al., 2005). Reduction of the amount of GluRS leads to reduced number and size of the chloroplasts and reduced Chl content.

B. Glutamyl-tRNA Reductase

Glutamyl-tRNA reductase (GluTR), the second enzyme of the pathway, reduces the activated α-carboxyl group of glutamyl-tRNA (Glu-tRNA) in the presence of NADPH and releases glutamate 1-semialdehyde (GSA) (Fig. 3.1). Pyridine nucleotides are required for this reaction (Hoober et al., 1988). The GluTR protein was purified from barley and consisted of five identical subunits of 54 kDa each (Pontoppidan and Kannangara, 1994).

This enzyme is subject to feedback regulation by heme and appears to be a major control point of porphyrin biosynthesis (Kannangara et al., 1988). Micromolar concentrations of Zn^{2+}, Cu^{2+} and Cd^{2+} inhibit barley GluTR (Pontoppidan and Kannangara, 1994). In green barley plants, this enzyme is stimulated by GTP (Kannagara et al., 1988).

GluTR is encoded by the *HEMA* gene. The cDNA for GluTR has been isolated from barley (Bougri and Grimm, 1996); cucumber (Tanaka et al. 1996), and *A. thaliana* (Kumar et al., 1996; Matsumoto et al., 2004). It has two isoforms in barley and in cucumber, whereas in *A. thaliana* it has three isoforms. In cucumber and *A. thaliana* plants, the *HEMA1* gene is expressed in photosynthetic tissues and is induced by illumination, but no transcripts are detectable in roots. Gene expression of *HEMA1*, and the corresponding protein abundance, increases in response to light treatment of dark-grown seedlings suggesting that increased demand for Chl biosynthesis stimulates its expression and the gene promoter may have light-responsive elements (Mohanty et al., 2006). Light-induced expression of *HEMA1* indicates the involvement of phytochrome (McCormac et al. 2001; McCormac and Terry, 2002a, b). On the other hand, *HEMA2* is preferentially expressed in non-photosynthetic tissues, and its expression is not altered with illumination. A third *HEMA* gene, *HEMA3*, has been identified in *A. thaliana*, but its expression is very low under all experimental conditions tested (Matsumoto et al., 2004). Northern blot analysis of *BHA1* (a cDNA clone from barley encoding GluTR) shows that the transcripts are present in roots and are elevated after cytokinin treatment, whereas *BHA 13* (another cDNA clone from barley encoding GluTR) is not present in roots. The *BHA1* levels show oscillations with a circadian rhythm. The *HEMA1* mRNA accumulates in response to a demand for Chl synthesis in photosynthesising tissues, whereas *HEMA2* mRNA is expressed in response to the demand of synthesis of porphyrins other than Chl (Bougri and Grimm, 1996).

A. thaliana plants, expressing antisense *HEMA1*, show decreased amounts of its mRNA, decreased ALA and reduced Chl content (Kumar and Soll, 2000). The RNAi silencing of genes encoding GluTR results in reduced levels of Chl and heme in young leaves (Hedtke et al., 2007). In *A. thaliana*, GluTR interacts with FLU, a negative

regulator of the Chl biosynthesis pathway (Meskauskiene et al., 2001, Meskauskiene and Apel, 2002). FLU is a nuclear-encoded chloroplastic protein and the *flu* mutant has a higher level of ALA synthesis and protochlorophyllide (Pchlide) accumulation than that of wild-type plants. Probably FLU is a component of a negative regulatory system for ALA synthesis when cells have a high Pchlide content. FLU-like protein is also present in barley (Lee et al., 2003).

C. Glutamate 1-Semialdehyde Aminotransferase

The formation of 5-aminolevulinate from GSA is catalyzed by glutamate 1-semialdehyde aminotransferase (GSA-AT). This enzyme is functionally an aminomutase, which transfers the amino group from carbon 2 of GSA to the neighboring carbon atom i.e., carbon 5 of ALA (Fig. 3.1). During the conversion of GSA to ALA, the amino group from pyridoxamine phosphate is donated to GSA, leading to the formation of an intermediate, 4, 5-diaminovalerate (Hoober et al., 1988). The enzyme then releases an amino group from position 4 of this intermediate, releasing 5-ALA. The enzyme is inhibited by gabaculine (Gough et al., 1992). With the exception of barley, most GSA-AT enzymes require either pyridoxamine 5′ phosphate or pyridoxal 5′ phosphate as a cofactor for the enzymatic conversion of GSA into ALA (Kannangara et al., 1988).

The *GSAT* gene encoding GSA-AT has been isolated from barley (Kannangara et al., 1994), *A. thaliana* (*GSAT1* and *GSAT2*) (Ilag et al., 1994), tomato (Polking et al., 1995); soybean (Sangwan and O'Brian, 1993), tobacco (*GSAT1* and *GSAT2*) (Höfgen et al., 1994) and brasicca (Tsang et al., 2003).

In *A. thaliana* light is reported to stimulate transcription of this gene (Ilag et al., 1994). The *GSAT* gene expression is also activated by the hormone kinetin (Yaronskaya et al., 2006). The gene expression of *GSAT*, and the protein abundance of GSAT-AT, increases when etiolated seedlings are transferred to light demonstrating that it is a light-inducible gene and significantly contributes to Chl synthesis (Mohanty et al., 2006). In soybean the *GSAT* gene is also light inducible. It contains a light-regulated cis element (containing GAGA) that is found to be involved in transcriptional control (Frustaci et al., 1995). Its message abundance is high in soybean leaves (Sangwan and O'Brian, 1993) whereas it is absent in roots (Frustaci et al., 1995). Antisense tobacco and brassica plants, expressing *GSAT*, show reduction in Chl levels (Höfgen et al., 1994; Tsang et al., 2003).

D. 5-Aminolevulinic Acid Dehydratase

5-Aminolevulinic acid dehydratase (ALAD), also known as porphobilinogen (PBG) synthase, is a homooctameric metalloenzyme that catalyzes the condensation of two ALA molecules to form PBG (Fig. 3.1). The mechanism of action of ALAD was first proposed by Shemin (1976). The aldol condensation between two ALA molecules involves the initial binding of two substrate molecules and a five-membered heterocyclic ring of PBG is formed with the help of a lysine and a histidine residue (Jordan and Shemin, 1980; Spencer and Jordan, 1994, 1995).

This enzyme has been isolated from wheat (Nandi and Waygood, 1967), tobacco (Shetty and Miller, 1969), radish (Shibata and Ochiai, 1977), spinach (Liedgens et al., 1980), pea (Smith, 1988) and tomato (Polking et al., 1995). Spinach enzyme is found to be a hexamer with molecular weight of 300 kDa (Liedgens et al., 1980) while radish leaves were found to have two isozymes of ALAD (Tchuinmogne et al., 1992). Enzymes from radish cotyledons showed a pH optimum of 8.0 (Shibata and Ochiai, 1977) and a requirement of Mg^{2+} and Mn^{2+} for activity. Using an artificially synthesized ALAD in pea, Kervinen et al. (2000) showed that this enzyme is most active at slightly alkaline pH and shows a maximal binding of three Mg (II) per subunit. The enzyme from tobacco leaves and radish cotyledons was found to be inhibited by Zn^{2+} and Fe^{2+} (Shetty and Miller, 1969; Shibata and Ochiai, 1977); furthermore, arsenic inhibits ALAD activity in maize leaves (Jain and Gadre, 2004), while $PbCl_2$ and $CdCl_2$ inhibit ALAD in *Amaranthus lividus* (Bhattacharjee and Mukherjee, 2003). The activity of ALAD significantly decreases during senescence (Hukmani and Tripathy, 1994) and in chill- and heat-stressed plants (Tewari and Tripathy, 1998).

The gene encoding ALAD has been isolated from pea (Boese et al., 1991), spinach (Schaumburg et al., 1992), soybean (Kaczor et al., 1994) and tomato (Polking et al., 1995). In pea, expression of *ALAD* was high in dark-grown as compared to light-grown tissues (Li et al., 1991). ALAD was detectable in embryonic leaves whether the plants were grown in darkness or under continuous white-light illumination (He et al., 1994).

In pea, *ALAD* transcript abundance was found to be highly dependent on leaf developmental age: the transcript abundance increased with increasing age until the leaf was fully expanded and after that the mRNA levels decreased sharply (He et al., 1994). However, a significant amount of protein was detected even in the matured leaves despite the mRNA expression of *ALAD* being extremely low. Additionally, even though the steady-state level of the *ALAD* mRNA was slightly higher in the dark than in the light, the corresponding protein level was significantly lower in the dark (He et al., 1994). The expression of *ALAD* was not regulated by light in soybean and its expression was 2–3 fold higher in symbiotic tissues as compared to the uninfected roots (Kaczor et al., 1994). On the other hand, in cucumber and wheat *ALAD* expression increased upon transfer of etiolated seedlings to light (Mohanty et al., 2006).

E. Porphobilinogen Deaminase

The enzyme porphobilinogen deaminase (PBGD) is a soluble chloroplastic protein (Castelfranco et al., 1988) that catalyzes the formation of the linear tetrapyrrole, hydroxymethylbilane, from four molecules of PBG (Fig. 3.1). PBGD has been isolated and purified from pea (Spano and Timko, 1991), wheat germ and spinach leaves (Higuchi and Bogorad, 1975) and *A. thaliana* (Jones and Jordan, 1994). Molecular weights from all these sources ranged from 34 kDa to 44 kDa.

The PBGD enzyme from pea chloroplasts was inhibited by Fe^{2+}, Mn^{2+} and Zn^{2+}, whereas Ca^{2+} and Mg^{2+} were only weakly inhibitory at physiological concentrations (Spano and Timko, 1991). PBGD is also inactivated by arginine-, histidine- and lysine-specific reagents as well as by the substrate analogue 2-bromoporphobilinogen (Jones and Jordan, 1994). The enzyme is heat stable and maintains its activity at temperatures ranging from 55^0C to 70^0C. Furthermore, PBGD activity rapidly declines during senescence (Hukmani and Tripathy, 1994) and is reduced by chill- or heat-stress (Tewari and Tripathy, 1998, 1999).

The *PBGD* gene has been isolated and cloned from pea (Witty et al., 1993) and *A. thaliana* (Lim et al., 1994). In *A. thaliana*, PBGD was found to be expressed both in the leaves and the roots (Lim et al., 1994). The steady-state level of *PBGD* mRNA was slightly higher in the dark than in the light, even though the protein level was significantly lower in dark (He et al., 1994). The *PBGD* transcript abundance was found to be highly dependent on leaf developmental age, i.e., the transcript abundance increased with increased age until the leaf was fully expanded and after that the mRNA levels decreased sharply (He et al., 1994). However, a significant amount of protein was detected even in matured leaves despite the mRNA expression of *PBGD* being extremely low.

Sequence comparison from different species shows that specific Arg and Cys residues are well conserved and these are implicated in catalysis and dipyrromethane cofactor binding (Witty et al., 1993). The synthesis and activity of PBGD are regulated by light and cell types (Smith, 1988; Shashidhara and Smith, 1991; Spano and Timko, 1991; He et al., 1994).

F. Uroporphyrinogen III Synthase

In concert with PBDG, the uroporphyrinogen III synthase (UROS) enzyme catalyses the formation of uroporphyrinogen III (Urogen III) from hydroxymethylbilane, a product of PBGD activity. This enzyme helps in maintaining the formation of biologically active isomer III by inverting the ring D; in its absence, hydroxymethylbilane spontaneously cyclizes to uroporphyrinogen I (Urogen I). Inversion of ring D probably involves the production of a spiro-cyclic intermediate (Crockett et al., 1991). This enzyme has been purified from wheat germ (Higuchi and Bogorad, 1975). The enzyme was found to be heat labile and the activity was enhanced by Na^+ and K^+. The enzymes PBGD and UROS may be present as a complex (Tsai et al., 1987). Tan et al. (2008) have isolated the *UROS* gene from *A. thaliana* and shown it to have in vitro activity. The localization of the protein in the chloroplast was confirmed by an in vitro protein import study and confocal microscopy (Tan et al., 2008). The barley *uros* mutant showed a necrotic phenotype in a developmental manner because of Urogen I accumulation (Ayliffe et al., 2009). The mutation in *UROS* also suppressed the expression of genes involved in the light reactions of photosynthesis (Ayliffe et al., 2009).

G. Uroporphyrinogen III Decarboxylase

The uroporphyrinogen III decarboxylase (UROD) enzyme catalyzes stepwise decarboxylation of Urogen III to yield coproporphyrinogen III (Coprogen III). The enzyme catalyzes decarboxylation of all four carboxyl residues of Urogen III to yield coproporphyrinogen. The order of Urogen III decarboxylation is substrate concentration dependent and under normal conditions enzymatic decarboxylation begins at the ring-D acetate group in a clockwise manner (Luo and Lim, 1993). Although all four isomers of uroporphyrinogen are accepted by the enzyme, aromatic porphyrins are not decarboxylated (Castelfranco and Beale, 1981). The discrimination between isomers Urogen I and Urogen III in conversion into coproporphyrinogen occurs principally at the first step. Porphyrins, especially oxidation products of the substrates, have been shown to inhibit the activity of UROD (Smith and Francis, 1981). The enzyme activity is inhibited by metals such as Fe^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+} and Mg^{2+} (Chen and Miller, 1974), but is stimulated by ATP (Manohara and Tripathy, 2000).

The *UROD* gene has been isolated from tobacco and barley (Mock et al., 1995). The in vitro translational product of *UROD* was imported into pea chloroplasts and processed to 39 kDa (Mock et al., 1995). Martins et al. (2001) reported the first crystal structure of a plant (tobacco) UROD. The expression of *UROD* and the corresponding protein expression both increase during illumination in the case of barley (Mock et al., 1995) and cucumber (Mohanty et al., 2006). Transgenic tobacco plants with reduced activity of UROD accumulate uroporphyrin and other photosensitizing tetrapyrrole intermediates. When UROD is antisensed, uroporphyrin accumulates in theses plants (Mock and Grimm, 1997; Shalygo et al., 1998). These plants also display increased pathogenesis-related protein expression (Mock et al., 1999). *UROD* impairment is responsible for the phenotype of a dominant disease lesion mimic mutant of maize (Hu et al., 1998).

H. Coproporphyrinogen Oxidase

Coproporphyrinogen oxidase (CPOX) catalyses the oxidative decarboxylation of propionate side chains on rings A and B of coprogen III to yield protoporphyrinogen IX (Protogen IX). In aerobic organisms, oxygen is utilized as the sole electron acceptor for enzymatic activity. The enzyme activity was found to be activated by $Fe^{2+}$and Mn^{2+} and inhibited by EDTA and o-phenanthroline (Hsu and Miller, 1970). The corresponding gene, *CPOX*, was isolated and characterized from soybean (Madsen et al., 1993), tobacco and barley (Kruse et al., 1995a, b), *A. thaliana* (Ishikawa et al., 2001) and maize (Williams et al., 2006).

The *CPOX* mRNA is highly expressed in soybean root nodules, but less in leaves. However, no mRNA for *CPOX* was detectable in soybean roots (Madsen et al., 1993). The level of mRNA reached its maximum in developing cells and decreased drastically when cells were completely differentiated. The *CPOX* gene expression is not affected by light or heat stress in wheat and cucumber; however, the gene expression is downregulated by chill stress (Mohanty et al., 2006). In vitro protein import assays of tobacco and barley CPOX protein showed that it was imported into the pea chloroplast and accumulated in its stroma. There are two isoforms of CPOX (Cpx1and Cpx2) found in maize. The Cpx1 fused with green fluorescent protein showed that it was localized in the plastid, whereas in the case of Cpx2, it appeared to localize to mitochondria (Williams et al., 2006). However, mitochondria lack CPOX activity (Smith, 1988).

Mock et al. (1999) have shown that tobacco plants containing antisense RNA for *CPOX* are more resistant to tobacco mosaic virus. Tobacco plants containing antisense *CPOX* RNA showed decreased enzyme levels of CPOX and were characterized by growth retardation and necrosis, showing that these plants were damaged due to oxidative stress (Kruse et al., 1995a). An *A. thaliana* mutant defective in the *LIN2* gene encoding CPOX develops lesions on leaves, in a developmentally regulated and light-dependent manner (Ishikawa et al., 2001).

I. Protoporphyrinogen Oxidase

Protoporphyrinogen oxidase (PPOX) catalyzes the oxygen-dependent aromatization of protogen IX to protoporphyrin IX (PPIX). This enzyme catalyses the six-electron oxidation of protogen using a flavin cofactor, and molecular oxygen as

terminal electron acceptor (Poulson and Polglasse, 1974). Protogen is unstable and spontaneously undergoes oxidation in the presence of oxygen and its oxidation is enhanced by light (Jacobs and Jacobs, 1979). PPOX is active only if there are no polar groups on rings A and B and is quite stable towards acids and bases. Koch et al. (2004) reported the crystal structure of mitochondrial PPOX from tobacco and revealed that it contains a FAD-binding domain, a substrate-binding domain and a membrane-binding domain (Koch et al., 2004). PPOX forms a loosely associated dimer that folds into a FAD-binding and substrate-binding domain. The substrate-binding domain of PPOX also helps in forming a complex with the ferrochelatase enzyme. PPOX has been purified from barley etioplasts (Jacobs and Jacobs, 1987) and localized in the envelope (stromal side) and thylakoid membranes (stromal side) of chloroplasts (Matringe et al., 1992a; Che et al., 2000).

Manohara and Tripathy (2000) showed that envelope and thylakoid membranes failed to synthesize PPIX from the substrate ALA, whereas the stromal fraction could synthesize a little of it; however, when all three components where mixed together, the PPIX synthesizing capacity increased. Moreover, the synthesizing capacity was reduced by the addition of oxidizing agents, and the reaction was reversed in the presence of reductants like dithiothreitol (DTT). ATP increased PPIX synthesis (Manohara and Tripathy, 2000).

