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Review Article

# The roles of a light-dependent protochlorophyllide oxidoreductase (LPOR), and ATP-dependent dark operative protochlorophyllide oxidoreductase (DPOR) in chlorophyll biosynthesis

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## Abstract

Chlorophyll is a green photosynthetic pigment, and photosynthesis drives the global carbon cycle. The reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) in the penultimate stage of biosynthesis of chlorophyll (Chl) is catalysed by light-independent protochlorophyllide reducatse (DPOR), and the light-dependent protochlorophyllide oxidoreductase (LPOR). The search was done to all manuscript sections according to terms chlorophyll, a light-dependent protochlorophyllide oxidoreductase, ATP-dependent dark operative protochlorophyllide oxidoreductase, chlorophyll, photosynthesis and chlorophyllide. Within the framework of photosynthesis and chlorophyll, this review article was aimed to provide an overview of the functional studies in chlorophyll biosynthesis, protein crystal structure, disclosure of action mechanisms, and possible future available direction of LPOR and DPOR in the biosynthesis of chlorophyll.

***Keywords:*** chlorophyll; chlorophyllide; DPOR; LPOR; protochlorophyllide oxidoreductase

## Introduction

### Chlorophyll and photosynthesis

Chlorophyll is the main driving engine for photosynthesis (Hunter *et al.*, 1994; Sun *et al.*, 2019; Lu *et al.*, 2020). It is the sole compartment containing green pigment where photosynthesis takes place within the cell (Humphrey, 1980; Mandal and Dutta, 2020; Sun et al., 2021). Chloroplast is one of the three kinds of plastids found only in plant cells (Mandal and Dutta, 2020). Chlorophyll has a tremendous capacity to trap light energy and apply in photolysis of water molecules to replenish the reducing power of the cells, which is necessary in carbon assimilation in subsequent steps of photosynthesis (van der Tol *et al.*, 2009; Soleymani *et al.*, 2016; Grajek *et al.*, 2020; Mandal and Dutta, 2020; Shahrajabian *et al.*, 2021).

Solar induced chlorophyll fluorescence (SIF) denotes reemitted light in the 650-850 nm range from the chlorophyll-a pigment, which is linked to initial steps in photosynthesis (Frankenberg and Berry, 2018). In some plants like peanut, *Arachis hypogaea* L. Golden2-like 1) (AhGLK1) activates the expression of AhPORA

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to promote chlorophyll biosynthesis, and that AhGLK1 physically interacts with *Arachis hypogaea* L. histone deacetylase 1) (Li *et al.*, 2019; Liu *et al.*, 2020). In photosynthesis, electrons are transported in the thylakoid from photosystem (PS) II to PSI and finally to $NADP^+$ in the storma, and electron transport results in the generation of the transthylakoid protein motive force that drives ATP synthesis (Gotoh *et al.*, 2010). The main photosynthesis is $6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$. The photosynthesis process is also influenced by micronutrients such as boron, iron, manganese, copper and zinc through various modes of actions.

Chlorophyll *a* (Chl *a*) is the universal photosynthetic pigment which is available in most oxygenic photosynthetic organisms, and performs all of the above functions (Bjorn *et al.*, 2009). Chl *b* and other accessory pigments are mainly active in light-harvesting complexes (LHCs), and are expected to improve light-harvesting efficiency (Nick *et al.*, 2013; Chen *et al.*, 2014; Voltsekhovskaja and Tyutereva, 2015). Chl*c* is another accessory pigment involved in light harvesting and is found in heterokont algae descended from secondary endosymbiosis (Green, 2011). In $C_3$ plants, the leaves do not have Kranz anatomy, Ribulose 1,5-bisphosphate (RUBP) is the first acceptor of $CO_2$, phosphoglycerate (PGA) is the first stable product, photosynthesis occurs in mesophyll cells, and photorespiration occurs while in $C_4$ plants, the leaves show Kranz anatomy, phosphoenolpyruvate (PEP) is the first acceptor of $CO_2$, oxaloacetate (OAA) is the first stable product, photosynthesis occurs in both mesophyll cells and bundle sheath cells, and photorespiration does not occur.

The chlorophyll content could depend on environmental and seasonal changes (Zhuang *et al.*, 2020). Photosynthesis, stomatal conductance, transpiration and yield were higher but sub-stomatal $CO_2$ concentration was lower under drought stress conditions than under control conditions in chickpea cultivars (Mafakheri *et al.*, 2010).

