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The role of photocatalysis as the ‘master switch’ of photomorphogenesis in early plant development.

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Enzymatic photocatalysis is seldom used in biology. Photocatalysis by protochlorophyllide oxidoreductase (LPOR) – one of only a few natural light-dependent enzymes – is an exception, and is responsible for the conversion of protochlorophyllide to chlorophyllide in chlorophyll biosynthesis. Photocatalysis by LPOR not only regulates biosynthesis of the most abundant pigment on Earth, it is also a ‘master switch’ in photomorphogenesis in early plant development. Upon illumination, LPOR promotes chlorophyll production, plastid membranes are transformed and the photosynthetic apparatus is established. Given these remarkable, light-induced pigment and morphological changes, the LPOR-catalyzed reaction has been studied extensively from catalytic, physiological and plant development perspectives, highlighting vital, and multiple, cellular roles of this intriguing enzyme. Here, we offer a perspective in which the link between LPOR photocatalysis and plant photomorphogenesis is explored. Notable have been breakthroughs in LPOR structural biology that have uncovered the structural-mechanistic basis of photocatalysis. These studies have clarified how photon absorption by the pigment protochlorophyllide – bound in a ternary LPOR–protochlorophyllide–NADPH complex – triggers photocatalysis, and a cascade of complex molecular and cellular events that lead to plant morphological change. Photocatalysis is therefore the ‘master switch’ responsible for early stage plant development, and ultimately life on Earth.

Main

As the cofactor responsible for harvesting and converting sunlight into photochemical energy in plants and other photosynthetic organisms, chlorophyll is the most abundant pigment and is crucial for life on Earth.¹ Reflecting this abundance, the rise and fall of chlorophyll levels across the globe is the only biochemical process visible from outer space.² The chlorophyll biosynthetic pathway consists of at least 12 different enzymatic steps (Figure 1). It is tightly regulated by a complex network of transcription factors, protein-protein interactions and putative allosteric interactions to prevent the accumulation of photoreactive intermediates.³⁻⁹ Many of the genes that encode the enzymes of the pathway are involved in a large coexpression network and several are controlled by photoreceptor (e.g. phytochrome, cryptochrome) mediated light-responsive signaling cascades that are also coupled to the expression of downstream genes required for photosynthesis.⁹ In plants, chlorophyll biosynthesis occurs in the chloroplast and starts from 5-aminolevulinic acid, a precursor for the biosynthesis of all tetrapyrroles.^{4,5} A series of enzymatic steps, common to the synthesis of most tetrapyrroles, then leads to the formation of a large, complex molecule, protoporphyrin IX, comprised of four pyrrole rings.^{4,5} Protoporphyrin IX lies at the branch-point of heme and chlorophyll biosynthesis, where either ferrochelatase inserts Fe^{2+} into the porphyrin macrocycle to form heme, or – along the chlorophyll branch – Mg^{2+} is incorporated through the action of ATP-dependent Mg-chelatase.^{4,5,10,11} This Mg-protoporphyrin IX complex is converted to chlorophyll by enzyme-catalyzed methylation,¹¹⁻¹³ formation of an additional isocyclic ring E,^{11,14,15} two subsequent reductions^{11,16} and a final esterification of the long phytol tail.^{11,17} Reduction of protochlorophyllide (Pchl_{id}) to chlorophyllide (Chl_{id}) is the penultimate step in this biosynthesis pathway.¹⁸⁻²² Control of this reaction is vital to regulated biosynthesis of chlorophyll biosynthesis and, importantly, early plant development.