PPOX has been isolated from spinach, tobacco and *A. thaliana* (Narita et al., 1996; Lermontova et al., 1997; Che et al., 2000; Watanabe et al., 2001). In all the above plant species, PPOX was encoded by two genes namely *PPOX1* and *PPOX2* that were found in the chloroplast and mitochondria, respectively. In *A. thaliana*, the transcripts of plastidal PPOX were very high in leaves, whereas they were low in roots and floral buds (Narita et al., 1996). In tobacco, both transcripts were expressed synchronously throughout tobacco plant development during diurnal and circadian growth (Lermontova et al., 1997). The spinach PPOX1 preferentially located itself on the stromal side of the thylakoid membrane and on the inner envelope membrane (Che et al., 2000). The spinach *PPOX2* codes for two proteins of molecular mass of 59 kDa (PPOX2 L) and of 55 kDa (PPOX2 S), by using two in-frame start codons. PPOX2 L is associated with the chloroplast inner envelope membrane and PPOX2 S is associated with the inner mitochondrial membranes (Watanabe et al., 2001).

PPOX is highly resistant to proteases (trypsin, endoproteinase Glu-C, or carboxypeptidases A, B and Y) because the protein is folded into an extremely compact form (Arnould and Camadro, 1998). However, structurally bicyclic herbicides, i.e., diphenyl ether-type herbicides, were shown to inhibit PPOX activity in chloroplasts (Camadro et al., 1991; Matringe et al., 1992b). Dayan et al. (2008) have shown that the fungal toxin Cyperin inhibits PPOX activity. The PPOX activity substantially decreases in response to chilling-stress (Tewari and Tripathy, 1998). Transgenic rice plants expressing a *Bacillus subtilis* PPOX gene (Lee et al., 2000), soybean plants overexpressing mitochondrial PPOX (Warabi et al., 2001) and tobacco plants overexpressing the plastidial PPOX of *A. thaliana* (Lermontova and Grimm, 2000) have been shown to confer resistance to acifluorfen herbicide. However, overexpression of human PPOX in rice resulted in accumulation of PPIX and as a result necrotic spots were observed (Jung et al., 2008). In tobacco, PPOX antisense plants had more necrotic leaf lesions under low- than under high-light growth conditions (Lermontova and Grimm, 2006**)**.

J. Mg-Chelatase

Mg-chelatase catalyzes the insertion of Mg^{2+} into PPIX to form Mg-protoporphyrin (Mg-PPIX). In photosynthetic organisms, Mg-chelatase has three subunits (CHLI, CHLD and CHLH) and catalyses the insertion of Mg^{2+} in two steps; an ATP-dependent activation that is followed by an ATP-dependent chelation step (Walker and Weinstein, 1994; Walker and Willows, 1997). The optimal ATP concentration for activation is found to be higher than that of the chelation step. Out of its three subunits, CHLI is an ATPase and its ATPase activity is repressed when it forms a complex with CHLD (Jensen et al., 1999). The N-terminal halves of subunits CHLD and CHLI share high sequence similarity that suggests the CHLD subunit is also an AAA + -protein (ATPases Associated with diverse cellular Activities); however, the ATPase activity has not been detected from CHLD (Jensen et al., 1999).

The activation step requires interaction of subunits CHLD and CHLI. Six CHLI subunits are

assembled into a hexameric ring structure, which is a Mg^{2+} and ATP-dependent process, and the hexameric ring formed by six CHLD subunits is an ATP-independent process. Both the hexameric rings form the Mg–ATP–I–D complex. Subsequently, the CHLH subunit along with the Mg–ATP–I–D complex forms the holocomplex that hydrolyses ATP to release Mg^{2+}. The Mg^{2+} is subsequently coordinated into the porphyrin macrocycle to form Mg-PPIX (Walker and Willows, 1997). It has been proposed that after the formation of Mg-PPIX, the complex disassembles. The accumulation of Mg-PPIX and pheophorbide inhibits Mg-chelatase activity in pea (Popperl et al., 1997). Mg-chelatase activity and the expression of the genes encoding this enzyme are upregulated by light (Mohanty et al., 2006). However, the activity of Mg-chelatase is severely downregulated by low and high temperatures (Tewari and Tripathy, 1998).

The *CHLI* gene has been cloned from soybean (Nakayama et al., 1995); barley (Jensen et al., 1996); *A. thaliana* (Gibson et al., 1996; Rissler et al., 2002); maize (Sawers et al., 2006) and rice (Zhang et al., 2006). This protein is localized in the stroma. The *CHLI* mRNA is induced by light (Gibson et al., 1996; Jensen et al., 1996; Nakayama et al., 1998) and constitutively expressed in matured leaves and also regulated by a diurnal rhythm but not regulated by a circadian rhythm (Matsumoto et al., 2004). In *A. thaliana*, most of the *chlI* homozygous mutants have a pale green phenotype (Rissler et al., 2002). A second *CHII* gene, *CHII-2* has been identified from *A. thaliana* (Rissler et al., 2002). By using the *A. thaliana* T-DNA knockout mutant line of *chlI*, it was observed that the second *CHII* gene also contributes to Chl synthesis. Recently it was also observed that *A. thaliana CHLI2* can substitute for *CHLI1* (Huang and Li, 2009). Using transformants of tobacco with sense and antisense mRNA for *CHLI*, it has been shown that both elevated and decreased levels of *CHLI* mRNA and CHLI protein led to reduced Mg-chelatase activity and in these plants Chl synthesis was also reduced (Papenbrock et al., 2000a). CHLI could be a target for chloroplastic thioredoxin and the in vivo reduction process is light dependent (Ikegami et al., 2007).

The N-terminus of CHLD shows structural similarities with the AAA domain of CHLI and therefore it is believed it contributes towards complex formation and interaction with CHLH (Fodje et al., 2001). The *CHLD* cDNA sequence has been isolated and cloned from tobacco (Papenbrock et al., 1997) and rice (Zhang et al., 2006). The *CHLD* expression changes with respect to the diurnal changes in tobacco (Papenbrock et al., 1999). Virus-induced gene silencing of *CHLH* in tobacco led to lowering of *CHLD* and *CHLI* mRNAs along with less Chl content (Hiriart et al., 2002).

In tobacco, *CHLH* is strongly expressed in young leaves and less expressed in mature leaves and only traces of both transcripts were found in flowering organs (Kruse et al., 1997). *CHLH* expression was found to be light inducible in soybean and rice and the transcript levels were under the control of a circadian oscillation (Nakayama et al., 1998; Jung et al., 2003). The *CHLH* transcripts undergo diurnal variation in *A. thaliana* and tobacco (Gibson et al., 1996; Papenbrock et al., 1999). Depending upon the concentration of Mg^{2+} in lysis buffer, the CHLH protein migrated between stroma and the envelope membranes and was localized in the envelope membrane at very high concentrations of Mg^{2+} (Nakayama et al., 1998).

Mutants of *CHLH* have been isolated from *A. thaliana* (Mochizuki et al., 2001). Transgenic tobacco plants expressing antisense RNA for Mg-chelatase *CHLH* were Chl deficient (Papenbrock et al., 2000b). In these plants, less PPIX and heme accumulated, and a decrease in ALA synthesizing capacity was seen. Virus-induced gene silencing of *CHLH* in tobacco led to lowering of *CHLD* and *CHLI* mRNAs along with less Chl content (Hiriart et al., 2002). The rice *CHLH* mutants also showed a Chl-deficient phenotype (Jung et al., 2003; Zhang et al., 2006). In *A. thaliana*, the Mg-chelatase subunit CHLH is also regulated by retrograde signaling (Mochizuki et al., 2001). Mutation in the *CHLH* gene had repressed expression of *LHCB*. *A. thaliana* protein GUN4 regulates Mg-chelatase activity (Larkin et al., 2003; Davison et al., 2005), and promotes the interactions between CHLH and chloroplast membranes (Adhikari et al., 2009).

K. S-Adenosyl-L-Methionine:Mg Protoporphyrin IX Methyltransferase

S-adenosyl-L-methionine:Mg-PPIX methyltransferase (SAM-MgProtoMTF) catalyzes the

conversion of Mg-PPIX to Mg-protoporphyrin monomethyl ester (MPE) by transferring a methyl group to the carboxyl group of the C13-propionate side chain of Mg-PPIX (Gibson et al., 1963) where, SAM acts as a methyl group donor. This enzyme belongs to the broad family of SAM-dependent methyltransferases (Kagan and Clarke, 1994), which contains the SAM-binding domain, a seven-stranded β-sheet (Jones, 1999). The gene (*CHLM*) encoding for the SAM-MgProtoMTF has been isolated from *A. thaliana* (Block et al., 2002) and tobacco (Alawady and Grimm, 2005). It was seen that the tobacco methyltransferase physically interacts with the CHLH subunit of Mg-chelatases (Alawady et al., 2005).

The *A. thaliana* CHLM protein contains an N-terminal plastid transit sequence. The mature protein (without transit peptide) contains two functional regions, the N-terminal hydrophobic region that enhances the association of the protein with the envelope and thylakoid membranes and the C-terminal region that binds to Ado-met (Block et al., 2002). The *A. thaliana chlm* T-DNA mutant shows albino phenotype, there is accumulation of Mg-PPIX and reduction in major Chl protein complexes (Pontier et al., 2007). Downregulation of the CHLM protein in antisense *CHLM* tobacco plants results in reduced ALA-synthesis and Mg-chelatase activities (Alawady and Grimm, 2005).

L. Mg-Protoporphyrin IX Monomethylester Cyclase

Mg-protoporphyrin IX monomethylester cyclase catalyzes the formation of an isocyclic ring E of the Mg-protoporphyrins and converts MPE to Pchlide. There are two pathways for the formation of the isocyclic ring, i.e., aerobic cyclization and anaerobic cyclization. The former pathway is predominant in plants, green algae and cyanobacteria where the ketone oxygen of divinyl Pchlide (DV-Pchlide) is derived from molecular oxygen (Walker et al., 1989). The pH optimum of the cyclase activity is approximately 9.0 and the enzyme activity was found to be inhibited by CN^- and N_3^- (Whyte and Castelfranco, 1993). The studies of the cyclase reaction with the two barley mutants *xantha l* and *viridis K* revealed the need of at least two plastidal proteins (a membrane bound protein and a soluble protein) for the cyclization reaction (Walker et al., 1991; Walker and Willows, 1997). Biochemical and genetic studies have demonstrated that the gene responsible for the *xantha-l* mutant encodes a membrane-bound cyclase subunit and it needs a soluble fraction for the cyclization reaction (Rzeznicka et al., 2005).

The gene responsible for the aerobic cyclization reaction has been isolated and characterized from different plants i.e., *CHL27* from *A. thaliana* and *XANTHA l* from barley (Tottey et al., 2003; Rzeznicka et al., 2005). Antisense *A. thaliana* and tobacco plants with reduced amounts of CHL27 show chlorotic leaves with reduced abundance of all Chl proteins and accumulate MPE (Tottey et al., 2003; Peter et al., 2010). The *A. thaliana chl27* T-DNA mutant is pale green with an elevated Chl *a*/*b* ratio, and has unstacked thylakoid membranes, reduced LHCII protein and the photosynthetic activity is reduced due to a damaged Photosystem II (PS II) reaction center (Bang et al., 2008; Hansson and Jensen, 2009). In tobacco plants the co-supression of the *NTZIP gene*, which includes coding for a diiron motif, resulted in a reduced Chl level and lower photosynthetic activity (Liu et al., 2004).

M. Protochlorophyllide Oxidoreductase

Protochlorophyllide oxidoreductase (POR) is the only light-requiring enzyme of the Chl biosynthesis pathway. It catalyses the conversion of Pchlide to chlorophyllide (Chlide) by using light as a substrate along with Pchlide and NADPH. POR converts Pchlide to Chlide, by adding two hydrogen atoms at C17 and C18 on ring D. In the POR catalytic cycle, a ternary enzyme-NADPH-Pchlide complex is formed. Light energy absorbed by the Pchlide in the complex may produce torsional strain in the molecule that provides a favorable condition for hydride/hydrogen transfer from NADPH (Begley and Young, 1989). POR is a member of a large family of enzymes known as short chain dehydrogenases/reductases (SDR) (Wilks and Timko, 1995) which generally catalyze NADP(H)- or NAD(H)-dependent reactions involving hydride and proton transfers. A tyrosine (Tyr) and a lysine (Lys) residue are both conserved throughout all members of the SDR family. In POR, it was also seen that Tyr and Lys residues are important for its activity (Wilks and Timko, 1995; Lebedev et al., 2001). The Tyr may be

deprotonated, acting as a general acid to facilitate hydride transfer to or from NAD(P)+/H (Bohren et al., 1994). The proton at the C-18 position of Pchlide is derived from Tyr and the hydride transferred to the C-17 position is derived from the *pro-S* face of NADPH. The close proximity of the Lys residue is thought to allow the deprotonation step to occur at physiological pH by lowering the apparent pK_a of the phenolic group of the Tyr (Wilks and Timko, 1995**)**. The mutation of either Tyr275 or Lys279 did not completely abolish the catalytic activity of POR. However, mutation of either residue impairs formation of the ground state ternary enzyme-substrate complex, indicating their key role in substrate binding. Both residues have multiple roles in catalysis, involving formation of the ground state ternary enzyme-substrate complex, stabilization of a Pchlide excited state species and proton transfer to the reaction intermediate formed after the light reaction (Menon et al., 2009). Recently it has been demonstrated that a light-activated conformational change of the protein is necessary to activate catalysis (Heyes et al., 2008; Sytina et al., 2008). The fact that POR is light activated means the enzyme–substrate complex can be formed in the dark. This has recently been exploited by studying Pchlide reduction at low temperatures to trap intermediates in the reaction pathway (Heyes et al., 2002, 2003; Heyes and Hunter, 2004). As a result, the reaction has been shown to consist of at least three distinct steps: an initial light-driven step, followed by a series of 'dark' reactions. An initial photochemical step can occur below 200 K (Heyes et al., 2002), whereas two 'dark' steps were identified for *Synechocystis* sp. PCC 6803 POR, which can only occur close to or above the 'glass transition' temperature of proteins (Heyes et al., 2003**)**. First, $NADP^+$ is released from the enzyme and then replaced by NADPH, before release of the Chlide product and subsequent binding of Pchlide have taken place (Heyes and Hunter, 2004). Monovinyl protochlorophyllide (MV-Pchlide) and DV-Pchlide don't influence differentially the enzyme kinetics or the steps involved in the reaction pathway (Heyes et al., 2006). The secondary structure analysis of POR reveals that it has 33% alpha helix, 19% beta-sheets, 20% turn and 28% random coil. A hydrophobic loop-region has been suggested to be involved in membrane anchoring (Birve et al., 1996). Mutation studies by Dahlin et al. (1999) found that mutation in predicted α-helical regions of the protein showed the least effect on enzyme activity, whereas mutations in the predicted β-sheet regions showed an adverse effect on enzyme function. The replacement of charged amino acids by alanine in the N- and C-terminal regions of the mature protein did not affect POR assembly, whereas mutations within the central core created protein incapable of proper attachment to the thylakoid.

POR is nuclear encoded, translated as a precursor protein in the cytosol and ultimately transported into plastids (Apel, 1981). It is a peripheral membrane protein that accumulates to high level in prolamellar bodies (PLBs), where it forms a ternary complex with Pchlide and NADPH (Oliver and Griffiths, 1982) and is present at low levels in the thylakoid membranes of developing and mature plastids. It is observed that the Cys residues of POR are crucial for its membrane association (Aronsson et al., 2001) and for NADPH and pigment binding (Townley et al., 2001; Reinbothe et al., 2006). The association of POR with Pchilde results in three different spectral forms of Pchlide based on their fluorescence emission maximum (in nm): Pchlide F631 (due to the pigment structural arrangements), Pchlide F644 (due to association of POR), and Pchlide F655 (due to localization in PLBs and/or prothylakoids) (Böddi et al., 1992, 1993). Spectroscopic studies of dark-grown bean seedlings produced the idea of two forms of Pchlide, a main component with a red absorption band at 650 nm and a minor component absorbing at 636 nm (Shibata, 1957). On the basis of flash illumination, two kinds of Pchlide can be categorized: one is transformed into Chlide and is called photoactive Pchlide, whereas the other remains unchanged and is called nonphotoactive Pchlide. The latter is assembled into various complexes with different molecular structure and spectral properties (Masuda and Takamiya, 2004; Schoefs and Franck, 2003). Plastids isolated from dark-grown wheat seedlings exhibit a smaller 77 K fluorescence emission peak at 632 nm due to nonphototransformable Pchlide and a larger peak at 657 nm due to phototransformable Pchlide. The non-phototransformable Pchlide emitting at 632 nm is due to a monomeric Pchlide complex or esterified Pchlide i.e., protochlorophyll (Lindsten et al. 1988), which spontaneously dimerizes to form

$(POR\text{-}Pchlide\text{-}NADPH)_2$. The short-wavelength, monomeric Pchlide is not flash-photoactive: instead it regenerates the long wavelength Pchlide forms (Schoefs and Franck, 1993; He et al., 1994; Schoefs et al., 1994, 2000a, b). The dimer has the absorption maximum at 638 nm and emission maximum at 645 nm (Lebedev and Timko, 1999). The dimeric POR-Pchlide-NADPH complex further polymerizes to form 16-mer or larger aggregates of POR-Pchlide-NADPH complex i.e., $(POR\text{-}Pchlide\text{-}NADPH)_n$ having absorption maximum at 650 nm and emission maximum at 657 nm (Böddi et al., 1989; Wiktorsson et al., 1993) and is flash photoactive (Böddi et al., 1991). However, long-term illumination i.e., more than a minute usually converts non-active Pchlide to photo-active Pchlide. Unpublished observations from our laboratory demonstrate that the photo-transformable Pchlide (F657) rapidly decreases when 5-day-old etiolated seedlings are transferred to 42°C in the dark for 24 h. In heat-stressed seedlings the Shibata shift is substantially arrested while in chill-stress conditions, the same is partially affected.

Full-length cDNA clones of *POR* were isolated from barley (Holtorf et al., 1995; Schulz et al., 1989), oat (Darrah et al., 1990), pea (Spano et al., 1992), wheat (Teakle and Griffiths, 1993), *A. thaliana* (Armstrong et al., 1995; Benli et al., 1991; Oosawa et al., 2000), tobacco (Masuda et al., 2002), cucumber (Kuroda et al., 1995) and banana (Coemans et al., 2005). The high degree of sequence similarity among PORs from different taxonomic group implies a common mechanism of enzyme action.