## Chlorophyllide

Chlorophyllide *a*is a metabolite late in the biosynthesis of bacteriochlorophylls and chlorophylls (Muller *et al.*, 2011). Chlorophyllide *a* is the prevailing form of chlorophyll in green algae and plants and the sole form of chlorophyll in many cyanobacteria; like most other chlorophylls, chlorophyll *a* includes of two moieties, the porphyrin macrocycle chlorophyllide *a* and a branched-carbon phytyl tail (Vavilin and Vermaas, 2007). Chlorophyll *b* is one of the main photosynthetic pigments of plants, and the regulation of chlorophyll *b* biosynthesis is principal for plants in order to acclimate to changing environmental conditions, and in the chloroplast, chlorophyll b is synthesized from chlorophyll *a* by chlorophyllide *a* oxygenase (CAO), a Rieske-type monooxygenase (Sakuraba *et al.*, 2009). Chlorophyll *b* is considered as the antenna chlorophyll, and the binding of it by antenna proteins is influential for the correct assembly of the antenna complexes in thylakoid membranes (Voitsekhovskaja and Tyutereva, 2015).

## Protochlorophyllide oxidoreductase (POR)

Protochlorophyllide (Pchlide) is a porphyrin dye, the main photosynthetic pigment, and one of the main intermediates in the biosynthetic pathway of chlorophyll (Chl) (Mysliwa-Kurdziel *et al.*, 2013). The enzyme protochlorophyllide oxidoreductase (POR) catalyzes a principle light-driven reaction which triggers a profound transformation in plant development, in the chlorophyll biosynthetic pathway (Knaust *et al.*, 1993; Armstrong *et al.*, 2000; Heyes and Hunter, 2005).

As POR is activated by light, it can provide information on the way in which light energy can be harnessed to power enzyme reactions and it shows a unique opportunity to study catalysis at low temperatures and on ultrafast timescales which are not available for most analyses of enzyme function (Heyes and Hunter, 2005). POR finds in multiple isoforms that share high level of homology, and silencing of POR results in accumulation of protochlorophyllide (Pchlide) and complete loss of chlorophyllide (Chlide) (Talaat, 2013). The importance of carotenoid for membrane organization of NADPH POR was found (Denev *et al.*, 2005).

POR is a major enzyme for the light-induced greening of angiosperms (Reinbothe *et al.*, 2003). Protochlorophyll (Pchl) and Protochlorophyllide (Pchlide) are naturally occurring prophyrins in plants; Pchl is more hydrophobic than Pchlide due to a long chain of phytol or its precursors attached to the tetrapyrrole ring (Mysliwa-Kurdziel *et al.*, 2013). The nature of the solvent environment determined the excited-state relaxation of Pchlide (Dietzek *et al.*, 2010). POR has a conserved Tyr and Lys residue in the enzyme active site like the other members of the short chain alcohol dehydrogenase/reductase family enzymes, which are involved in a proposed reaction mechanism involving proton transfer from the Tyr hydroxyl group to Pchlide (Menon *et al.*, 2009). POR, together with DNA photolyase, is one of only the two enzymes that show a direct, natural requirement for light and because mixing techniques are no longer needed to initiate the reaction, it is possible to trigger catalysis at cryogenic temperatures and on very fast time scales (Mees *et al.*, 2004); consequently, POR has proven to be an unique model system for studying the function of protein dynamics in driving enzyme catalysis (Heyes and Hunter, 2005).

It is reported that phytochrome A (phyA) down-regulates the synthesis of NADPH: protochlorophyllide (Pchlide) oxidoreductase and active $Pchlide^{655}$ under far-red light (FR) (Sineshchekov *et al.*, 2004). Dark-operative protochlorophyllide (Pchlide) oxidoreductase (DPOR) is a nitrogenase-like enzyme catalyzing a reduction of the C17=C18 double bond of Pchlide to form chlorophyllide a (Chlide) in bacteriochlorophyll biosynthesis, and DPOR contains of an ATP-dependent reducatse component, L-protein (a BchL dimer), and a catalytic constituent, NB-protein (a BchN-BchB heterotetramer), and the L-protein transfers electrons to the NB-protein to reduce Pchlide, which is associated with ATP hydrolysis (Nomata *et al.*, 2016).

The NADPH-protochlorophyllide oxidoreductase (NPR) is a well-documented enzyme catalyzing photoconversion of protochlorophyllide to chlorophyllide *a* and its expression is negatively adjusted by light (Kuroda *et al.*, 1995). Oosawa *et al.* (2000) demonstrated that *Arabidopsisthaliana* are controlled by three POR isoforms, which are differentially controlled by development and light. Zhang *et al.* (2019) indicated that how the POR active site promotes light-driven reduction of protochlorophyllide by localized hydride transfer NADPH and long-range proton transfer along structurally defined proton-transfer pathways.