A characteristic feature of *POR* accumulating in darkness is its sensitivity to illumination. The *POR* mRNA expression was also decreased (Santel and Apel, 1981). Red and far-red light treatment also inhibits *POR* mRNA expression indicating that *POR* expression is controlled by phytochrome (Apel, 1981; Batschauer and Apel 1984; Mosinger et al., 1985). The negative effect of light on the POR enzyme and its mRNA was observed in different dicotyledons like bean, pea, tomato and *A. thaliana* (Forreiter et al., 1991; Spano et al., 1992; Armstrong et al., 1995) and in the monocotyledonous plants maize and barley (Forreiter et al., 1991; Holtorf et al., 1995). However, some flowering plants have isoforms of POR. In *A. thaliana*, (Armstrong et al., 1995; Oosawa et al., 2000; Su et al., 2001; Pattanayak and Tripathy, 2002), barley (Holtorf et al., 1995; Holtorf and Apel, 1996a, b) and tobacco (Masuda et al., 2002) there are different PORs present. The N-terminus of PORA and PORB of barley etioplasts have recently been characterized (Ploscher et al., 2009). In *A. thaliana* there are three isoforms of POR, namely PORA, PORB and PORC. These three isoforms are differentially regulated by light. The level of *PORA* mRNA and protein decreases on illumination of etiolated plants (Holtroff and Apel, 1996a) while that of *PORC* increases and was dominantly expressed in both mature and immature tissues (Oosawa et al., 2000). *PORB* transcript and PORB protein levels remain constant in both dark and on illumination (Armstrong et al., 1995, Holtrof et al., 1995; Holtroff and Apel, 1996a). Both *PORB* and *PORC* of *A. thaliana* exhibit diurnal fluctuation but only the *PORB* mRNA of *A. thaliana* exhibits circadian regulation (Su et al., 2001). *PORC* mRNA and PORC protein expression also increased under high light intensity (Su et al., 2001; Masuda et al., 2003). In cucumber the levels of the *POR* mRNA increased in etiolated cotyledons when they were illuminated with continuous light (Kuroda et al., 1995; Fusada et al., 2000). The plant hormone cytokinin regulates cucumber *POR* gene expression by binding to the *cis*-elements present at the 5' region of the *POR* promoter (Fusada et al., 2005). In tobacco, two POR isoforms have been isolated, the expression of which was not negatively regulated by light, persisted in mature green tissue and showed diurnal fluctuations with a similar oscillation phase (Masuda et al., 2002).

A plant specific downstream element in the 3' untranslated region of the *PORA* transcript confers *PORA* mRNA instability, whereas it was not responsible for *PORB* mRNA degradation (Holtorf and Apel, 1996a). *POR* gene expression in cucumber is regulated by phytohormone, particularly by cytokinins and abscissic acid (Kuroda et al., 2001). In the *lip1* mutant of pea, cytokinins restored the formation of PLB and photoactive Pchlide in the dark (Seyedi et al., 2001a), but in *A. thaliana* its application results in loss of PLBs (Chory et al., 1994). In lupine, *POR* expression is also regulated by cytokinins and abscissic acid (Kusnetsov et al., 1998).

POR gene expression is also organ specific. *A. thaliana PORB* and *PORC* are expressed in all photosynthetic tissues of the mature plants but

not in root (Armstrong et al., 1995; Oosawa et al., 2000). Cucumber *POR* expression is also observed in photosynthetic tissues (Kuroda et al., 1995). Plant age also plays a crucial role in *POR* gene expression. In *A. thaliana* and barley *PORA* expression is only observed in young seedlings whereas *PORB* is expressed both in young and matured green tissue (Armstrong et al., 1995; Schunmann and Ougham, 1996). In *A. thaliana* both *PORB* and *PORC* expression is observed in green tissue (Oosawa et al., 2000; Su et al., 2001). In the leaves of dark-grown seedlings, the highest level of expression is observed 8–10 days post germinated seedlings (Spano et al., 1992). The transcript level of pea *POR* did not decrease after 48 h of light exposure. But immunoblot analysis showed there was no POR protein after 48 h of light exposure. These results suggested that pchlide reductase activity in pea is primarily regulated post-transcriptionally, most likely at the level of translation initiation/elongation or protein turnover (Spano et al., 1992).

Degradation of PORA is specific and controlled by nuclear-encoded proteases. The mechanism of light-activation of protease expression is unknown. Mapleston and Griffiths (1980) observed that POR activity decreased after illumination. Reinbothe et al. (1995) showed that the barley precursor of PORA and Pchlide (pPORA-Pchlide) complex was resistant to protease treatment and independent of the presence or absence of NADPH. In contrast, the pPORA-Chlide complex was rapidly degraded. The pPORA protein without its substrate or products was less sensitive to proteolysis than the pPORA-Chlide complex suggesting that both substrate binding and product formation had caused differential changes in protein conformation (Reinbothe et al., 1995). PORB was not degraded by the protease. The PORA degrading protein is assumed to be nuclear encoded, energy dependent and a plastid localized protein in barley (Reinbothe et al., 1995). But the study of post-import degradation of radiolabeled barley pPORA and pPORB on incubation with stroma enriched fractions from etiolated and light grown barley or wheat did not show any protease activity (Dahlin et al., 2000).

In vivo functions of each POR isoform have been extensively studies in *A. thaliana* seedlings. In the *det340* (de-etiolated) mutant of *A. thaliana*, *PORA* is constitutively down regulated, resulting in a lack of PORA and photoactive Pchlide-F655 in dark-grown seedlings and the plants are susceptible to photooxidative damage at extremely low light intensities (Lebedev et al., 1995). The photoprotective mechanism of PORA is also described by Buhr et al. (2008). *A. thaliana* etiolated seedlings grown under continuous far-red light are unable to green when subsequently transferred to white light, which is called far-red blocking of the greening process. This process involves depletion of PORA, partial depletion of PORB and the concomitant loss of PLBs resulting in photo-oxidative damage (Barnes et al., 1996; Runge et al., 1996). From these studies, PORA has been proposed to play a special role in the formation of POR ternary complexes containing photoactive Pchlide-F655, PLB assembly, and protection against photo-oxidative damage caused by non-photoactive Pchlide (Reinbothe et al., 1999). However, overexpression of *PORA* and *PORB* in specific mutants overcame the photo-oxidative damage (Sperling et al., 1997, 1998). Franck et al. (2000) examined in detail redundant roles of PORA and PORB in etioplast differentiation by manipulating the total POR content and the PORA to PORB ratio of *A. thaliana* seedlings using antisense and overexpression approaches.

It was recently observed that overexpression of a cyanobacterial POR protein in the *A. thaliana porA* mutant could restore PLB formation. However, the amount of photoactive Pchlide in the etioplasts of the complementing lines was retained at a low level as in the parent *PORA* knockdown mutant (Masuda et al., 2009). The *lip1* mutant of pea lacked PLBs but could store PLBs if treated with cytokinin (Seyedi et al., 2001a): but unlike the *A. thaliana* mutant, it did not undergo photooxidative damage (Seyedi et al., 2001b). The physiological function of specific POR isoforms in vivo has been well characterized in knockout mutants of *A. thaliana* (Frick et al., 2003; Masuda et al., 2003). Single *POR* mutants display no obvious phenotypes at the whole plant or chloroplast ultrastructural levels, except that *PORB* mutants have less extensive etioplast inner membranes. However, the *PORB/PORC* double mutant, which displayed a seedling-lethal *xantha* phenotype at the cotyledon stage, contained only a small amount of Chl *a*, and possessed chloroplasts with mostly unstacked thylakoid membranes (Frick et al., 2003). Masuda et al. (2003)

focused on the greening process of *por* mutants, and showed that the etiolated *PORB* mutant seedling was able to green to a similar extent as the wild type, and the greening of the *porc* mutant was repressed under high light conditions.

From a molecular evolutionary perspective, the light-dependent POR (LPOR) enzymes are extraordinarily highly conserved. Comparative analysis of complete plastid genome sequences indicate that LPOR genes were lost from the plastid at some point during early evolution (Martin et al., 1998, 2002), and analysis of LPOR proteins in species of conifer show evidence for loss of enzyme activity (Kusumi et al., 2006). The discovery of genes for LPOR in the plastid genomes of diverse cryptophyte algae suggests that these genes have been lost relatively recently.

N. Divinyl Reductase

In all photosynthetic organisms Pchlide and Chlide are originally formed as 3,8-divinyl derivates. The 8-vinyl reductase reduces the 8-vinyl group on the tetrapyrrole to an ethyl using NADPH as the reductant. This enzymatic activity has been detected in isolated chloroplasts of barley (Tripathy and Rebeiz, 1988), plastid membranes from cucumber (Parham and Rebeiz, 1995), and also in solubilized crude extracts derived from etiolated barley leaves (Kolossov and Rebeiz, 2001). It has been demonstrated in vitro that the monovinyl (MV) and divinyl (DV) Chl biosynthesis reactions may operate in parallel (Tripathy and Rebeiz, 1986). However, the mutant of maize (*Zea mays*) that accumulated only DV-Chl instead of MV-Chl and capable of photosynthetic growth with DV-Chl suggests that a single gene product is responsible for the reduction of the vinyl group of Chlide (Bazzaz, 1981). Nagata et al. (2005) followed by Nakanishi et al. (2005) isolated a mutant of *A. thaliana* which accumulates DV-Chl. By map-based cloning they found out that the gene is 8-vinyl reductase. The recombinant protein was successfully tested for the conversion of the C8-vinyl group of Chlide to an ethyl group on ring B. The 3,8-divinyl-chlide *a* is the major substrate of divinyl reductase (DVR) (Nagata et al., 2007). The mutant is pale green and the Chl *a*/*b* ratio varies in between 6 and 10 depending on the developmental stage and growth conditions. This mutant is capable of photosynthesizing and growing under low-light conditions (70–90 μmole photons m^{-2} s^{-1}); but rapidly dies under high light conditions (1,000 μmole photons m^{-2} s^{-1}) (Nagata et al., 2005). The thylakoid membranes were organized in a disorderly fashion having no distinct grana stacks in the mutant but no distinct differences in the size and the number of chloroplasts between the wild type and the mutant were observed. Starch granules were not found in the mutant chloroplasts, suggesting the reduction of photosynthetic activity in the mutant (Nakanishi et al., 2005). The transcript level of *DVR* expression is high in leaves, stems and flower buds, and low in roots.

O. Chlorophyllide a Oxygenase

Chlorophyllide *a* oxygenase (CAO) converts chlorophyllide (Chlide) *a* to Chlide *b*. During conversion of Chlide *a* to Chlide *b* the electron is transferred from the Rieske center to the mononuclear iron with subsequent activation of molecular oxygen for oxygenation of the Chlide *a* methyl group (Beale and Weinstein, 1990; Porra et al., 1993). Chlide *b* is synthesized by oxidation/conversion of the methyl group on the D ring of the porphyrin molecule to a formyl group at that position. The CAO enzyme contains domains for a [2Fe-2S] Rieske center and for a mononuclear nonheme iron-binding site and has a tyrosine radical (Eggink et al., 2004). The conserved Rieske center and non-heme-iron binding motifs of CAO are likely to be involved in electron transport from ferredoxin to molecular oxygen. The recombinant CAO protein catalyzes Chlide *a* to Chlide *b* in the presence of NADPH and reduced ferredoxin (Oster et al., 2000). However, Pchlide *a* is not a substrate for the CAO enzyme (Oster et al., 2000).

The *CAO* gene was first isolated from *Chlamydomonas reinhardtii* by Tanaka et al. (1998) and has also been isolated from *A. thaliana* (Espineda et al., 1999) and rice (Lee et al., 2005). Both transcript and protein levels of CAO increased when *A. thaliana* plants were transferred from moderate to shade light (Harper et al., 2004). Rice has two CAO isoforms encoded by *OsCAO1* and *OsCAO2* that are differentially regulated in light and dark. The *OsCAO1* transcript is less in the dark and induced by light whereas the *OsCAO2* mRNA levels are higher in dark conditions, and its transcripts are reduced by exposure to light (Lee et al., 2005).

Overexpression of the *CAO* gene in *A. thaliana* led to an increase in the Chl *b* level leading to reduction of the Chl *a*:*b* ratio from 2.85 to 2.65 in full green rosette leaves and at the same time there is 10–20% increase in antenna size (Tanaka et al., 2001). Overexpression of *A. thaliana CAO* in *Synechosystis* sp. PCC 6803 resulted in production of Chl *b* up to about 10% of total Chl content and the resulting Chl *b* pigments efficiently incorporated into the Photosystem I Chl-protein complex (Satoh et al., 2001). Simultaneous overexpression of both *CAO* and *LHCII* genes in *Synechosystis* sp. PCC 6803 resulted in an increase in Chl *b* content up to 80% of total Chl (Xu et al., 2001). Overexpression of *CAO* in tobacco plants resulted in a decreased Chl *a*/*b* ratio i.e., from 3.38 in wild-type plants to 2.33 in transgenic plants when grown in high light (Pattanayak et al., 2005). High light grown transgenic *A. thaliana* plants also showed a decreased Chl *a*/*b* ratio under high light (Tanaka and Tanaka, 2005). When the *CAO* gene of *Prochlorothrix holandica* was overexpressed in *A. thaliana* it was observed that approximately 40% of Chl *a* of the core antenna complexes was replaced by Chl *b* in both photosystems (Hirashima et al., 2006). The CAO sequence has been classified into four parts, the N-terminal sequence predicted to be a transit peptide, the subsequent conserved sequence unique in land plants (A-domain), a less-conserved sequence (B-domain) and the C-terminal conserved sequence common in chlorophytes and prochlorophytes (C-domain) (Nagata et al., 2004). The C-domain is sufficient for catalytic activity and the N-terminal 'A' domain confers protein instability by sensing the presence of Chl *b* and regulates the accumulation of the CAO protein (Yamasato et al., 2005). Chloroplast Clp protease is involved in regulating Chl *b* biosynthesis through the destabilization of CAO in response to the accumulation of Chl *b* (Nakagawara et al., 2007). The B domain alone is not involved in the regulation of CAO protein levels (Sakuraba et al., 2007). Further work on domain analysis also indicated that transgenic *A. thaliana* plants overexpressing the A-domain-deleted CAO accumulated an excess amount of Chl *b* during greening and the etiolated transgenic plants either died or were retarded when exposed to continuous light immediately after etiolation (Yamasato et al., 2008). This was most likely due to deregulated Chl b synthesis that reduced the energy transfer rate between photosynthetic pigments (Sakuraba et al., 2010).

P. Chlorophyll b Reductase

Chl *b* reductase catalyzes the conversion of Chl *b* to Chl *a*. It reduces the formyl group of Chl *b* to a hydroxymethyl group. It was observed that barley etioplasts had Chlide *b* reductase activity and the enzyme needs NADPH and reduced ferredoxin for its activity (Scheumann et al., 1996; 1999). The gene encoding Chl *b* reductase was isolated from rice and it belongs to a family of short-chain dehydrogenase/reductases (Kusaba et al., 2007). It encodes a protein of 504 amino acids and contains a dinucleotide binding motif (TGXXXGXG) and a catalytic site (YXXXK) and uses NADPH as a cofactor. Interestingly, two genes for Chl *b* reductase were found in the genomes of *A. thaliana* and rice (Kusaba et al., 2007; Sato et al., 2009). It was also observed that disruption of the genes encoding Chl *b* reductase in *A. thaliana* resulted in non-degradation of Chl *b* and LHCII (Horie et al., 2009).

The interconversion of Chl *b* and Chl *a* form a "chlorophyll cycle" which starts with Chlide *a* (Fig. 3.2). It has been observed that in presence of recombinant CAO enzyme, the Chlide *a* gets converted to Chlide *b* where NADPH, molecular oxygen and ferredoxin are used (Oster et al., 2000). In this in vitro assay, even a small amount of 7-hydroxymehtyl Chlide *a* was formed. When the 7-hydroxymehtyl Chlide *a* was used as a substrate for the in vitro enzymatic assay, the recombinant enzyme also efficiently converted 7-hydroxymehtyl Chlide *a* to Chlide *b* (Oster et al., 2000). Then, Chl synthase converts Chlide *b* into Chl *b*. Chl b is then converted to hydroxymethyl Chl *a* by the enzyme Chl *b* reductase (Kusaba et al., 2007). The enzyme converts the formyl group of Chl *b* to a hydroxymethyl group using NADPH as a reductant. 7-Hydroxymethyl-Chl *a* reductase catalyzes the reduction of the hydroxylmethyl group of 7-hydroxymethyl Chl *a* to a methyl group to form Chl *a*. The gene for this enzyme is yet to be identified.

Q. Geranyl-Geranyl Reductase

Geranyl-geranyl reductase catalyses the reduction of geranyl geranyl diphosphate to phytyl diphosphate. The cDNA encoding a pre-geranyl-geranyl reductase from *A. thaliana* has been isolated and characterized (Keller et al., 1998). The recombinant protein catalyzed the reduction

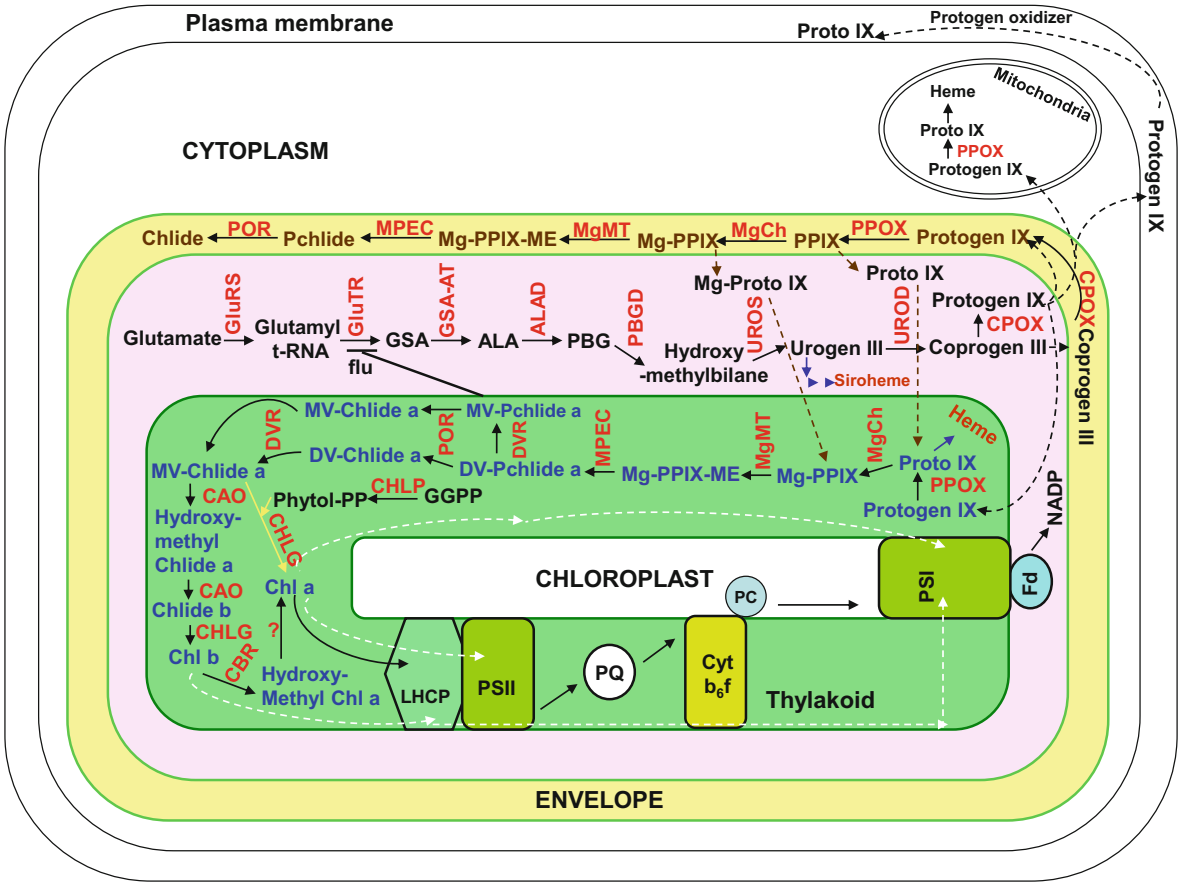

Fig. 3.2. Intraplastidic chlorophyll biosynthesis route in green plants. All abbreviations in this figure are defined in the *Abbreviations* list for this chapter.

of geranyl-geranyl-Chl *a* into phytyl-Chl *a* as well as the reduction of free geranyl-geranyl diphosphate into phytyl diphosphate, suggesting that this is a multifunctional gene. The transcript level is up-regulated during etioplast to chloroplast and chloroplast to chromoplast development (Keller et al., 1998).

The decrease in the expression of *CHLP* coding for geranyl-geranyl reductase affects the Chl and tocopherol contents in tobacco (Tanaka et al., 1999). The reduced tochopherol and Chl contents in *CHLP* antisense plants resulted in the reduction of electron transport chains and PS II activity. There is also more lipid peroxidation product in *CHLP* antisense plants. Havaux et al. (2003) found the accumulation of xanthophylls cycle pigments in *CHLP* antisense plants which could be a compensatory mechanism for tochopherol deficiency. The *CHLIP* transcript levels in peach was abundant in Chl-containing tissues and flower organs but barely detected in roots and mesocarp of the ripening fruits (Giannino et al., 2004).

R. Chlorophyll Synthase

Chlorophyll synthase catalyzes the esterification of Chlide *a* and Chlide *b* to Chl (Rüdiger et al., 1980). Pchlide is not the substrate for this enzyme, which indicates that reduction of the 17, 18 double bond on ring D is essential for esterification (Benz and Rüdiger, 1981b). Compounds which have the 13(2)-carbomethoxy group at the same side of the macrocycle as the propionic side chain of ring D are neither substrates nor competitive inhibitors (Helfrich et al., 1994). Only compounds having the 13(2)-carbomethoxy group at the opposite site are substrates for the enzyme. Esterification kinetics of Chlide is a rapid phase, leading to esterification of 15% of total Chlide within 15–30 s, followed by a lag-phase

of nearly 2 min and a subsequent main phase (Schmid et al., 2002; Domanskii et al., 2003). It has been shown that the conversion of Chlide to Chl is a four-step process including three intermediates i.e., Chlide geranylgeraniol, Chlide dihydrogeranylgeraniol and Chlide tetrahydrogeranylgeraniol before the formation of Chlide phytol or Chl (Shoefs and Bertrand, 2000).

In etioplasts, geranyl-geranyl pyrophosphate (GGPP) is used as a substrate (Rüdiger et al., 1980), while in chloroplasts the preferential substrate is phytyl diphosphate (PhPP) (Soll et al., 1983). Chl synthase in chloroplast thylakoid membranes incorporates phytol in the presence of ATP and a stromal kinase (Benz and Rüdiger, 1981a). The enzyme was not affected by the developmental stage of the plastids. In etiolated wheat, the enzyme was found in latent form in PLBs (Lindsten et al., 1990).

The *CHLG* gene, encoding Chl synthase, was isolated from *A. thaliana*, *Avena sativa*, rice and tobacco. (Gaubier et al. 1995; Schmid et al., 2001; Wu et al., 2007; Shalygo et al., 2009). In *A. thaliana*, the *CHLG* transcript is only detected in green or greening tissues (Gaubier et al., 1995), whereas in *A. sativa*, this gene is expressed equally both in dark- and light-grown seedlings (Schmid et al., 2001). Sequence analysis of EST cDNAs from rice yielded a putative Chl synthase homolog (Scolnik and Bartley, 1996); however, the biochemical properties and physiological functions remained unknown until Wu et al. (2007) characterized a rice mutant with inactivated *CHLG*. The young rice Chl synthase mutant plants show yellow-green leaves with decreased Chl synthesis (Wu et al., 2007). In the mutated plants, there is accumulation of tetrapyrrole intermediates, reduced expression of *lhcb1* and delayed chloroplast development. However, the antisense expression of the tobacco *CHLG* gene did not result in the accumulation of Chlide, but there was reduced ALA synthesizing capacity (Shalygo et al., 2009).

III. Intraplastidic Route of Chlorophyll Biosynthesis

Chloroplasts have two limiting envelope membranes, the outer membrane in contact with the cytoplasm of the cell and the inner membrane surrounding the stroma. The inner envelope membrane is essential for the biosynthesis of plastid components such as glycolipids and prenylquinones (Douce and Joyard, 1990). In addition, the envelope membrane plays a key role in the sorting of plastid proteins that are coded by the nuclear genome. The envelope also plays a significant role in Chl degradation. Chlorophyllase and Mg-dechelatase are present in the inner envelope membrane (Matile et al., 1996, 1999).

Chl is bound to pigment-protein complexes of thylakoid membranes. Chl and its precursors are essential for chloroplast development and nuclear gene expression (Eichacker et al., 1990; Jilani et al., 1996; Kropat et al., 1997, 2000). Interplay of envelope, stroma and thylakoids is shown for PPIX (Proto IX in Fig. 3.2) synthesis and enzymes responsible for conversion of ALA to PPIX i.e., ALA dehydratase, porphobilinogen deaminase, uroporphyrinogen decarboxylase and coproporphyrinogen oxidase are mostly located in the stromal phase (Manohara and Tripathy, 2000). All subsequent steps of Chl biosynthesis are catalyzed by membrane-bound or membrane-associated enzymes (Joyard et al., 1990; Matringe et al., 1992a; Manohara and Tripathy, 2000). Pchlide (Pineau et al., 1986) and PPIX (Mohapatra and Tripathy, 2002, 2003, 2007) are present both in the thylakoid and envelope membranes. Out of the total plastidic Pchlide, envelope membranes contained 1.5%, thylakoids have the maximum 98.48% and stroma has a trace fraction of 0.02%. Distribution of the Mg-PPIX and its monoester was 89.0% in thylakoids, 10.0% in stroma and 1.0% in envelope. A substantial fraction (33.77%) of plastidic PPIX was partitioned into stroma. Envelope contained 0.66% and thylakoids had 65.57% of the total plastidic PPIX pool. Had there been trafficking of Pchlide from the envelope to thylakoids or vice versa, significant amounts of Pchlide should have been present in the stroma. Therefore, in the developed chloroplast, Pchlide present in the envelope may be synthesized de novo independent of thylakoids. During greening Pchlide content (mg protein)$^{-1}$ decreased in thylakoids and increased in envelope membranes (Barthelemy et al., 2000).

ALA biosynthetic enzymes are located in the stroma (Kannangara et al., 1994). Enzymes responsible for conversion of ALA to protogen IX i.e., ALA dehydratase, porphobilinogen deaminase,

and uroporphyrinogen decarboxylase are mostly located in the stromal phase (Manohara and Tripathy, 2000). Coproporphyrinogen oxidase activity is predominantly observed in the stroma and a small fraction in the envelope (M.S. Manohara and B.C. Tripathy, unpublished). Once synthesized in the stroma, protogen IX may associate either to the envelope or thylakoid membranes where it is oxidized to PPIX. The presence of PPOX is shown both in the envelope and thylakoid membranes (Matringe et al., 1992a). Part of plastidic protogen IX migrates to mitochondria where it is oxidised by protoporphyrinogen oxidase II to PPIX, the substrate of heme synthesis (Lermontova et al., 1997). A substantial amount of PPIX present in the stroma may be due to its own diffusion from the site of its synthesis i.e., envelope and thylakoids. The next step in Chl biosynthesis is the conversion of PPIX to Mg-PPIX by Mg-chelatase. The association of MPE with envelope membranes suggests that Mg-chelatase is functional in envelope membranes. It is often argued that Chl biosynthetic enzymes present in envelope membranes are protein translocation intermediates. As there is an obligate requirement of three subunits of Mg-chelatase i.e., CHLD, CHLH and CHLI to assemble in a definite proportion to form the functional enzyme (von Wettstein et al., 1995; Kannangara et al., 1997; Papenbrock et al., 1997), it is unlikely that translocation intermediates of Mg-chelatase enzyme could mediate the synthesis of Mg-porphyrin. PPIX is converted to Mg-PPIX in the stroma in close association with inner envelope membrane or thylakoid. This explains the presence of MPE in the envelope membrane, stroma and thylakoids.

It is likely that the amphiphilic tetrapyrrole Mg-PPIX subsequently migrates to both envelope and thylakoids where it is independently esterified to MPE and subsequently metabolised to Pchlide. The POR protein is present both in the envelope and thylakoid membranes and phototransforms Pchlide to Chlide. As Chl synthetase is absent from envelope memembrane, Chl synthesis is not advanced in the envelope membrane. Chlide present in the envelope membrane may play an important role in the stabilization of light-harvesting proteins during their post-translational protein import. Although envelope membranes participate in tetrapyrrole biosynthesis leading to the synthesis of Chlide, the major role is played by the thylakoid membranes during late steps of Chl biosynthesis. The detailed intraplastidic Chl biosynthesis pathway is shown in Fig. 3.2.

IV. Regulation of Mg and Fe Branches of Tetrapyrrole Biosynthesis

In photosynthetic organisms Chl and heme biosynthesis is tightly regulated at various levels in response to environmental adaptation and plant development. The formation of ALA is the key regulatory step and provides adequate amounts of the common precursor molecule for the Mg and Fe branches of tetrapyrrole biosynthesis. ALA formation declines immediately after transition from light to dark and is correlated with an immediate accumulation of Pchlide in darkness. Pathway control prevents accumulation of metabolic intermediates and avoids photo-oxidative damage. Dark repression of ALA formation relies more on rapid post-translational regulation in response to accumulating Pchlide than on changes in nuclear gene expression (Stobart and Ameenbukhari, 1984; 1986; Richter et al., 2010).

PPIX may be acted upon by ferro-chelatase to form Fe-PPIX leading to heme synthesis. This is the second known branch point of tetrapyrrole biosynthesis after methylation of Urogen III leading to siroheme synthesis (Fig. 3.2). Ferrochelatase is inhibited by ATP (Cornah et al., 2002). During the day when ATP levels are higher, the magnesium branch of the pathway would be favored as Mg-chelatase needs ATP for Mg-PPIX synthesis (see below). Conversely, in the night the steady-state level of total heme increases in tobacco plants during the dark period and corresponds to the Fe-chelatase activity (Papenbrock et al., 1999). In *A. thaliana*, the Mg-chelatase subunit CHLH reaches a peak at the beginning of the light phase and ferrochelatase reaches a peak at the end of the light phase, indicating a diurnal regulation of Mg and Fe branches of tetrapyrrole biosynthesis (Harmer et al., 2000). NADPH-dependent thioredoxin reductase could be involved in redox regulation or protection of chlorophyll biosynthetic enzymes (Stenbaek and Jensen, 2010). The most important external modulator of the tetrapyrrole pathway in plants is light. In angiosperms, it plays a direct role in the Chl branch as light-dependent POR

phototransforms most of Pchlide to Chlide and this removes Pchlide-mediated feedback inhibition of ALA biosynthesis leading to increased availability of PPIX for the augmented ATP-dependent (light-dependent) Mg-chelation reaction. DELLAs (gibbralic acid-INSENSITIVE [GAI], REPRESSOR OF GA1-3 [RGA], RGA-LIKE1 [RGL1], RGL2, and RGL3) are a subfamily of transcriptional regulators that repress GA-mediated responses, and GA overcomes this DELLA-mediated restraint by stimulating the polyubiquitination of DELLAs that integrate many other environmental signal inputs in addition to light through their interaction with phytochrome interacting factors (PIFs) (Achard et al., 2006). DELLAs may regulate the levels of POR and protochlorophyllide in the dark to protect etiolated seedlings against photooxidative damage during initial light exposure (Cheminant et al., 2011).

V. Heterogeneity of Chlorophyll Biosynthesis

In higher plants i.e., angiosperms, Chls are synthesized during the day. Most of the DV-Pchlide (Tripathy and Rebeiz, 1985) synthesized during the day is immediately acted upon by POR and phototransformed to DV-Chlide. The latter is immediately fully converted to MV-Chlide (Duggan and Rebeiz, 1982; Nagata et al., 2007) that is subsequently phytylated to form MV-Chl. During the day under steady-state conditions even in the presence of POR and light, not all of the divinyl Pchlide pool is phototransformed to DV-Chlide. This pool of DV-Pchlide is slowly acted upon by divinyl reductase and converted to MV-Pchlide. The latter is subsequently phototransformed by POR to MV-Chlide that is esterified to form MV-Chl. During the night most of the DV-Pchlide pool that accumulates is slowly converted to MV-Pchlide by DVR (Tripathy and Rebeiz, 1988). At day break MV-Pchlide is immediately photo-transformed by POR to MV-Chlide. DVR activity varies between different species. It reduces DV-Pchlide to MV-Pchlide very quickly in barley and wheat but does so very slowly in cucumber and a number of other plant species (Carey et al., 1985). Therefore, various species have different MV- to DV-Pchlide ratios. As DVR speedily converts DV-Chlide to MV-Chlide, we do not see a mixture of MV- and DV-Chlide or MV- and DV-Chl in green plants. This Chl biosynthetic heterogeneity needs to be further understood.

VI. Evolution of Chlorophylls

Porphyrins are found in early rocks and meteorites (Hodgson and Baker, 1964). Synthesis of porphyrins, from pyrroles and aldehydes in an abiotic environment in aqueous clay suspension, has demonstrated the potential for non-enzymatic anabolic events that may have taken place before life originated (Cady and Pinnavaia, 1978). These porphyrins present in early Earth history were available for life when life came into existence from matter. As several porphyrins were needed for the sustenance of life, their synthesis was enhanced by the advent of enzymes (Larkum, 1991). Among tetrapyrroles, uroporphyrinogen, coproporphyrinogen, protoporphyrinogen and protoporphyrin were probably the earliest evolved pigments as they are needed for heme, cytochrome, biliproteins, phytochrome, vitamin B12, and F430 synthesis to support respiration and several metabolic processes (Hodgson and Ponamperuma, 1968). Early reaction centers in anaerobic prokaryotes could be porphyrins synthesized from oxidation of porphyrinogens mediated by UV light. On the early Earth, in the absence of oxygen, a lot of UV light could penetrate the atmosphere and UV light and blue light could have driven early photoreactions.

The Fe^{2+} ion was abundant in the anaerobic conditions early in Earth's history and Fe^{2+} could have been spontaneously inserted into a PPIX moiety to form heme. Formation of Bchl or Chl required the insertion of Mg^{2+} rather than Fe^{2+} to PPIX and this may have taken place non-enzymatically in places of high Mg^{2+} availability leading to the synthesis of Mg-PPIX, a first branch point leading to Bchl or Chl synthesis. Mg-porphyrins were favored over Fe-porphyrins because of their longer excited lifetime and were more efficient in inter-molecular energy transfer. With the advent of enzymes such the bacterial proteins BchlD, BchlH and BchlI or the eukaryotic enzymes CHLD, CHLH and CHLI during

the course of evolution, the enzymatic catalysis of Mg-insertion into a PPIX moiety by the assembly of these proteins to form the holoenzyme Mg-chelatase that required ATP hydrolysis was favored. Moreover, a fifth isocyclic ring was added to synthesize Pchlide, favoring capture of solar energy from a broader visible spectral range. Pchlide under early anaerobic conditions may have been converted to form the chlorin, Chlide by a light-independent Pchlide reductase enzyme made up of 3 subunits i.e., Bchl, BchN and BchB or CHLL, CHLN and CHLB. It is likely that from here Bchl *a* and Chl *a* branches diverged. It is possible that the early oxygenic photosynthetic bacteria i.e., chloroxybacteria evolved from an ancient group of anoxygenic photosynthetic bacteria and contained both Bchl and Chl (Larkum, 1999). Later the Chl branch was favored in the oxygen rich atmosphere.