## Light-Dependent NADPH Protochlorophyllide Oxidoreductase (LPOR)

The development of chloroplasts up to the stage of etioplasts which consist characteristic structures recognized as prolamellar bodies (PLBs) which have tubules joined together in a regular network and have a particular paracrystalline symmetry (Kowalewska *et al.*, 2016). Protochlorophyllide (Pchlide) is a porphyrin dye and one of the main intermediates in the biosynthetic pathway of chlorophyll (Chl), the key photosynthetic pigment (Mysliwa-Kurdziel *et al.*, 2013). Conversion of pchlide to chl includes two reactions, first is reduction of one double-bond in the porphyrin ring, leading to chlorophyllide (Chlide) formation, and the second is esterification of chlide by phytol or its unsaturated precursors (Willows, 2003; Eckhardt *et al.*, 2004; Bollivar, 2006; Masuda, 2008; Masuda and Fujita, 2008). The enzyme protochlorophyllide oxidoreductase (POR) has a significant function in plant development, and it catalyzes one of the later steps in chlorophyll synthesis, the light-induced reduction of protochlorophyllide (PChlide) into chlorophyllide (Chlide) in the presence of NADPH (Garrone *et al.*, 2015).

In angiosperms, Pchlide reduction to Chlide is completely light-dependent and catalyzed by a protochlorophyllide oxidoreductase (LPOR, EC 1.3.1.33), a photoenzyme (Mysliwa-Kurdziel *et al.*, 2013), and angiosperms accumulate Pchlide in the dark but do not synthesize Chl (Kruk, 2005; Schoefs, 2005; Belyaeva and Litvin, 2007; Masuda, 2008; Reinbothe *et al.*, 2010). In the absence of light, Pchlide accumulates in etioplasts, which develops instead of chloroplasts and may consist a regular paracrystalline lipid structure called as a prolamellar body (PLB) (Solymosi and Schoefs, 2008; Solymost and Schoef, 2010), and among different proteins recognized in PLB (Blomqvist *et al.*, 2008), the most abundant is LPOR, which is found specially in the form of ternary Pchlide: LPOR: NADPH complexes (Ryberg and Sundqvist, 1982).

LPOR is ubiquitous amongst eukaryotic phototrophs and is included of nucleus-encoded subunits that are post-translationally targeted to the chloroplasts (Aronsson *et al.*, 2003); moreover, it is present in almost all chlorophyll manufacturing organisms but missing in photosynthetic bacteria (Adamson *et al.*, 1997). LPOR belongs to the class of proteins known as short chain alcohol dehydrogenases and appears to have originally evolved in cyanobacteria because of strong evolutionary pressure and high oxygen sensitivity of DPOR (Yang and Cheng, 2004).

PLBs contain two spectral forms of the Pchlide-LPOR complexes with absorption maxima ~640 and 650 (Selstam *et al.*, 2002). Schoefs and Frank (2004) concluded that only Pchlide, which forms ternary complexes with LPOR and NADPH (e.g. having the fluorescence maxima at 645 and 656 nm at 77K) can be reduced to Chlide by a millisecond flash illumination of saturating intensity. Short-wavelength forms were assigned to Pchlide which was unbound to the LPOR enzyme and mostly discovered in PTs (Ryberg and Sundqvist, 1982), and long-wavelength forms, found in PLBs, were attributed to aggregates of Pchlide: LPOR: NADPH complexes of different sizes that are stabilized by interaction of $\pi$ electrons of the neighboring pigment molecules (Boddi *et al.*, 1989).

Two isoenzymes of the LPOR have been recognized: the LPOR-A which is accumulated in the dark and LPOR-B which is believed to work in plants under light exposure (Boddi *et al.*, 1998). The redox state of NADPH in Pchlide: LPOR: NADPH complexes also have effect on PChlide spectral properties and may contribute to the heterogeneity (Frank *et al.*, 1999). Mysliwa-Kurdziel *et al.* (2013) found the part of galactolipids of PLB *in vivo* in regulating the proportion between Pchlide aggregates and monomers, which is important for the proper assembly of ternary photoactive Pchlide: LPOR: NADPH complexes and for well-organized PChlide to Chlide photoreduction.

The paracrystalline nature of PLBs is considered to be due to the aggregation of a complex containing protochlorophyllide (Pchlide), light-dependent protochlorophyllide oxidoreductase (LPOR), and NADPH (Pchlide-LPOR-NADPH) (Ryberg and Sundqvist, 1982). The role of the large pigment-protein complex Pchlide-LPOR in the formation of PLB membranes with a cubic phase structure is possibly because of the ability of this pigment-protein complex to form an oligomer and to anchor the LPOR protein into the membrane (Selstam, 1998). It has been reported that as a consequence of light-requirement of this enzyme, when plants are grown in darkness (etiolation), chlorophyll biosynthesis is terminated at the Pchlide phase and its accumulation is happened (Kruk *et al.*, 2005). Park *et al.* (2002) observed that the proper composition of carotenoids in the PLB membrane can play an important function in the formation and maintenance of its paracrystalline structure. Grzyb *et al.* (2013) reported that AFM seems a promising method for scrutinizing PLB formation and the changes in PLB structure and it provides a notable chance to observe PLBs at a physiological temperature without the necessity fixation.