Chl *d* probably evolved in *Acaryochloris marina* (Miyashita et al., 1996) from early Chl biosynthetic intermediates to harness solar energy at a longer wavelength than that of Chl *a* probably being dictated by its niche. Among prokaryotes, the prochlorophytes contain both Chl *a* and Chl *b* and have a relatively larger proportion of Chl *b* leading to a reduced Chl *a*/*b* ratio typically found in eukaryotic green algae and plants. The early marine environment that had reduced light intensity in deeper layers probably favored the synthesis of more accessory pigments such as Chl *b*. In eukaryotic Chl *b*-containing organisms, including chlorophytes and higher plants, the Chl *a*/*b* ratio is larger than in prochlorophytes due to reduced synthesis of Chl *b*. This was achieved by adding the N-terminal regulatory Chl *b*-sensing A domain to CAO that leads the enzyme into the degradation pathway. This modulates the level of CAO that converts Chlide *a* to Chlide *b* (Nagata et al., 2004; Nakagawara et al., 2007; Tanaka and Tanaka, 2007).

VII. Future Prospects

Although we have a fairly complete understanding of the mechanisms required for Chl biosynthesis, apart from knowing all the genes involved in the synthesis of the cyclopentanone ring (ring E), Chl *d* and conversion of Chl *b* to Chl *a*, our knowledge of Chl biosynthetic heterogeneity, regulation of Chl biosynthesis, their modulated insertion to light-harvesting complexes and different photosystems is far from complete. Chls and their biosynthetic intermediates are directly involved in the generation of $^1O_2$ or O_2^- via type II or type I photosensitization reactions (Chakraborty and Tripathy, 1992; Tripathy et al., 2007). Understanding the regulation of Chl biosynthesis and the insertion Chl into photosynthetic protein complexes will be important for the future minimization of the generation of reactive oxygen species in plants. Besides it will enhance our knowledge of the biology of energy capture and transfer among photosynthetic pigments that could be utilized in artificial photosynthesis research. Evolution of Chl synthesis is little understood and needs to be studied further.

Acknowledgements

Authors wish to thank Professor Govindjee for critically reading the manuscript and encouragement and help in editing the manuscript.

References

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J and Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 311: 91–94

Adhikari ND, Orler R, Chory J, Froehlich JE and Larkin RM (2009) Porphyrins promote the association of genomes uncoupled 4 and a MG-chelatase subunit with chloroplast membranes. J Biol Chem 284: 24783–24796

Alawady AE and Grimm B (2005) Tobacco Mg protoporphyrin IX methyltransferase is involved in inverse activation of Mg porphyrin and proto heme synthesis. Plant J 41: 282–290

Alawady A, Reski R, Yaronskaya E and Grimm B (2005) Cloning and expression of the tobacco CHLM sequence encoding Mg protoporphyrin IX methyltransferase and its interaction with Mg chelatase. Plant Mol Biol 57: 679–91

Apel K (1981) The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). Phytochrome-induced decrease of translatable mRNA coding for the NADPH: protochlorophyllide oxidoreductase. Eur J Biochem 120: 89–93

Armstrong GA, Runge S, Frick G, Sperling U and Apel K (1995) Identification of NADPH: protochlorophyllide oxidoreductase A and B: A branched pathway for light-dependent chlorophyll biosynthesis in *Arabidopsis thaliana*. Plant Physiol 108: 1505–1517

Arnould S and Camadro JM (1998) The domain structure of protoporphyrinogen oxidase, the molecular target of diphenyl ether-type herbicides. Proc Natl Acad Sci USA 95: 10553–10558

Aronsson H, Sundqvist C, Timko MP and Dahlin C (2001) The importance of the C-terminal region and Cys residues for the membrane association of the NADPH:protochlorophyllide oxidoreductase in pea. FEBS Lett 502: 11–15

Ayliffe MA, Agostino A, Clarke BC, Furbank R, von Caemmerer S and Pryor AJ (2009) Suppression of the barley uroporphyrinogen III synthase gene by a Ds activation tagging element generates developmental photosensitivity. Plant Cell 21: 814–831

Bang WY, Jeong IS, Kim DW, Im CH, Ji C, Hwang SM, Kim SW, Son YS, Jeong J, Shiina T and Bahk JD (2008) Role of *Arabidopsis* CHL27 protein for photosynthesis, chloroplast development and gene expression profiling. Plant Cell Physiol 49:1350–63

Barnes SA, Nishizawa NK, Quaggio RB, Whitelam GC and Chua N-H (1996) Far-red light blocks greening of *Arabidopsis* seedlings via a phytochrome A-mediated change in plastid development. Plant Cell 8: 601–615

Barthélemy X, Bouvier G, Radunz A, Docquier S, Schmid GH and Franck F (2000) Localization of NADPH-protochlorophyllide reductase in plastids of barley at different greening stages. Photosynth Res 64: 63–76

Batschauer A and Apel K (1984) An inverse control by phytochrome of the expression of two nuclear genes in barley (*Hordeum vulgare* L.). Eur J Biochem 143: 593–597

Bazzaz MB (1981) New chlorophyll chromophores isolate from a chlorophyll deficient mutant of maize. Photobiochem Photobiophys 2: 199–207

Begley TP and Young H (1989) Protochlorophyllide reductase. 1. Determination of the regiochemistry and the stereochemistry of the reduction of protochlorophyllide to chlorophyllide. J Am Chem Soc 111: 3095–3096

Beale SI and Weinstein JD (1990) Tetrapyrrole metabolism in photosynthetic organisms. In: Dailey HA (eds) Biosynthesis of Heme and Chlorophyll. pp 287–291. McGraw-Hill, New York

Benli M, Schulz R and Apel K (1991) Effect of light on the NADPH-Pchlide oxidoreductase (POR) of *A. thaliana*. Plant Mol Biol 16: 615–625

Benz J and Rüdiger W (1981a) Incorporation of 1-14 C-isopentenyldiphosphate, geraniol and farnesol into chlorophyll in plastid membrane fractions of *Avena sativa* L. Z Pflanzenphysiol 102: 95–100

Benz J and Rüdiger W (1981b) Chlorophyll biosynthesis: various chlorophyllides as exogenous substrates for chlorophyll synthetase. Z Naturforsch 36c: 51–57

Bhattacharjee S and Mukherjee AK (2003) Heavy metals alter photosynthetic pigment profiles as well as activities of chlorophyllase and 5-aminolevulinic acid dehydratase (ALAD) in *Amaranthus lividus* seedlings. J Environ Biol 24: 395–399

Birve SJ, Selstam E and Johansson LB (1996) Secondary structure of NADPH: protochlorophyllide oxidoreductase examined by circular dichroism and prediction methods. Biochem J 317: 549–555

Block MA, Tewari AK, Albrieux C, Marechal E and Joyard J (2002) The plant S-adenosyl-L-methionine: Mg-protoporphyrin IX methyl transferase is isolated in both envelope and thylakoid chloroplast membranes. Eur J Biochem 269: 240–248

Böddi B, Lindsten A, Ryberg M and Sundqvist C (1989) On the aggregational states of protochlorophyllide and its protein complexes in wheat etioplasts. Physiol Plant 76: 135–143

Böddi B, Ryberg M and Sundqvist C (1991) The formation of a short-wavelength chlorophyllide form at partial phototransformation of protochlorophyllide in etioplast inner membranes. Photochem Photobiol 53: 667–673

Böddi B, Ryberg M and Sundqvist C (1992) Identification of four universal protochlorophyllide forms in dark-grown leaves by analyses of the 77 K fluorescence emission spectra. J Photochem Photobiol B Biol 12: 389–401

Böddi B, Ryberg M and Sundqvist C (1993) Analysis of the 77 K fluorescence emission and excitation spectra of isolated etioplast inner membranes. J Photochem Photobiol B Biol 21: 125–133

Boese QF, Spano AJ, Li JM and Timko MP (1991) Aminolevulinic acid dehydratase in pea (*Pisum sativum* L.) Identification of an unusual metal binding in the plant enzyme. J Biol Chem 266: 17060–17066

Bohren KM, Grimshaw CE, Lai CJ, Harrison DH, Ringe D, Petsko GA and Gabbay KH (1994) Tyrosine-48 is the proton donor and histidine-110 directs substrate stereochemical selectivity in the reduction reaction of human aldose reductase: enzyme kinetics and crystal structure of the Y48H mutant enzyme. Biochemistry 33: 2021–2032

Bougri O and Grimm B (1996) Members of a low-copy number gene family encoding glutamyl-tRNA reductase are differentially expressed in barley. Plant J 9: 867–878

Bruyant P and Kannangara CG (1987) Biosynthesis of 5-aminolevulinate in greening barley leaves. VII. Purification and characterization of the glutamate-tRNA ligase. Carlsberg Res Comm 52: 99–109

Buhr F, El Bakkouri M, Valdez O, Pollmann S, Lebedev N, Reinbothe S and Reinbothe C (2008) Photoprotective role of NADPH:protochlorophyllide oxidoreductase A. Proc Natl Acad Sci USA 105: 12629–12634

Cady SS and Pinnavaia TJ (1978) Porphyrin intercalation in mice-type silicates. Inorg Chem 17: 1501–1507

Camadro JM, Matringe M, Scalla R and Labbe P (1991) Kinetic studies on protoporphyrinogen oxidase inhibition by diphenyl ether herbicides. Biochem J 277: 17–21

Carey EE, Tripathy BC and Rebeiz CA (1985) Chloroplast biogenesis 51: Modulation of monovinyl and divinyl protochlorophyllide biosynthesis by light and darkness in vitro. Plant Physiol 79: 1059–1063

Castelfranco PA and Beale SI (1981) Chlorophyll biosynthesis. In: Stumpf PK and Conn EE (eds) The Biochemistry of Plants: A Comprehensive Treatise, Vol 8, pp 375–421, Academic Press, New York

Castelfranco PA, Thayer SS, Wilkinson JQ and Bonner BA (1988) Labeling of porphobilinogen deaminase by radioactive 5-aminolevulinic acid in isolated developing pea chloroplasts. Arch Biochem Biophys 266: 219–226

Chakraborty N and Tripathy BC (1992) Involvement of singlet oxygen in photodynamic damage of isolated chloroplasts of cucumber (*Cucumis sativus* L.) cotyledons. Plant Physiol 98: 7–11

Che FS, Watnabe N, Iwano M, Inokuchi H, Takayama S, Yoshida S and Isogai A (2000) Molecular characterization and subcellular localization of protoporphyrinogen oxidase in spinach chloroplasts. Plant Physiol 124: 59–70

Cheminant S, Wild M, Bouvier F, Pelletier S, Renou JP, Erhardt M, Hayes S, TerryMJ, Genschik P and Achard P (2011) DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photooxidative damage during seedling deetiolation in Arabidopsis. Plant Cell May 27. [Epub ahead of print]

Chen TC and Miller GW (1974) Purification and characterization of uroporphyrinogen decarboxylase from tobacco leaves. Plant Cell Physiol 15: 993–1005

Chory J, Reinecke D, Sim S, Washburn T and Brenner M (1994) A role of cytokinins in de-etiolation in *Arabidopsis det* mutants have an altered response to cytokinins. Plant Physiol 104: 339–347

Coemans B, Matsumura H, Terauchi R, Remy S, Swennen R and Sagi L (2005) SuperSAGE combined with PCR walking allows global gene expression profiling of banana (*Musa acuminata*), a non-model organism. Theor Appl Genet 111: 1118–1126

Cornah JE, Roper JM, Pal Sing D and Smith AG (2002) Measurement of ferrochelatase activity using a novel assay suggests that plastids are the major site of haem biosynthesis in both photosynthetic and non-photosynthetic cells of pea (*Pisum sativum* L.). Biochem J 362: 423–32

Crockett N, Alefounder PR, Battersby AR and Abell C (1991) Uroporphyrinogen III synthase: Studies on its mechanism of action molecular biology and biochemistry. Tetrahedron 47: 6003–6014

Dahlin C, Aronsson H, Wilks HM, Lebedev N, Sundqvist C and Timko MP (1999) The role of protein surface charge in catalytic activity and chloroplast membrane association of the pea NADPH:protochlorophyllide oxidoreductase (POR) as revealed by alanine scanning mutagenesis. Plant Mol Biol 39: 309–323

Dahlin C, Aronsson H, Almkvist J and Sundqvist C (2000) Protochlorophyllide-independent import of two NADPH: Pchlide oxidoreductase proteins (PORA and PORB) from barley into isolated plastids. Physiol Plant 109: 298–303

Darrah PM, Kay SA, Teakle GR and Griffiths WT (1990) Cloning and sequencing of protochlorophyllide reductase. Biochem J 265: 789–798

Davison PA, Schubert HL, Reid JD, Iorg CD, Heroux A, Hill CP and Hunter CN (2005) Structural and biochemical characterization of Gun4 suggests a mechanism for its role in chlorophyll biosynthesis. Biochemistry 44: 7603–7612

Day IS, Golovkin M and Reddy AS (1998) Cloning of the cDNA for glutamyl-tRNA synthetase from *Arabidopsis thaliana*. Biochim Biophys Acta 1399: 219–224

Dayan FE, Ferreira D, Wang YH, Khan IA, McInroy JA and Pan Z (2008) A pathogenic fungi diphenyl ether phytotoxin targets plant enoyl (acyl carrier protein) reductase. Plant Physiol 147: 1062–1071

Domanskii V, Rassadina V, Gus-Mayer S, Wanner G, Schoch S and Rudiger W (2003) Characterization of two phases of chlorophyll formation during greening of etiolated barley leaves. Planta 216: 475–483

Douce R and Joyard J (1990) Biochemistry and function of the plastid envelope. Annu Rev Cell Biol 6: 173–216

Duggan JX and Rebeiz CA (1982) Chloroplast biogenesis. 37. Induction of chlorophyllide *a* (E459F675) accumulation in higher plants. Plant Sci Lett 24: 27–37

Eggink LL, LoBrutto R, Brune DC, Brusslan J, Yamasato A, Tanaka A and Hoober JK (2004) Synthesis of chlorophyll *b*: localization of chlorophyllide a oxygenase and discovery of a stable radical in the catalytic subunit. BMC Plant Biol 4: 5

Eichacker LA, Soll J, Lauterbach P, Rudiger W, Klein RR and Mullet JE (1990) In vitro synthesis of chlorophyll *a* in the dark triggers accumulation of chlorophyll *a* apoproteins in barley etioplasts. J Biol Chem 265:13566–13571

Espineda CE, Linford AS, Devine D and Brusslan JA (1999) The AtCAO gene, encoding chlorophyll *a* oxygenase, is required for chlorophyll *b* synthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 96: 10507–10511

Fodje MN, Hansson A, Hansson M, Olsen JG, Gough S, Willows RD and Al-Karadaghi S (2001) Interplay between an AAA module and an integrin I domain may regulate the function of magnesium chelatase. J Mol Biol 311: 111–122

Forreiter C, Van Cleve B, Schmidt A and Apel K. (1991) Evidence for a general light-dependent negative control of NADPH–protochlorophyllide oxidoreductase in angiosperms. Planta 183: 126–132

Franck F, Sperling U, Frick G, Pochert B, Van Cleve B, Apel K and Armstrong GA (2000) Regulation of etioplast pigment–protein complexes, inner membrane architecture, and protochlorophyllide *a* chemical heterogeneity by light-dependent NADPH: protochlorophyllide oxidoreductases A and B. Plant Physiol 124: 1678–1696

Frank HA, Young AJ, Britton G and Cogdell RJ (eds) (1999) The Photochemistry of Carotenoids, Advances in Photosynthesis, Vol 8. Kluwer Academic Publishers, Dordrecht

Frick G, Su Q, Apel K and Armstrong GA (2003) An *Arabidopsis porB porC* double mutant lacking light-dependent NADPH: protochlorophyllide oxidoreductases B and C is highly chlorophyll-deficient and developmentally arrested. Plant J 35: 141–153

Frustaci JM, Sangwan I and O'Brian MR (1995) gsa1 is a universal tetrapyrrole synthesis gene in soybean and is regulated by a GAGA element. J Biol Chem 270: 7387–7393

Fusada N, Masuda T, Kuroda H, Shiraishi T, Shimada H, Ohta H and Takamiya K (2000) NADPH–protochlorophyllide oxidoreductase in cucumber is encoded by a single gene and its expression is transcriptionally enhanced by illumination. Photosynth Res 64: 147–154

Fusada N, Masuda T, Kuroda H, Shimada H, Ohta H and Takamiya K (2005) Identification of a novel cis-element exhibiting cytokinin-dependent protein binding in vitro in the 5'-region of NADPH-protochlorophyllide oxidoreductase gene in cucumber. Plant Mol Biol 59: 631–645

Gaubier P, Wu HJ, Laudie M, Delseny M and Grellet F (1995) A chlorophyll synthetase gene from *Arabidopsis thaliana*. Mol Gen Genet 249: 673–676

Giannino D, Condello E, Bruno L, Testone G, Tartarini A, Cozza R, Innocenti AM, Bitonti MB and Mariotti D (2004) The gene geranylgeranyl reductase of peach (*Prunus persica* [L.] Batsch) is regulated during leaf development and responds differentially to distinct stress factors. J Exp Bot 55: 2063–2073

Gibson LC, Marrison JL, Leech RM, Jensen PE, Bassham DC, Gibson M and Hunter CN (1996) A putative Mg chelatase subunit from *Arabidopsis thaliana* cv C24. Sequence and transcript analysis of the gene, import of the protein into chloroplasts, and in situ localization of the transcript and protein. Plant Physiol 111: 61–71

Gibson KD, Neuberger A and Tait GH (1963) Studies on the biosynthesis of porphyrin and bacteriochlorophyll by *Rhodopseudomonas spheriodes*. 4. S-adenosylmethionine-magnesium protoporphyrin methyltransferase. Biochem J 88: 325–334

Gough SP, Kannangara CG and von Wettestein D (1992) Glutamate 1-semialdehyde aminotransferase as a target for herbicides. In: Boger P and Sandmann G (eds) Target Assays for Modern Herbicides and Related Phytotoxic Compounds, pp 21–27. Lewis Publishers, Chelesa

Grimm B, Porra RJ, Rüdiger W and Scheer H (eds) (2006) Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications, Advances in Photosynthesis and Respiration, Vol 25. Springer, Dordrecht

Hansson A and Jensen PE (2009) Chlorophyll limitation in plants remodels and balances the photosynthetic apparatus by changing the accumulation of photosystems I and II through two different approaches. Physiol Plant 135: 214–228

Harmer SL, Hogenesch JB, Straume M, Chang H-S, Han B, Zhu T, Wang X, Kreps JA and Kay SA (2000) Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290: 2110–2113

Harper AL, von Gesjen SE, Linford AS, Peterson MP, Faircloth RS, Thissen MM and Brusslan JA (2004) Chlorophyllide *a* oxygenase mRNA and protein levels correlate with the chlorophyll *a*/*b* ratio in *Arabidopsis thaliana*. Photosynth Res 79: 149–159

Havaux M, Lutz C and Grimm B (2003) Chloroplast membrane photostability in chlP transgenic tobacco plants deficient in tocopherols. Plant Physiol 132: 300–310

He ZH, Li J, Sundqvist C and Timko MP (1994) Leaf development age controls expression of genes encoding enzymes of chlorophyll and heme biosynthesis in pea (*Pisum sativum* L.). Plant Physiol 106: 537–546

Hedtke B, Alawady A, Chen S, Börnke F and Grimm B (2007) HEMA RNAi silencing reveals a control mechanism of ALA biosynthesis on Mg chelatase and Fe chelatase. Plant Mol Biol 64: 733–742

Helfrich M, Schoch S, Lempert U, Cmiel E and Rudiger W (1994) Chlorophyll synthetase cannot synthesize chlorophyll *a*. Eur J Biochem 219: 267–275

Heyes DJ and Hunter CN (2004) Identification and characterization of the product release steps within the catalytic cycle of protochlorophyllide oxidoreductase. Biochemistry 43: 8265–8271

Heyes DJ, Ruban AV, Wilks HM and Hunter CN (2002) Enzymology below 200 K: the kinetics and thermodynamics of the photochemistry catalyzed by protochlorophyllide oxidoreductase. Proc Natl Acad Sci USA 99: 11145–11150

Heyes DJ, Ruban AV and Hunter CN (2003) Protochlorophyllide oxidoreductase: spectroscopic characterization of the 'dark' reactions. Biochemistry 42: 523–528

Heyes DJ, Kruk J and Hunter CN (2006) Spectroscopic and kinetic characterization of the light-dependent enzyme protochlorophyllide oxidoreductase (POR) using monovinyl and divinyl substrates Biochem J 394: 243–248

Heyes DJ, Menon BR, Sakuma M and Scrutton NS (2008) Conformational events during ternary enzyme-substrate complex formation are rate limiting in the catalytic cycle of the light-driven enzyme protochlorophyllide oxidoreductase. Biochemistry 47: 10991–10998

Higuchi M and Bogorad L (1975) The purification and properties of uroporphyrinogen I synthase and uroporphyrinogen III cosynthase. Interactions between the Enzymes. Ann NY Acad Sci 244: 401–418

Hirashima M, Satoh S, Tanaka R and Tanaka A (2006) Pigment shuffling in antenna systems achieved by expressing prokaryotic chlorophyllide *a* oxygenase in *Arabidopsis*. J Biol Chem 281: 15385–15393

Hiriart JB, Lehto K, Tyystjarvi E, Junttila T and Aro EM (2002) Suppression of a key gene involved in chlorophyll biosynthesis by means of virus-inducing gene silencing. Plant Mol Biol 50: 213–224

Hodgson GW and Baker GL (1964) Evidence for porpyrin in the Orgueil meteorite. Nature 202:125–127

Hodgson GW and Ponnamoeruma C (1968) Prebiotic porphyrin genesis: Porphyrin from electric discharge in methane, ammonia and water vapour. Proc Natl Acad Sci USA 31:153–158

Höfgen R, Axelsen KB, Kannangara CG, Schuttke I, Pohlenz, Willmitzer L, Grimm B and von Wettestein D (1994) A visible marker for antisense mRNA expression in plants: Inhibition of chlorophyll biosynthesis with a glutamate 1-semialdehyde aminotransferase antisense gene. Proc Natl Acad Sci USA 91: 1726–1730

Holtorf H and Apel K (1996a) Transcripts of the two NADPH–protochlorophyllide oxidoreductase genes PorA and PorB are differentially degraded in etiolated barley seedlings. Plant Mol Biol 31: 387–392

Holtorf H and Apel K (1996b) The regulation of NADPH–protochlorophyllide oxidoreductase *a* and *b* in green barley plants kept under a diurnal light dark cycle. Planta 199: 289–295

Holtorf H, Reinbothe S, Reinbothe C, Bereza B and Apel K (1995) Two routes of chlorophyllide synthesis that are differentially regulated by light in barley (*Hordeum vulgare* L.). Proc Natl Acad Sci USA 92: 3254–3258

Hoober JK, Kahn A, Ash DE, Gough SP and Kannagara CG (1988) Biosynthesis of 5-aminolevulinate in greening barley leaves.IX. Structure of the substrate, mode of gabaculine inhibition, and the catalytic mechanism of glutamate-1-semialdehyde aminotransferase. Carlsberg Res Com 53:11–25

Horie Y, Ito H, Kusaba M, Tanaka R and Tanaka A (2009) Participation of chlorophyll *b* reductase in the initial step of the degradation of light-harvesting chlorophyll *a*/*b*-protein complexes in *Arabidopsis*. J Biol Chem 284:17449–17456

Hsu WP and Miller GW (1970) Coproporphyrinogenase in tobacco (*Nicotiana tabacum* L.). Biochem J 117: 215–220

Hu G, Yalpani N, Briggs SP and Johal GS (1998) A porphyrin pathway impairment is responsible for the phenotype of a dominant disease lesion mimic mutant of maize. Plant Cell 10: 1095–1105

Huang DD, Wang WY, Gough SP and Kannangara CG (1984) Delta-Aminolevulinic acid-synthesizing enzymes need an RNA moiety for activity. Science 225: 1482–1484

Huang YS and Li HM (2009) *Arabidopsis* CHLI2 can substitute for CHLI1. Plant Physiol 150: 636–645

Hukmani P and Tripathy BC (1994) Chlorophyll biosynthetic reactions during senescence of excised barley (*Hordeum vulgare* L. cv IB 65) Leaves. Plant Physiol 105: 1295–1300

Ikegami A, Yoshimura N, Motohashi K, Takahashi S, Romano PG, Hisabori T, Takamiya K and Masuda T (2007) The CHLI1 subunit of *Arabidopsis thaliana* magnesium chelatase is a target protein of the chloroplast thioredoxin. J Biol Chem 282: 19282–19291

Ilag LL, Kumar AM and Soll D (1994) Light reduction of chlorophyll biosynthesis at the level of 5-aminolevulinate formation in *Arabidopsis*. Plant Cell 6: 265–275

Ishikawa A, Okamoto H, Iwasaki Y and Asahi T (2001) A deficiency of coproporphyrinogen III oxidase causes lesion formation in *Arabidopsis*. Plant J 27: 89–99

Jacobs NJ and Jacobs JM (1979) Microbial oxidation of protoporphyrinogen: an intermediate in heme and chlorophyll biosynthesis. Arch Biochem Biophys 197: 396–403

Jacobs JM and Jacobs NJ (1987) Oxidation of protoporphyrinogen to protoporphyrin, a step in chlorophyll and haem biosynthesis. Purification and partial characterization of the enzyme from barley organelles. Biochem J 244: 219–224

Jain M and Gadre RP (2004) Inhibition of 5-amino levulinic acid dehydratase activity by arsenic in excised etiolated maize leaf segments during greening. J Plant Physiol 161: 251–255

Jensen PE, Willows RD, Petersen BL, Vothknecht UC, Stummann BM, Kannangara CG, von Wettstein D and Henningsen KW (1996) Structural genes for Mg-chelatase subunits in barley: Xantha-f, -g, and -h. Mol Gen Genet 250: 383–394

Jensen PE, Gibson LCD and Hunter CN (1999) ATPase activity associated with the magnesium-protoporphyrin IX chelatase enzyme of *Synechocystis* PCC6803: evidence for ATP hydrolysis during Mg^{2+} insertion, and MgATP dependent interaction of ChlI and ChlD subunits. Biochem J 339:127–134

Jilani A, Kar S, Bose S and Tripathy BC (1996) Regulation of the carotenoid content and chloroplast development by levulinic acid. Physiol Plant 96: 139–145

Jones DT (1999) GenTHREADER: an efficient and reliable protein fold recognition method for genomic sequences. J Mol Biol 287: 797–815

Jones RM and Jordan PM (1994) Purification and properties of porphobilinogen deaminase from *Arabidopsis thaliana*. Biochem J 299: 895–902

Jordan PM and Shemin D (1980) Mechanism of action of 5-aminolevulinic acid dehydratase: Stepwise order of addition of the two molecules of 5-aminilevulinic acid in the enzymatic synthesis of porphobilinogen. J Chem Soc Chem Comm 240–242

Joyard J, Block M, Pineau B, Albrieux C and Douce R (1990) Envelope membranes from mature spinach chloroplasts contain a NADPH:protochlorophyllide reductase on the cytosolic side of the outer membrane. J Biol Chem 265: 21820–21827

Jung KH, Hur J, Ryu CH, Choi Y, Chung YY, Miyao A, Hirochika H and An G (2003) Characterization of a rice chlorophyll-deficient mutant using the T-DNA gene-trap system. Plant Cell Physiol 44: 463–472

Jung S, Lee HJ, Lee Y, Kang K, Kim YS, Grimm B and Back K (2008) Toxic tetrapyrrole accumulation in protoporphyrinogen IX oxidase-overexpressing transgenic rice plants. Plant Mol Biol 67: 535–546

Kaczor CM, Smith MW, Sangwan I and O'Brian MR (1994) Plant delta-aminolevulinic acid dehydratase. Expression in soybean root nodules and evidence for a bacterial lineage of the Alad gene. Plant Physiol 104: 1411–1417

Kagan RM and Clarke S (1994) Widespread occurrence of three sequence motifs in diverse S-adenosylmethionine-dependent methyltransferases suggests a common structure for these enzymes. Arch Biochem Biophys 310: 417–427

Kannangara CG, Gough SP, Oliver RP and Rasmussen SK (1984) Biosynthesis of 5-ALA in greening barley leaves. VI. Activation of glutamate by ligation to RNA. Carlsberg Res Com 49: 417–437

Kannagara CG, Gough SP, Bruyant P, Hoober JK, Kahn A and Wettestein DV (1988) $tRNA^{glu}$ as a cofactor on δ-aminolevulinate biosynthesis: Steps that regulatechlorophyll synthesis. Trends Biol Sci 13: 139–143

Kannangara CG, Andersen RV, Pontoppidan B, Willows R and von Wettstein D (1994) Enzymic and mechanistic studies on the conversion of glutamate to 5-aminolaevulinate. Ciba Found Symp 180: 3–20

Kannangara CG, Vothknecht UC, Hansson M and von Wettstein D (1997) Magnesium chelatase: association with ribosome and mutant complementation studies identify barley subunit Xantha-G as a functional counterpart of *Rhodobacter* subunit BchD. Mol Gen Genet 254: 85–92

Keller Y, Bouvier F, D'`Harlingue A and Camara B (1998) Metabolic compartmentation of plastid prenyllipid biosynthesis. Evidence for the involvement of a multifunctional geranylgeranyl reductase. Eur J Biochem 251: 413–417

Kervinen J, Dunbrack Rl Jr, Litwin S, Martins J, Scarrow RC, Volin M, Yeung AT, Yoon E and Jaffe EK (2000) Porphobilinogen synthase from pea: Expression from an artificial gene, kinetic characterisation and novel implication for subunit interactions. Biochemistry 39: 9018–9029

Kim YK, Lee JY, Cho HS, Lee SS, Ha HJ, Kim S, Choi D and Pai HS (2005) Inactivation of organellar glutamyl- and seryl-tRNA synthetases leads to developmental arrest of chloroplasts and mitochondria in higher plants. J Biol Chem 280: 37098–38106

Koch M, Breithaupt C, Kiefersauer R, Freigang J, Huber R and Messerschmidt A (2004) Crystal structure of protoporphyrinogen IX oxidase: a key enzyme in haem and chlorophyll biosynthesis EMBO J 23: 1720–1728

Kolossov VL and Rebeiz CA (2001) Chloroplast biogenesis 84: Solubilization and partial purification of membrane-bound [4-vinyl] chlorophyllide *a* reductase from etiolated barley leaves. Anal Biochem 295: 214–219

Kropat J, Oster U, Rüdiger W and Beck CF (1997) Chlorophyll precursors are signals of chloroplast origin involved in light induction of nuclear heat-shock genes. Proc Natl Acad Sci USA 94: 14168–14172

Kropat J, Oster U, Rüdiger W and Beck CF (2000) Chloroplast signalling in the light induction of nuclear HSP70 genes requires the accumulation of chlorophyll precursors and their accessibility to cytoplasm/nucleus. Plant J 24: 523–531

Kruse E, Mock HP and Grimm B (1995a) Reduction of coproporphyrinogen oxidase level by antisense RNA synthesis leads to deregulated gene expression of plastid proteins and affects the oxidative defense system. EMBO J 14: 3712–3720

Kruse E, Mock HP and Grimm B (1995b) Coproporphyrinogen III oxidase from barley and tobacco-sequence analysis and initial expression studies. Planta 196: 796–803

Kruse E, Mock HP and Grimm B (1997) Isolation and characterization of tobacco (*Nicotiana tabacum*) cDNA clones encoding proteins involved in magnesium chelation into protoporphyrin IX. Plant Mol Biol 35: 1053–1056

Kumar AM and Söll D (2000) Antisense HEMA1 RNA expression inhibits heme and chlorophyll biosynthesis in *Arabidopsis*. Plant Physiol 122: 49–56

Kumar AM, Csankovszki G and Söll D (1996) A second and differentially expressed glutamyl-tRNA reductase gene from *Arabidopsis thaliana*. Plant Mol Biol 30: 419–426

Kuroda H, Masuda T, Ohta H, Shioi Y and Takamiya K (1995) Light-enhanced gene expression of NADPH–protochlorophyllide oxidoreductase in cucumber. Biochem Biophys Res Commun 210: 310–316

Kuroda H, Masuda T, Fusada N, Ohta H and Takamiya K (2001) Cytokinin-induced transcriptional activation of NADPH–protochlorophyllide oxidoreductase gene in cucumber. J Plant Res 114: 1–7

Kusnetsov V, Herrmann RG, Kulaeva ON and Oelmüller R (1998) Cytokinin stimulates and abscisic acid inhibits greening of etiolated *Lupinus luteus* cotyledons by affecting the expression of the light-sensitive protochlorophyllide oxidoreductase. Mol Gen Gene 259: 21–28

Kusaba M, Ito H, Morita R, Iida S, Sato Y, Fujimoto M, Kawasaki S, Tanaka R, Hirochika H, Nishimura M and Tanaka A (2007) Rice NON-YELLOW COLORING1 is involved in light-harvesting complex II and grana degradation during leaf senescence. Plant Cell 19: 1362–1375

Kusumi J, Sato A and Tachidi H (2006) Relaxation of functional constraint on light-independent protochlorophyllide reductase in *Thuja*. Mol Biol Evol 23: 941–948

Larkin RM, Alonso JM, Ecker JR and Chory J (2003) GUN4, a regulator of chlorophyll synthesis and intracellular signaling. Science 299: 902–906

Larkum AWD (1991) The evolution of chlorophylls. In: Scheer H (ed) Chlorophylls, pp 367–383. CRC Press, Boca Raton

Larkum AWD (1999) The evolution of algae. In: Seckbach J (ed) Enigmatic Microorganisms and Life in Extreme Environments, pp 31–48. Kluwer Academic Publishers, Dordrecht

Lebedev N and Timko MP (1999) Protochlorophyllide oxidoreductase B-catalysed protochlorophyllide photoreduction in vitro: insight into the mechanism of chlorophyll formation in light-adapted plants. Proc Natl Acad Sci USA 96: 9954–9959

Lebedev N, Van Cleve B, Armstrong G and Apel K (1995) Chlorophyll synthesis in a deetiolated (*det340*) mutant of *Arabidopsis* without NADPH–protochlorophyllide (PChlide) oxidoreductase (POR) *a* and photoactive PChlide-F655. Plant Cell 7: 2081–2090

Lebedev N, Karginova O, McIvor W and Timko MP (2001) Tyr275 and Lys279 stabilize NADPH within the catalytic site of NADPH: protochlorophyllide oxidoreductase and are involved in the formation of the enzyme photoactive state. Biochemistry 40: 12562–12574

Lee HJ, Lee SB, hung JS, Han SU, Han O, Guh JO, Jeon JS, An G and Back K (2000) Transgenic rice plants expressing a *Bacillus subtilis* protoporphyrinogen oxidase gene are resistant to diphenyl-ether herbicide acifluorfen. Plant Cell Physiol 41: 743–749

Lee KP, Kim C, Lee DW and Apel K (2003) TIGRINA d, required for regulating the biosynthesis of tetrapyrolles in barley, is an ortholog of FLU gene of *Arabidopsis thaliana*. FEBS Lett 553: 119–124

Lee S, Kim JH, Yoo ES, Lee CH, Hirochika H and An G (2005) Differential regulation of chlorophyll *a* oxygenase genes in rice. Plant Mol Biol 57: 805–818

Lermontova I and Grimm B (2000) Overexpression of plastidic protoporphyrinogen IX oxidase leads to resistance to the diphenyl-ether herbicide acifluorfen. Plant Physiol 122: 75–84

Lermontova I and Grimm B (2006) Reduced activity of plastid protoporphyrinogen oxidase causes attenuated photodynamic damage during high-light compared to low-light exposure. Plant J 48: 499–510

Lermontova I, Kruse E, Mock HP and Grimm B (1997) Cloning and characterisation of a plastidal and a mitochondrial isoform of tobacco protoporphyrinogen IX oxidase. Proc Natl Acad Sci USA 94: 8895–8900

Li J, Spano AJ and Timko MP (1991) Isolation and characetisation of nuclear genes encoding the ALA dehydratase of pea (*Pisum sativum* L.). Plant Physiol 96: 125–127

Liedgens W, Grutzmann R and Schneider HAW (1980) Highly efficient purification of the labile plant enzyme 5-aminolevulinate dehydratase (EC 4.2.1.24) by means of monoclonal antibodies. Z Naturforsch 35c: 958–962