There is the evidence that heavy metals can interact with LPOR enzyme and inhibit Chl biosynthesis at the level of Pchlide to Chlide photoreduction (Stobart *et al.*, 1985; Boddi *et al.*, 1995; Berska *et al.*, 2001). Mysliwa-Kurdziel and Strzalka (2005) concluded that the stability of PLB membranes may additionally be influenced because of the heavy metal treatment. Sperling *et al.* (1997) stated that highly regular structure of PLB membranes is thought to play a part in the protection of plants against photooxidation caused by intensive illumination of dark-adapted plants which is not appropriate for young plants.

LPORs were suggested to have evolved about 2 billion years ago in cyanobacteria as a consequence of increasing atmospheric oxygen levels (Yamazaki *et al.*, 2006), and therefore, they are supposed to be absent in anoxygenic phototrophs (Kaschner *et al.*, 2014). Kaschner *et al.* (2014) observed that *in vitro* and *in vivo* functional assays unequivocally prove light-dependent protochlorophyllide reduction by *D. shibae* DFL12$^{T}$ and reveal LPORs are not restricted to cyanobacteria and plants. The three different isoforms of LPOR from *A. thaliana* were all proved to form Pchlide-LPOR-NADPH complexes with similar kinetic and spectral properties (Gabruk *et al.*, 2015). Kaschner *et al.* (2014) also concluded that anoxygenic phototrophs only contain oxygen-sensitive dark-operative PORs (DPORs), which catalyze protochlorophyllide reduction independent of the presence of light, however, oxygenic phototrophs additionally contains oxygen-insensitive

but light-dependent PORs (LPORs). Reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) by dark operative DPOR and light-dependent LPOR protochlorophyllide oxidoreductases is shown in Figure 1.

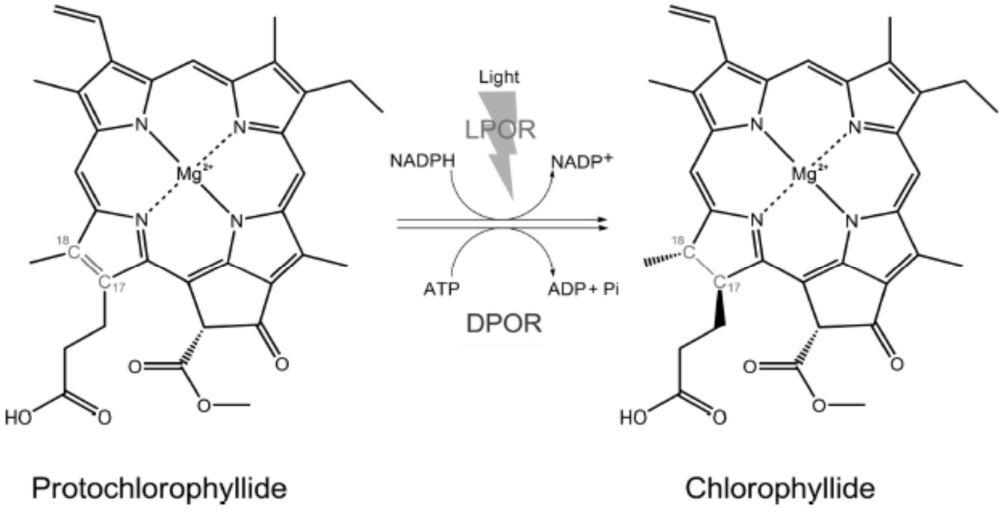


**Figure 1.** Reduction of protochlorophyllide (Pchlide) to chlorophyllide (Chlide) by dark operative DPOR and light-dependent LPOR protochlorophyllide oxidoreductases
The C17 = C18 double bond of Pchlide which is reduced by the respective protochlorophyllide reductase is shown in grey (Kaschner *et al.*, 2014)

Ourteam worked together with the University of Manchester andShanghai Jiao Tong Hospital/Faculty of Basic Medicineand reportedcrystal structures of the apo-POR enzyme (Thermosynechococcus. elongatus, PDB code 6RNV), and NADPH-bound POR (*Synechocystis* and *T. elongatus*, PDB code 6G08 and 6RNW), solved at 1.3 Å, 1.9Å and 1.9 Å resolution, respectively (Zhang *et al.*, 2019). This study reveals how the POR active site facilitates light-driven reduction of Pchlide by localized hydride transfer from NADPH and long-range proton transfer along structurally defined proton transfer pathways. Our study provides a structural basis for harnessing light energy to drive catalysis in this important chlorophyll biosynthetic enzyme, which is crucial for light-to-chemical energy conversion and unidirectional energy flow in the biosphere.

## Light-independent Pchlide Reductase (DPOR)

In comparison with the dependence on light for the greening (Chl synthesis) of angiosperms, some photosynthetic organisms like non-flowering land plants, cyanobacteria, algae, and anoxygenic photosynthetic bacteria are capable of synthesizing Chls and BChls in the dark (Beigbeder *et al.*, 1995; Nomata *et al.*, 2005). These organisms have an unassociated Pchlide reductase enzyme called dark-operative Pchlide oxidoreductase (DPOR: light-independent Pchlide oxidoreductase). This enzyme catalyzes double-bond reduction in a light-independent manner (Fujita, 1996; Armstrong *et al.*, 1998; Fujita and Bauer, 2003), which has been well-known as a mysterious enzyme because of the absence of a reliable assay system, which has hampered attempts at purification (Armstrong, 1998; Fujita and Bauer, 2003).

Nomata *et al.* (2006) reported that dark-operative protochlorophyllide reductase in bacteriochlorophyll biosynthesis is a nitrogenase-like enzyme including of L-protein (BchL-dimer) as a reductase component and NB-protein (BchN-BchB-heterotetramer) as a catalytic component. DPOR plays an important part in chlorophyll biosynthesis of gymnosperms, algae, mosses, ferns, and photosynthetic bacteria in the absence of

light; although, DPOR shares notable amino acid sequence homologies with nitrogenase, only the initial catalytic steps look like nitrogenase catalysis (Brocker *et al.*, 2008).

DPOR is made of electron donor (BchL) and acceptor (BchNB) component proteins; BchNB is further consisted of two subunits each of BchN and BchB arranged as an $\alpha_2\beta_2$ heterotetramer with two active sites for substrate reduction (Corless *et al.*, 2020). Organisms containing DPOR can have functional chloroplasts in the dark and start photosynthesis upon exposure in light (Kusumi *et al.*, 2006). A series of genetic researches that indicated three genes, *bchL*, *bchN*, and *bchB*, encode subunits of DPOR in *Rhodobacter capsulatus*, *Plectonema boryanum*, and *Chlamydomonas reinhardtii* (Fujita, 1996; Suzuki *et al.*, 1997; Fujita and Bauer, 2003; Maximova and Slovakova, 2014).

The DPOR subunits, BchL, BchN, and BchB, reveal significant sequence similarity to the nitrogenase subunits, NifH, NifD, and NifK, respectively, which exhibit that the mechanism of reducing the D-ring of Pchlide may be similar to the reduction of dinitrogen by nitrogenase (Fujita and Bauer, 2003). Two other DPOR subunits, BchN/ChlN, and BchB/ChlB show sequence similarity to both of NifD and NifK proteins, although at a lower level of 25-30% similarity (including three Cys residues in NifD and one Cys residue in NifK) (Fujita, 1996; Fujita and Bauer, 2003).

DPOR includes of two separable components, L-protein (BchL), and NB-protein (BchN-BchB), and DPOR activity is dependent on ATP and a reducing agent such as dithionite, and L-protein was considered to function as an ATP-dependent electron donor to NB-protein, which provides the catalytic site for Pchlide reduction (Fujita and Bauer, 2000). L-protein and NB-protein of DPOR resemble Fe protein and MoFe protein of nitrogenase, respectively, in crystal structure (Sarma *et al.*, 2008; Brocker *et al.*, 2010; Muraki *et al.*, 2010). The [4Fe-4S] cluster of the L-protein is decreased by ferredoxin *in vivo* or dithionite *in vitro* (Fujita and Bauer, 2000; Nomata *et al.*, 2005), and the electron is transferred to the NB-cluster in the NB-protein through a transient complex formation between the L-protein and the NB-protein (Nomata *et al.*, 2013).

Takano *et al.* (2011) stated that the redox character of the NB cluster is responsible for why Asp36 is vital in DPOR, and also the unknown coordinating ligand of the [4Fe-4S] cluster in the D36A mutant of DPOR is a chloride ion. Nomata *et al.* (2008) showed that together with the Fe and sulfide contents, NB-protein carries two oxygen-tolerant [4Fe-4S] clusters. Kondo *et al.* (2011) suggested that Asp36 contributes to the low redox potential necessary to reduce protochlorophyllide. Yamamoto *et al.* (2009) suggested that the DPOR from an oxygenic photosynthetic organism did not obtain oxygen tolerance during evolution; but that the cyanobacterial cell developed a mechanism to protect DPOR from oxygen. Wu *et al.* (2001) found that a *chlL*-deletion mutant of *Synerchocystis* sp. PCC 6803 designated as *chlL*$^-$ was inadequate to make significant amounts of chlorophyll in darkness, and PCR analysis confirmed that the *chlL*$^-$ pseudorevertant mutant still lacked the *chlL* gene. Nazi and Khan (2013) found that the activation of dark-operative pathway requisites additional factors/genes to *chlL* and *chlN* genes to develop chlorophyll, and therefore photosynthetically competent chloroplasts.