Lim SH, Witty M, Wallace-Cook AD, Ilag LI and Smith AG (1994) Porphobilinogen deaminase is encoded by a single gene in *Arabidopsis thaliana* and is targeted to the chloroplasts. Plant Mol Biol 26: 863–872

Lindsten A, Ryberg M and Sundqvist C (1988) The polypeptide composition of highly purified prolamellar bodies and prothylakoids from wheat (*Tritium aestivum*) as revealed by silver staining. Physiol Plant 72: 167–176

Lindstein A, Welch CJ, Schoch S, Ryberg M, Rüdiger W and Sundqvist C (1990) Chlorophyll synthatase is latent in well preserved prolamellar bodies of etiolated wheat. Physiol Plant 80: 277–285

Liu N, Yang YT, Liu HH, Yang GD, Zhang NH and Zheng CC (2004) NTZIP antisense plants show reduced chlorophyll levels. Plant Physiol Biochem 42: 321–327

Luo J and Lim CK (1993) Order of urogen III decarboxylation on incubation of PBG and urogen III with erythrocyte UDC. Biochem J 289: 529–532

Madsen O, Sandal L, Sandal NN and Marcker KA (1993) A soybean coproporphyrinogen oxidase gene is highly expressed in root nodules. Plant Mol Biol 23: 35–43

Manohara MS and Tripathy BC (2000) Regulation of protoporphyrin IX biosynthesis by intraplastidic compartmentalization and adenosine triphosphate. Planta 212: 52–59

Mapleston RE and Griffiths WT (1980) Light modulation of the activity of protochlorophyllide reductase. Biochem J 189: 125–133

Martin W, Stoebe B, Goremykin V, Hansmann S, Hasegawa M and Kowallik KV (1998). Gene transfer to the nucleus and the evolution of chloroplasts. Nature 393: 162–165

Martin BM, Grimm B, Mock HP, Huber R and Messerschmidt A (2001) Crystal structure and substrate binding modeling of the uroporphyrinogen-III decarboxylase from *Nicotiana tabacum*. Implications for the catalytic mechanism. J Biol Chem 276: 44108–44116

Martin W, Rujan T, Richly E, Hansen A, Cornelsen S, Lins T, Leister D, Stoebe B, Hasegawa M and Penny D (2002) Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci USA 99: 12246–12251

Masuda T and Takamiya K (2004) Novel insights into enzymology, regulation and physiological functions of light-dependant Protochlorophyllide oxidoreductase in angiosperms. Photosynth Res 81: 1–29

Masuda T, Fusada N, Shiraishi T, Kuroda H, Awai K, Shimada H, Ohta H and Takamiya K (2002) Identification of two differentially regulated isoforms of protochlorophyllide oxidoreductase (POR) from tobacco revealed a wide variety of light and development-dependent regulations of POR gene expression among angiosperms. Photosynth Res 74: 165–172

Masuda T, Fusada N, Oosawa N, Takamatsu K, Yamamoto YY, Ohto M, Nakamura K, Goto K, Shibata D, Shirano Y, Hayashi H, Kato T, Tabata S, Shimada H, Ohta H and Takamiya K (2003) Functional analysis of isoforms of NADPH: protochlorophyllide oxidoreductase (POR), PORB and PORC, in *Arabidopsis thaliana*. Plant Cell Physiol 44: 963–974

Masuda S, Ikeda R, Masuda T, Hashimoto H, Tsuchiya T, Kojima H, Nomata J, Fujita Y, Mimuro M, Ohta H and Takamiya K (2009) Prolamellar bodies formed by cyanobacterial protochlorophyllide oxidoreductase in *Arabidopsis*. Plant J 58: 952–960

Matringe M, Camadro JM, Block MA, Joyard J, Scalla R, Labbe P and Douce R (1992a) Localisation within the chloroplasts of protoporphyrinogen oxidase, the target enzyme for diphenylether like herbicides. J Biol Chem 267: 4646–4651

Matringe M, Mornet R and Scalla R. (1992b) Characterization of [3 H] acifluorfen binding to purified pea etioplasts and evidence that protogen oxidase specifically binds acifluorfen. Eur J Biochem 209: 861–868

Matile P, Hortensteiner S, Thomas H and Krautler B (1996) Chlorophyll breakdown in senescent leaves. Plant Physiol 12: 1403–1409

Matile P, Hortensteiner S and Thomas H (1999) CHLOROPHYLL DEGRADATION. Annu Rev Plant Physiol Plant Mol Biol 50: 67–95

Matsumoto F, Obayashi T, Sasaki-Sekimoto Y, Ohta H, Takamiya K and Masuda T (2004) Gene expression profiling of the tetrapyrrole metabolic pathway in *Arabidopsis* with a mini-array system. Plant Physiol 135: 2379–2391

McCormac AC and Terry MJ (2002a) Light-signalling pathways leading to the coordinated expression of HEMA1 and Lhcb during chloroplast development in *Arabidopsis thaliana*. Plant J 32: 549–559

McCormac AC and Terry MJ (2002b) Loss of nuclear gene expression during the phytochrome A-mediated far-red block of greening response. Plant Physiol 130: 402–414

McCormac AC, Fischer A, Kumar AM, Soll D and Terry MJ (2001) Regulation of HEMA1 expression by phytochrome and a plastid signal during de-etiolation in *Arabidopsis thaliana*. Plant J 25: 549–561

Menon BR, Waltho JP, Scrutton NS and Heyes DJ (2009) Cryogenic and laser photoexcitation studies identify multiple roles for active site residues in the light-driven enzyme protochlorophyllide oxidoreductase. J Biol Chem 284: 18160–18166

Meskauskiene R and Apel K (2002) Interaction of FLU, a negative regulator of tetrapyrrole biosynthesis, with the glutamyl–tRNA reductase requires the tetratricopeptide repeat domain of FLU. FEBS Lett 532: 27–30

Meskauskiene R, Nater M, Goslings D, Kessler F, op den Camp R and Apel K (2001) FLU: a negative regulator of chlorophyll biosynthesis in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 98: 12826–12831

Miyashita H, Ikemoto H, Kurano N, Adachi K, Chilara M and Miyachi S (1996) Chlorophyll *d* as a major pigment. Nature 383: 402

Mochizuki N, Brusslan JA, Larkin R, Nagatani A and Chory J (2001) *Arabidopsis* genomes uncoupled 5 (GUN5) mutant reveals the involvement of Mg-chelatase H subunit in plastidto- nucleus signal transduction. Proc Natl Acad Sci USA 98: 2053–2058

Mock HP and Grimm B (1997) Reduction of uroporphyrinogen decarboxylase by antisense RNA expression affects activities of other enzymes involved in tetrapyrrole biosynthesis and leads to light dependent necrosis. Plant Physiol 113: 1101–1112

Mock HP, Trainotti L, Kruse E and Grimm B (1995) Isolation, sequencing and expression of cDNA sequences encoding uroporphyrinogen decarboxylase from tobacco and barley. Plant Mol Biol 28: 245–256

Mock HP, Heller W, Molina A, Neubohn B, Sandermann H and Grimm B (1999) Expression of uroporphyrinogen decarboxylase or coproporphyrinogen oxidase antisense RNA in tobacco induces pathogen defence responses conferring increased resistance to tobacco mosaic virus. J Biol Chem 274: 4231–4238

Mohanty S, Grimm B and Tripathy BC (2006) Light and dark modulation of chlorophyll biosynthetic genes in response to temperature. Planta 224: 692–699

Mohapatra A and Tripathy BC (2002) Detection of protoporphyrin IX in envelope membranes of pea chloroplasts. Biochem Biophys Res Commun 299: 751–754

Mohapatra A and Tripathy BC (2003) Developmental changes in sub-plastidic distribution of chlorophyll biosynthetic intermediates in cucumber (*Cucumis sativus* L.). J Plant Physiol 160: 9–15

Mohapatra A and Tripathy BC (2007) Differential distribution of chlorophyll biosynthetic intermediates in stroma, envelope and thylakoid membranes in *Beta vulgaris*. Photosynth Res 94: 401–410

Mosinger E, Batschauer A, Schafer E and Apel K (1985) Phytochrome control of in vitro transcription of specific genes in isolated nuclei from barley (*Hordeum vulgare*). Eur J Biochem 147: 137–142

Nakagawara E, Sakuraba Y, Yamasato A, Tanaka R and Tanaka A (2007) Clp protease controls chlorophyll *b* synthesis by regulating the level of chlorophyllide *a* oxygenase. Plant J 49: 800–809

Nagata N, Satoh S, Tanaka R and Tanaka A (2004) Domain structures of chlorophyllide *a* oxygenase of green plants and *Prochlorothrix hollandica* in relation to catalytic functions. Planta 218: 1019–1025

Nagata N, Tanaka R, Satoh S and Tanaka A (2005) Identification of a vinyl reductase gene for chlorophyll synthesis in *Arabidopsis thaliana* and implications for the evolution of *Prochlorococcus* species. Plant Cell 17: 233–240

Nagata N, Tanaka R and Tanaka A (2007) The major route for chlorophyll synthesis includes [3,8-divinyl]-chlorophyllide a reduction in *Arabidopsis thaliana*. Plant Cell Physiol 48: 1803–1808

Nakanishi H, Nozue H, Suzuki K, Kaneko Y, Taguchi G and Hayashida N (2005) Characterization of the *Arabidopsis thaliana* mutant *pcb2* which accumulates divinyl chlorophylls. Plant Cell Physiol 46: 467–473

Nakayama M, Masuda T, Sato N, Yamagata H, Bowler C, Ohta H, Shioi Y and Takamiya K (1995) Cloning, subcellular localization and expression of CHLI, a subunit of magnesium- chelatase in soybean. Biochem Biophys Res Com 215: 422–428

Nakayama M, Masuda T, Bando T, Yamagata H, Ohta H and Takamiya K (1998) Cloning and expression of the soybean chlH gene encoding a subunit of Mg-chelatase and localization of the Mg^{++} concentration-dependent chlH protein within the chloroplast. Plant Cell Physiol 39: 275–284

Nandi DL and Waygood ER (1967) Biosynthesis of porphyrins in wheat leaves. II. 5-aminolevulinate hydrolyase. Canad J Biochem 45: 327–336

Narita S, Tanaka R, Ito T, Okada K, Taketani S and Inokuchi H (1996) Molecular cloning and characterisation of a cDNA that encodes protoporphyrinogen oxidase of *Arabidopsis thaliana*. Gene 182: 169–175

Oliver RP and Griffiths WT (1982) Pigment-protein complexes of illuminated etiolated leaves. Plant Physiol 70: 1019–1025

Oosawa N, Masuda T, Awai K, Fusada N Shimada H, Ohta H and Takamiya K (2000) Identification and light-induced expression of a novel gene of NADPH-potochlorophyllide oxidoreductase isoform in *Arabidopsis thaliana*. FEBS Lett 474: 133–136

Oster U, Tanaka R, Tanaka A and Rudiger W (2000) Cloning and functional expression of the gene encoding the key enzyme for chlorophyll *b* biosynthesis (CAO) from *Arabidopsis thaliana*. Plant J 21: 305–310

Papenbrock J, Gräfe S, Kruse E, Hanel F and Grimm B (1997) Mg-chelatase of tobacco: identification of a Chl D cDNA sequence encoding a third subunit, analysis of the interaction of the three subunits with the yeast two-hybrid system, and reconstitution of the enzyme activity by co-expression of recombinant CHL D, CHL H and CHL I. Plant J 12: 981–990

Papenbrock J, Mock HP, Kruse E and Grimm B (1999) Expression studies on tetrapyrrole biosynthesis: inverse maxima of magnesium chelatase and ferrochelatase activity during cyclic photoperiods. Planta 208: 264–273

Papenbrock J, Pfundel E, Mock HP and Grimm B (2000a) Decreased and increased expression of the subunit CHL I diminishes Mg chelatase activity and reduced chlorophyll synthesis in transgenic tobacco plants. Plant J 22: 155–164

Papenbrock J, Mock HP, Tanaka R, Kruse E and Grimm B (2000b) Role of magnesium chelatase activity in the early steps of the tetrapyrrole biosynthetic pathway. Plant Physiol 122: 1161–1169

Parham R and Rebeiz CA (1995) Chloroplast biogenesis 72: a [4-vinyl] chlorophyllide *a* reductase assay using divinyl chlorophyllide *a* as an exogenous substrate. Anal Biochem 231: 164–169

Pattanayak GK and Tripathy BC (2002) Catalytic function of a novel protein protochlorophyllide oxidoreductase C of *Arabidopsis thaliana*. Biochem Biophys Res Commun 291: 921–924

Pattanayak GK, Biswal AK, Reddy VS and Tripathy BC (2005) Light-dependent regulation of chlorophyll *b* biosynthesis in chlorophyllide *a* oxygenase overexpressing tobacco plants. Biochem Biophys Res Commun 326: 466–471

Peter E, Rothbart M, Oelze ML, Shalygo N, Dietz KJ and Grimm B (2010) Mg protoporphyrin monomethylester cyclase deficiency and effects on the tetrapyrrole metabolism in different light conditions. Plant Cell Physiol 2010 May 11. [Epub ahead of print]

Pineau B, Dubertret G, Joyard J and Douce R (1986) Fluorescence properties of the envelope membranes from spinach chloroplasts. Detection of protochlorophyllide. J Biol Chem 261: 9210–9215

Polking GF, Hannapel DJ and Gladon RJ (1995) A cDNA clone for 5-aminolevulinic acid dehydratase from tomato (*Lycopersicon esculentum* Mill.). Plant Physiol 107: 1033–1034

Plöscher M, Granvogl B, Reisinger V and Eichacker LA (2009) Identification of the N-termini of NADPH: protochlorophyllide oxidoreductase A and B from barley etioplasts (*Hordeum vulgare* L.) FEBS J 276: 1074–1081

Pontier D, Albrieux C, Joyard J, Lagrange T and Block MA (2007) Knock-out of the magnesium protoporphyrin IX methyltransferase gene in *Arabidopsis*. Effects on chloroplast development and on chloroplast-to-nucleus signaling. J Biol Chem 282: 2297–2304

Pontoppidan B and Kannangara CG (1994) Purification and partial characterization of barley glutamyl-tRNAglu reductase, the enzyme that directs glutamate to chlorophyll biosynthesis. Eur J Biochem 225: 529–537

Popperl G, Oster U, Blos I and Rudiger W (1997) Magnesium chelatase of *Hordeum vulgare* L. is not activated by light but inhibited by pheorphorbide. Z Naturforsch 52c: 144–152

Porra RJW, Schäfer E, Cmiel IK and Scheer H (1993) Derivation of the formyl group oxygen of chlorophyll *b* from molecular oxygen in greening leaves of a higher plant (*Zea mays*). FEBS Lett 371: 21–24

Poulson R and Polglasse WJ (1974) Aerobic and anaerobic coproporphyrinogen oxidase activities in extract from *Saccharomyces cerevisiae*. J Biol Chem 249: 6367–6371

Ratinaud MH, Thomes JC and Julien R (1983) Glutamyl-tRNA synthetases from wheat. Isolation and characterization of three dimeric enzymes. Eur J Biochem 135: 471–477

Rebeiz CA, Benning C, Bohnert HJ, Danielle H, Hoober JK, Lichtenthaler HK, Portis AR and Tripathy BC (eds) (2010) The Chloroplast: Basics and Applications, Advances in Photosynthesis and Respiration, Vol 31. Springer, Dordrecht

Reinbothe C, Apel K and Reinbothe S (1995) A light-induced protease from barley plastids degrades NADPH:protochlorophyllide oxidoreductase complexed with chlorophyllide. Mol Cell Biol 15: 6206–6212

Reinbothe C, Lebedev N and Reinbothe S (1999) A protochlorophyllide light-harvesting complex involved in de-etiolation of higher plants. Nature 397: 80–84

Reinbothe C, Buhr F, Bartsch S, Desvignes C, Quigley F, Pesey H and Reinbothe S (2006) In vitro-mutagenesis of NADPH:protochlorophyllide oxidoreductase B: two distinctive protochlorophyllide binding sites participate in enzyme catalysis and assembly. Mol Genet Genomics 275: 540–552

Richter A, Peter E, Pörs Y, Lorenzen S, Grimm B and Czarnecki O (2010) Rapid dark repression of 5-aminolevulinic acid synthesis in green barley leaves. Plant Cell Physiol 51: 670–681

Rissler HM, Collakova E, DellaPenna D, Whelan J and Pogson BJ (2002) Chlorophyll biosynthesis. Expression of a second chl I gene of magnesium chelatase in *Arabidopsis* supports only limited chlorophyll synthesis. Plant Physiol 128: 770–779

Rudiger W, Benz J and Guthoff C (1980) Detection and partial characterization of activity of chlorophyll synthetase in etioplast membranes. Eur J Biochem 109: 193–200

Runge S, Sperling U, Frick G, Apel K and Armstrong GA (1996) Distinct roles for light-dependent NADPH:protochlorophyllide oxidoreductases (POR) A and B during greening in higher plants. Plant J 9: 513–523

Rzeznicka K, Walker CJ, Westergren T, Kannangara CG, von Wettstein D, Merchant S, Gough SP and Hansson M (2005) Xantha-l encodes a membrane subunit of the aerobic Mg-protoporphyrin IX monomethyl ester cyclase involved in chlorophyll biosynthesis. Proc Natl Acad Sci USA 102: 5886–5891

Sakuraba Y, Yamasato A, Tanaka R and Tanaka A (2007) Functional analysis of N-terminal domains of Arabidopsis chlorophyllide *a* oxygenase. Plant Physiol Biochem 45: 740–749

Sakuraba Y, Yokono M, Akimoto S, Tanaka R, Tanaka A. (2010) Deregulated chlorophyll *b* synthesis reduces the energy transfer rate between photosynthetic pigments and induces photodamage in *Arabidopsis thaliana*. Plant Cell Physiol 51: 1055–1065

Sangwan I and O'Brian MR (1993) Expression of the soybean (*Glycine max*) glutamate 1-semialdehyde aminotransferase gene in symbiotic root nodules. Plant Physiol 102: 829–834

Santel HJ and Apel K (1981) The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). The effect of light on the NADPH: protochlorophyllide oxidoreductase. Eur J Biochem 120: 95–103

Sato Y, Morita R, Katsuma S, Nishimura M, Tanaka A and Kusaba M (2009) Two short-chain dehydrogenase/reductases, NON-YELLOW COLORING 1 and NYC1-LIKE, are required for chlorophyll b and light-harvesting complex II degradation during senescence in rice. Plant J 57: 120–131

Satoh S, Ikeuchi M, Mimuro M and Tanaka A (2001) Chlorophyll *b* expressed in cyanobacteria functions as a light-harvesting antenna in photosystem I through flexibility of the proteins. J Biol Chem 276: 4293–4297

Sawers RJ, Farmer PR, Moffett P and Brutnell TP (2006) In planta transient expression as a system for genetic and biochemical analyses of chlorophyll biosynthesis. Plant Methods 2:15

Schaumburg A, Schneider-Poetsch HA and Eckerskorn C (1992) characterization of plastid 5-aminolevulinate dehydratase (ALAD; EC 4.2.1.24) from spinach (*Spinacia oleracea* L.) by sequencing and comparison with non-plant ALAD enzymes. Z Naturforsch C 47: 77–84

Scheumann V, Ito H, Tanaka A, Schoch S and Rüdiger W (1996) Substrate specificity of chlorophyll(ide) *b* reductase in etioplasts of barley (*Hordeum vulgare* L.). Eur J Biochem 242: *163–170*

Scheumann V, Klement H, Helfrich M, Oster U, Schoch S and Rüdiger W (1999) Protochlorophyllide *b* does not occur in barley etioplasts. FEBS Lett 445: 445–448

Schmid HC, Oster U, Kögel J, Lenz S and Rüdiger W (2001) Cloning and characterisation of chlorophyll synthase from *Avena sativa*. J Biol Chem 382: 903–911

Schmid HC, Rassadina V, Oster U, Schoch S and Rudiger W (2002) Pre-loading of chlorophyll synthase with tetraprenyl diphosphate is an obligatory step in chlorophyll biosynthesis. J Biol Chem 383: 1769–1778

Schoefs B and Franck F (1993) Photoreduction of protochlorophyllide to chlorophyllide in 2-d-old dark-grown bean (*Phaseolus vulgaris* cv. Commodore) leaves. Comparison with 10-d-old dark-grown (etiolated) leaves. J Expt Bot 44: 1053–1057

Schoefs B and Bertrand M (2000) The formation of chlorophyll from chlorophyllide in leaves containing proplastids is a four-step process. FEBS lett 486: 243–246

Schoefs B and Franck F (2003) Protochlorophyllide reduction: mechanism and evolution. Photochem Photobiol 78: 543–557

Schoefs B, Garnir HP and Bertrand M (1994) Comparison of the photoreduction protochlorophyllide to chlorophyllide in leaves and cotyledons from dark-grown beans as a function of age. Photosynth Res 41: 405–417

Schoefs B, Bertrand M and Franck F (2000a) Spectroscopic properties of protochlorophyllide analyzed in situ in the course of etiolation and in illuminated leaves. Photochem Photobiol 72: 85–93

Schoefs B, Bertrand M and Franck F (2000b) Photoactive protochlorophyllide regeneration in cotyledons and leaves from higher plants. Photochem Photobiol 72: 660–668

Schon A, Krupp G, Gough S, Berry-Lowe S, Kannangara CG and Soll D (1986) The RNA required in the first step of chlorophyll biosynthesis is a chloroplast glutamate tRNA. Nature 322: 281–284

Schon A, Kannangara CG, Gough S and Soll D (1988) Protein biosynthesis in organelles requires misaminoacylation of tRNA. Nature 331: 187–190

Schulz R, Steinmuller K, Klaas M, Forreiter C, Rasmussen S, Hiller C and Apel K (1989) Nucleotide sequence of a cDNA coding for the NADPH–protochlorophyllide oxidoreductase (PCR) of barley (*Hordeum vulgare* L.) and its expression in *Escherichia coli*. Mol Gen Genet 217: 355–361

Schunmann PH and Ougham HJ (1996) Identification of three cDNA clones expressed in the leaf extension zone and with altered patterns of expression in the slender mutant of barley: a tonoplast intrinsic protein, a putative structural protein and protochlorophyllide oxidoreductase. Plant Mol Biol 31: 529–537

Scolnik P and Bartley GE (1996) A table of some cloned plant genes involved in isoprenoid biosynthesis. Plant Mol Biol Rep 14: 305–319

Seyedi M, Selstam E, Timko MP and Sundqvist C (2001a) The cytokinin 2-isopentenyladenine causes partial reversion to skotomorphogenesis and induces formation of prolamellar bodies and protochlorophyllide657 in the *lip1* mutant of pea. Physiol Plan. 112: 261–272

Seyedi M, Timko MP and Sundqvist C (2001b) The distribution of protochlorophyllide and chlorophyll within seedlings of the *lip1* mutant of pea. Plant Cell Physiol 42: 931–941

Shalygo NV, Mock HP, Averina NG and Grimm B (1998) Photodynamic action of uroporphyrin and protochlorophyllide in greening barley leaves treated with cesium chloride. J Photochem Photobiol B 42: 151–158

Shalygo N, Czarnecki O, Peter E, Grimm B (2009) Expression of chlorophyll synthase is also involved in feedback-control of chlorophyll biosynthesis. Plant Mol Biol 71: 425–436

Shashidhara LS and Smith AG (1991) Expression and subcellular location of the tetrapyrrole synthesis enzyme porphobilinogen deaminase in light-grown *Euglena gracilis* and three nonchlorophyllous cell lines. Proc Natl Acad Sci USA 88: 63–67

Shemin D (1976) 5-Aminolevulinic acid dehydratase: Structure, function and mechanism. Philos Trans R Soc London 273: 109–115

Shetty AS and Miller GW (1969) Purification and general properties of 5-aminolevulinate dehydratase from *Nicotiana tabaccum* L. Biochem J 114: 331–337

Shibata K (1957) Spectroscopic studies of chlorophyll formation in intact leaves. J Biochem Tokyo, Japan 44: 147–173

Shibata H and Ochiai H (1977) Purification and properties of δ-aminolevulinic acid dehydratase from radish cotyledons. Plant Cell Physiol 18: 420–429

Smith AG (1988) Subcellular localization of two porphyrin-synthesis enzymes in *Pisum sativum* (pea) and Arum (cuckoo-pint) species. Biochem J 249: 423–428

Smith AG and Francis JE (1981) Investigations of rat liver uroporphyrinogen decarboxylase. comparisons of porphyrinogens I and III as substrate nad inhibition by porphyrins. Biochem J 195: 241–250

Soll J, Schultz G, Rüdiger W and Benz J (1983) Hydrogenation of geranylgeraniol. Two pathways exist in spinach chloroplasts. Plant Physiol 71: 849–854

Spano AJ and Timko MP (1991) Isolation, characterization and partial amino acid sequence of a chloroplast-localized porphobilinogen deaminase from pea (*Pisum sativum* L.). Biochim Biophys Acta 1076: 29–36

Spano AJ, He Z, Michel H, Hunt DF and Timko MP (1992) Molecular cloning, nuclear gene structure, and developmental expression of NADPH:protochlorophyllide oxidoreductase in pea (*Pisum sativum* L.). Plant Mol Biol 18: 967–972

Spencer P and Jordan PM (1994) 5-Aminolevulinic acid dehydratase: Characterization of the α and ß metal-binding sites of the *Escherichia coli* enzyme. In: Chadwick DJ and Ackrill K (eds) The Biosynthesis of Tetrapyrrole Pigments, Ciba Foundation Symposium 180, pp 50–64. John Weily and Sons, Chichester

Spencer P and Jordan PM (1995) Characterization of the two 5-aminolevulinic acd binding sites of 5-aminolevulinic acid dehydratase from *Escheichia coli*. Biochem J 305: 151–158

Sperling U, Van Cleve B, Frick G, Apel K and Armstrong GA (1997) Overexpression of light-dependent PORA or PORB in plants depleted of endogenous POR by far-red light enhances seedling survival in white light and protects against photooxidative damage. Plant J 12: 649–658

Sperling U, Franck F, Cleve BV, Frick G, Apel K and Armstrong GA (1998) Etioplast differentiation in *Arabidopsis*: Both PORA and PORB restore the prolamellar body and photoactive protochlorophyllide-F655 to the cop1 photomorphogenic mutant. Plant Cell 10: 283–296

Stenbaek A and Jensen PE. (2010) Redox regulation of chlorophyll biosynthesis. Phytochem 71: 853–859

Stobart AK and Ameen-Bukhari I (1984) Regulation of δ-aminolaevulinic acid synthesis and protochlorophyllide regeneration in the leaves of dark-grown barley (*Hordeum vulgare*) seedlings. Biochem J 222: 419-426

Stobart AK and Ameen-Bukhari I (1986) Photoreduction of protochlorophyllide and its relationship to δ-aminolaevulinic acid synthesis in the leaves of dark-grown barley (*Hordeum vulgare*) seedlings. Biochem J 236: 741-748

Su Q, Frick G, Armstrong G and Apel K (2001) POR C of *Arabidopsis thaliana*: a third light- and NADPH-dependent protochlorophyllide oxidoreductase that is differentially regulated by light. Plant Mol Biol 47: 805–813

Sytina OA, Heyes DJ, Hunter CN, Alexandre MT, Van Stokkum IH, Van Grondelle R and Groot ML (2008) Conformational changes in an ultrafast light-driven enzyme determine catalytic activity. Nature 456: 1001–1004

Tan FC, Cheng Q, Saha K, Heinemann IU, Jahn M, Jahn D and Smith AG (2008) Identification and characterization of the *Arabidopsis* gene encoding the tetrapyrrole biosynthesis enzyme uroporphyrinogen III synthase. Biochem J 410: 291–299

Tanaka R and Tanaka A (2005) Effects of chlorophyllide *a* oxygenase overexpression on light acclimation in *Arabidopsis thaliana*. Photosynth Res 85: 327–340

Tanaka R and Tanaka A (2007) Tetrapyrrole biosynthesis in higher plants. Annu Rev Plant Biol 58: 321–46

Tanaka R, Yoshida K, Nakayashiki T, Masuda T, Tsuji H, Inokuchi H and Tanaka A (1996) Differential expression of two *hemA* mRNAs encoding glutamyl-tRNA reductase proteins in greening cucumber seedlings. Plant Physiol 110: 1223–1230

Tanaka A, Ito H, Tanaka R, Tanaka NK, Yoshida K and Okada K (1998) Chlorophyll *a* oxygenase (CAO) is involved in chlorophyll *b* formation from chlorophyll *a*. Proc Natl Acad Sci USA 95: 12719–12723

Tanaka R, Oster U, Kruse E, Rudiger W and Grimm B (1999) Reduced activity of geranylgeranyl reductase leads to loss of chlorophyll and tocopherol and to partially geranylgeranylated chlorophyll in transgenic tobacco plants expressing antisense RNA for geranylgeranyl reductase. Plant Physiol 120: 695–704

Tanaka R, Koshino Y, Sawa S, Ishiguro S, Okada K and Tanaka A (2001) Overexpression of chlorophyllide *a* oxygenase (CAO) enlarges the antenna size of photosystem II in *Arabidopsis thaliana*. Plant J 26: 365–373

Tchuinmogue SJ, Bruyant P and Balange AP (1992) Immunological characterization of two 5-aminolevulinate dehydratase in radish leaves. Plant Physiol Biochem 30: 255–261

Teakle GR and Griffiths WT (1993) Cloning, characterization and import studies on protochlorophyllide reductase from wheat (*Triticum aestivum*). Biochem J 296: 225–230

Tewari AK and Tripathy BC (1998) Temperature-stress-induced impairment of chlorophyll biosynthetic reactions in cucumber and wheat. Plant Physiol 117: 851–858

Tewari AK and Tripathy BC (1999) Acclimation of chlorophyll biosynthetic reactions to temperature stress in cucumber (*Cucumis sativus* L.). Planta 208: 431–437

Tottey S, Block MA, Allen M, Westergren T, Albrieux C, Scheller HV, Merchant S and Jensen PE (2003) *Arabidopsis* CHL27, located in both envelope and thylakoid membranes, is required for the synthesis of protochlorophyllide. Proc Natl Acad Sci USA 100: 16119–16124

Townley HE, Sessions RB, Clarke AR, Dafforn TR and Griffiths WT (2001) Protochlorophyllide oxidoreductase: a homology model examined by site-directed mutagenesis. Proteins 44: 329–335

Tripathy BC and Rebeiz CA (1985) Chloroplast biogenesis: quantitative determination of monovinyl and divinyl Mg-protoporphyrins and protochlorophyll(ides) by spectrofluorometry. Anal Biochem 149: 43–61

Tripathy BC and Rebeiz CA (1986) Chloroplast biogenesis. Demonstration of the monovinyl and divinyl monocarboxylic routes of chlorophyll biosynthesis in higher plants. J Biol Chem 261: 13556–13564

Tripathy BC and Rebeiz CA (1988) Chloroplast biogenesis 60: Conversion of divinyl protochlorophyllide to monovinyl protochlorophyllide in green(ing) barley, a dark monovinyl/light divinyl plant species. Plant Physiol 87: 89–94

Tripathy BC, Mohapatra A and Gupta I (2007) Impairment of the photosynthetic apparatus by oxidative stress induced by photosensitization reaction of protoporphyrin IX. Biochim Biophys Acta 1767: 860–868

Tsai S, Bishop DF and Desnick RJ (1987) Purification and properties of uroporphyrinogen III synthase from human erythrocytes. J Biol Chem 262: 1268–1273

Tsang EW, Yang J, Chang Q, Nowak G, Kolenovsky A, McGregor DI and Keller WA (2003) Chlorophyll reduction in the seed of *Brassica napus* with a glutamate 1-semialdehyde aminotransferase antisense gene. Plant Mol Biol 51: 191–201

von Wettstein D, Gough S and Kannangara CG (1995) Chlorophyll biosynthesis. Plant Cell 7: 1039–1057

Walker CJ and Weinstein JD (1994) The magnesium-insertion step of chlorophyll biosynthesis is a two-stage reaction. Biochem J 299: 277–284

Walker CJ and Willows RD (1997) Mechanism and Regulation of Mg-chelatase. Biochem J 327: 321–333

Walker CJ, Mansfield KE, Smith KM and Castelfranco PA (1989) Incorporation of atmospheric oxygen into the carbonyl functionality of the protochlorophyllide isocyclic ring. Biochem J 257: 599–602

Walker CJ, Castelfranco PA and Whyte BJ (1991) Synthesis of divinyl protochlorophyllide. Enzymological properties of the Mg-protoporphyrin IX monomethyl ester oxidative cyclase system. Biochem J 276: 691–697

Warabi E, Usui K, Tanaka Y and Matsumoto H (2001) Resistance of a soybean cell line to oxifluorfen by overproduction of mitochondrial protoporphyrinogen oxidase. Pest Manag Sci 57: 743–748

Watanabe N, Che F-S, Iwano M, Takayama S, Yoshida S and Isogai A (2001) Dual targeting of spinach protoporphyrinogen oxidase II to mitochondria and chloroplast by alternative use in-frame initiation codons. J Biol Chem 276: 20474–20481

Whyte BJ and Castelfranco PA (1993) Further observations on the Mg-protoporphryin IX monomethyl ester (oxidative) cyclase system. Biochem J 290: 355–359

Wiktorsson B, Engdahl S, Zhong LB, Böddi B, Ryberg M and Sundqvist C (1993) The effect of cross-liking of the subunits of NADPH-protochlorophyllide oxidoreductase of the aggregational state of protochlorophyllide. Photosynthtica 29: 205–218

Wilks HM and Timko MP (1995) A light-dependent complementation system for analysis of NADPH:protochlorophyllide oxidoreductase: Identification and mutagenesis of two conserved residues that are essential for enzyme activity. Proc Natl Acad Sci USA 92: 724–728

Williams P, Hardeman K, Fowler J and Rivin C (2006) Divergence of duplicated genes in maize: evolution of contrasting targeting information for enzymes in the porphyrin pathway. Plant J 45: 727–739

Wise RR and Hoober JK (eds) (2006) The Structure and Function of Plastids, Advances in Photosynthesis and Respiration, Vol 23. Springer, Dordrecht

Witty M, Wallace-Cook AD, Albrecht H, Spano AJ, Michel H, Shabanowitz J, Hunt DF, Timko MP and Smith AG (1993) Structure and expression of chloroplast-localized porphobilinogen deaminase from pea (*Pisum sativum* L.) isolated by redundant polymerase chain reaction. Plant Physiol 103: 139–147

Wu Z, Zhang X, He B, Diao L, Sheng S, Wang J, Guo X, Su N, Wang L, Jiang L, Wang C, Zhai H and Wan J (2007) A chlorophyll-deficient rice mutant with impaired chlorophyllide esterification in chlorophyll biosynthesis. Plant Physiol 145: 29–40

Xu H, Vavilin D and Vermaas W (2001) Chlorophyll *b* can serve as the major pigment in functional photosystem II complexes of cyanobacteria. Proc Natl Acad Sci USA 98: 14168–14173

Yamasato A, Nagata N, Tanaka R and Tanaka A (2005) The N-terminal domain of chlorophyllide *a* oxygenase confers protein instability in response to chlorophyll *b* accumulation in *Arabidopsis*. Plant Cell 17: 1585–1597

Yamasato A, Tanaka R and Tanaka A (2008) Loss of the N-terminal domain of chlorophyllide *a* oxygenase induces photodamage during greening of *Arabidopsis* seedlings. BMC Plant Biol 8: 64

Yaronskaya E, Vershilovskaya I, Poers Y, Alawady AE, Averina N and Grimm B (2006) Cytokinin effects on tetrapyrrole biosynthesis and photosynthetic activity in barley seedlings. Planta 224: 700–709

Zhang H, Li J, Yoo JH, Yoo SC, Cho SH, Koh HJ, Seo HS and Paek NC (2006) Rice *Chlorina-1* and *Chlorina-9* encode ChlD and ChlI subunits of Mg-chelatase, a key enzyme for chlorophyll synthesis and chloroplast development. Plant Mol Biol 62: 325–337