Fujita *et al.* (1998) concluded that both LPOR and DPOR contribute to Chl synthesis in the cells growing in the light, the extent of the contribution by LPOR increases with increasing light intensity. Wei *et al.* (2004) also observed that at low light intensities, the Chl content in the mutant lacking DPOR was very low and its growth rate was retarded, but, under low light conditions, cyanobacteria might have the possibility to make efficient utilize of the small amount of light to boost photosynthetic efficiency for survival. Nomata *et al.* (2013) demonstrated that nicotinamide (NA) inhibits DPOR activity by blocking the electron transfer from L-protein to NB-protein, a reaction scheme of DPOR, in which the binding of protochlorophyllide (Pchlide) to the NB-protein precedes the electron transfer from the L-protein is proposed based on the NA impacts. Nomata *et al.* (2014) considered DPOR as a special iron-sulphur enzyme to form substrate radicals along with sequential proton- and electron-transfer steps with the protein folding very similar to that of nitrogenase.

The reaction of Pchlide reduction and inhibition of DPOR activity by NA, DPOR assay was carried out with purified L-protein and NB-protein in the absence (black line) or presence (500 μM, red line) of NA are indicated in Figure 2.

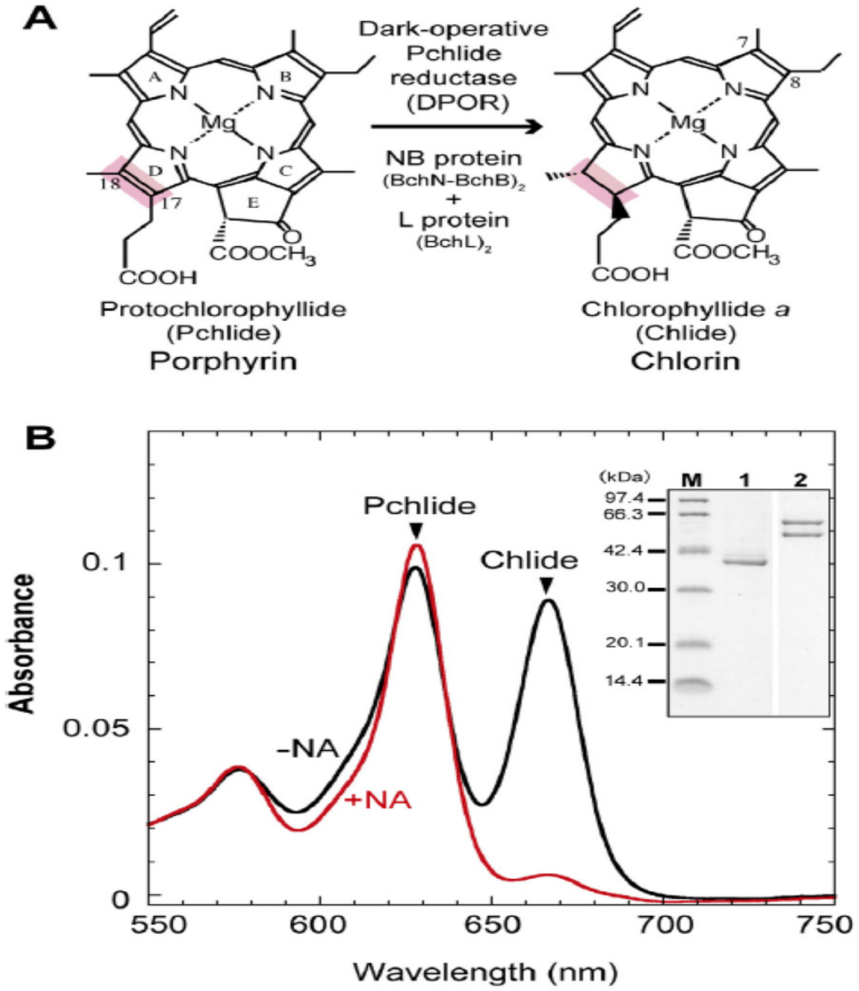


**Figure 2.** (A) The reaction of Pchlide reduction
The C17=C18 double bond of Pchlide is stereo specifically reduced by DPOR to form Chlide, the direct precursor of Chl a in oxygenic photosynthetic organisms. In Bchl a biosynthesis Chlide is further reduced to 3-vinyl bacteriochlorophyllide a by Chlide oxidoreductase, another nitrogenase-like enzyme. (B) Inhibition of DPOR activity by NA, DPOR assay was carried out with purified L-protein and NB-protein in the absence (black line) or presence (500 μM, red line) of NA. The reactions were stopped by the addition of acetone and the absorption spectra were recorded. Inset. SDS-PAGE profile of the affinity purified L-protein (lane 1:1 μg) and NB-protein (lane 2:2.5 μg) (Reinbothe *et al.*, 2010; Nomata *et al.*, 2013).

Fujita and Bauer (2000) reported that ring D of pchlide can be reduced in the stereo-specific manner by two different enzymes, LPOR or DPOR, and the side chain of B-ring indicated by R is either vinyl or ethyl. Yamazaki *et al.* (2006) also showed that although these two enzymes carry out the same stereo-specific reduction of the double bond of the D-ring to produce chlorophyllide a (Chlide), the direct precursor for Chl a, they are structurally very dissimilar and use completely unalike mechanisms. So, DPOR and LPOR are analogous enzymes (Galperin *et al.*, 1998). Shui *et al.* (2009) reported that transcripts levels of all DPOR genes are up-regulated approximately 2-fold in green light (GL) relative to levels in red light (RL), whereas LPOR transcript levels are reduced in GL.

Yamamoto *et al.* (2014) found that both dark operative protochlorophyllide oxidoreductase (DPOR) and chlorophyllide a oxidoreductase (COR) include of two components, Fe protein and MoFe protein cognates, and COR catalyzes 8-vinyl reduction of 8-vinyl chlorophyllide a in addition to the known activity of

C7=C8 double bond reduction. The sequential operation of DPOR and COR in the conversion of Pchlide to MV-Bchlide is presented in Figure 3. Two structurally unrelated Pchlide reductases are shown in Figure 4.

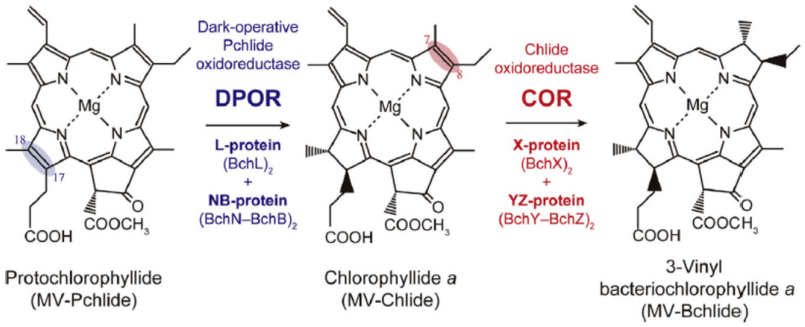


**Figure 3.** The sequential operation of DPOR and COR in the conversion of Pchlide to MV-Bchlide
DPOR reduces the C17=C18 double bond (blue) of Pchlide to produce Chlide, and then COR reduces the C7=C8 double bond (red) of Chlide to produce MV-Bchlide (Yamamoto *et al.*, 2014).

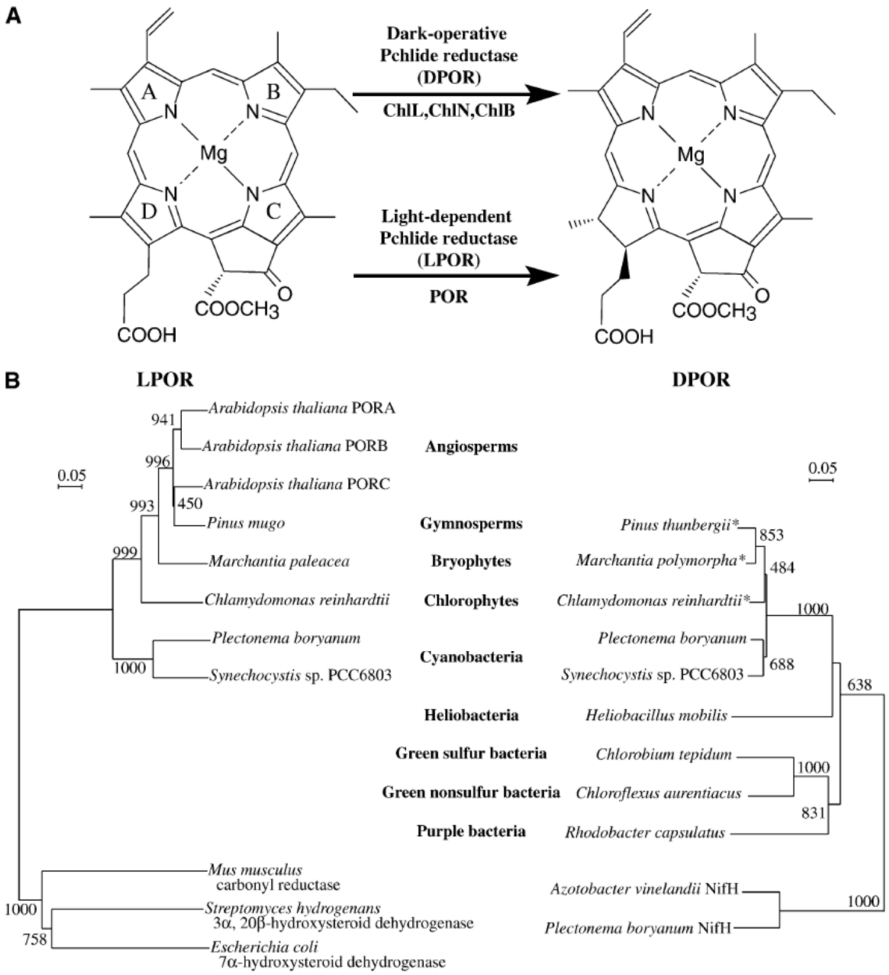


**Figure 4.** Two structurally unrelated Pchlide reductases
**A**. Pchlide reduction. The Pchlide D-ring is reduced by two different enzymes, DPOR and LPOR. **B**. Phylogenetic trees and distribution in extant phototrophs of two Pchlide reductases. The amino acid sequences of LPOR and DPOR (Bchl/ChlL) were recovered from GenBank as illustrative for each main taxon of the extant phototrophs. **Asterisks**

indicate that the genes are encoded in chloroplast genomes in these organisms. Each phylogenetic tree was constructed based on multiple sequence alignment by using ClustalX (version 1.81) and njplot. The three enzymes belonging to the SDR family and nitrogenase Fe-protein (NifH) were used as outgroups for the LPOR and DPOR trees, respectively (Yamazaki *et al.*, 2006).

## Conclusions

Photosynthesis happens in mesophyll cell of the green leaves in a cell organelle called chloroplast, and within the chloroplast there is a membranous system including grana, the stroma lamellae and the fluid stroma. The membrane system traps the light energy and synthesizes ATP and NADPH, and this set of reaction which relies on light is called light reaction. In stroma, enzymatic reactions incorporate $CO_2$ into the plant leading to the synthesis of sugar which in turn forms starch, and this set of reactions which are not directly dependent on light but are dependent on the products of light reactions, furthermore, ATP and NADPH is called dark reaction. Conversion of protochlorophyllide (Pchlide) into chlorophyllide (Chlide), a key step in chlorophyll biosynthesis, is mediated by a light-dependent NADPH: protochlorophyllide oxidoreducatse (POR). Two unrelated enzyme complexes have evolved to handle biosynthesis of chlorophyll, a light-dependent protochlorophyllide oxidoreductase (LPOR), and an ATP-dependent dark operative protochlorophyllide oxidoreductase (DPOR). POR has a conserved Tyr and Lys residue in the enzyme active site as the same as the other members of the short chain alcohol dehydrogenase/reductase family enzymes, which are active in a proposed reaction mechanism involving proton transfer from the Tyr hydroxyl group to Pchlide. Together with DNS photolyase, POR is one of the only two enzymes which show a natural, direct requirement for light. The enzyme POR has a significant function in plant development, and it catalyzes one of the later steps in chlorophyll synthesis, the light-induced reduction of PChlide into Chlide in the presence of NADPH. DPOR plays an important role in chlorophyll biosynthesis of gymnosperms, ferns, algae, mosses, and photosynthetic bacteria in the absence of light, although, DPOR shares considerable amino acid sequence homologies with nitrogenase, only the initial catalytic steps look like nitrogenase catalysis. DPOR is made of electron donor (BchL) and acceptor (BchNB) component proteins; BchNB is further consisted of two subunits each of BchN and BchB arranged as an $\alpha_2\beta_2$ heterotetramer with two active sites for substrate reduction. The DPOR subunits, BchL, BchN, and BchB, reveal significant sequence similarity to the nitrogenase subunits, NifH, NifD, and NifK, respectively, which exhibit that the mechanism of reducing the D-ring of Pchlide may be similar to the reduction of dinitrogen by nitrogenase. DPOR has been considered as a special iron-sulphur enzyme to form substrate radicals along with sequential proton- and electron-transfer steps with the protein folding very similar to that of nitrogenase. The study of the structure of LPOR has implications for deep understanding of light-to-chemical energy conversion in the biosphere and ultimately, for the design of new chemical / biological photocatalysts. Perhaps in near future scientists will be able to apply existing LPOR and DPOR structural data to design new nitrogen-fixing enzymes that can express and function in eukaryotic cells.

## Authors' Contributions

All authors read and approved the final manuscript.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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