In nature, two routes have evolved to reduce Pchl_{id}, specifically at the C17-C18 double bond, to form Chl_{id} (Figure 1).²³ In evolutionary terms, a light-independent (or dark-operative) Pchl_{id} oxidoreductase (DPOR) is the older enzyme.^{24,25} It is a multi-subunit enzyme comprising three separate subunits.²⁶⁻²⁸ DPOR closely resembles the enzyme nitrogenase. It catalyzes Pchl_{id} reduction in an ATP-dependent manner and is sensitive to oxygen.²⁶⁻²⁸ DPOR arose in anoxygenic

photosynthetic bacteria and can also be found in non-flowering land plants, algae and cyanobacteria.^{24,25} Light-dependent protochlorophyllide oxidoreductase (LPOR) is distinct from DPOR. LPOR also catalyzes reduction of Pchl_{id} and is found in all plants, algae and cyanobacteria. Phylogenetic analyses indicate that LPOR appeared first in cyanobacteria at the onset of oxygenic photosynthesis.^{24,25,29} However, LPOR enzymes have also recently been discovered in anoxygenic phototrophic bacteria, which most likely obtained the enzyme by horizontal gene transfer from cyanobacteria, and suggests that LPORs are more widespread than first thought.²⁹⁻³¹ Some organisms, including non-flowering land plants, algae and cyanobacteria, possess DPOR and LPOR and, although the role of both enzymes in these species is still not fully understood, it is proposed that they enable optimal production of chlorophyll under variable conditions.^{24,25} As the DPOR enzyme is highly sensitive to oxygen the presence of both enzymes may help the photosynthetic organism to adapt to changes in the light and oxygen conditions in the environment.²⁵ However, LPOR is the only enzyme responsible for Pchl_{id} reduction in angiosperms and has a vital regulatory role. It is known to interact with several upstream proteins involved in the import of LPOR to the plastid (*e.g.* Pchl_{id} *a* oxygenase, tyrosine aminotransferase, chaperone-like protein of POR1),^{32,33} in addition to a number of regulatory proteins (*e.g.* FLU protein, CHL27, phycocyanobilin:ferredoxin oxidoreductase) and the neighbouring cyclase enzyme to control flux through the entire chlorophyll biosynthetic pathway.^{7,34,35} Moreover, LPOR also interacts with downstream proteins, such as light-harvesting like proteins (LILs) and the POR-interacting TPR protein, involved in the assembly pathway for the formation of photosynthetic complexes.^{7,36,37} Hence, it acts as a light-activated trigger to control the chlorophyll content and subsequent development of the photosynthetic apparatus in plant cells.¹⁸⁻²² Photocatalysis by LPOR is therefore a ‘master switch’ in early plant development triggering formation of the light-dependent photosynthetic apparatus. These plant development changes occur on timescales many orders of magnitude slower than LPOR photocatalysis. Across these timescales, advances in physiological, mechanistic and structural appreciation of LPOR function are leading to a new understanding of the biological importance of LPOR. Further exploration of these interconnected functional relationships is therefore warranted.

Photocatalysis is the ‘master switch’ in early plant development

Active LPOR complexes were first discovered in etiolated leaves over 70 years ago.³⁸ This has led to a long, intensive research history, initially on dark-drown seedlings and leaves, followed by isolated membranes, cell fragments and more recently purified protein samples, to establish the pivotal role of LPOR in plant physiology and development (reviewed in detail in ^{18,39}). In the dark (*e.g.* under fallen leaves or stones) angiosperm seedlings undergo skotomorphogenesis, attributed to the accumulation of Pchl_{ide} by the chlorophyll biosynthesis pathway.⁴⁰ In this scenario, development of a proplastid into a chloroplast is stalled at the etioplast – plastids that develop in darkness.³⁹ In such etiolated plants and seedlings, LPOR and Pchl_{ide} accumulate as a ternary complex with NADPH to form highly organized, paracrystalline structures, known as prolamellar bodies (PLBs) (Figure 2).⁴¹⁻⁴⁴ These PLBs are located within the membranes of etioplasts and are surrounded by prothylakoid membranes, which develop ultimately into the thylakoids of the chloroplast. PLBs are rich in lipid – mainly monogalactosyldiacylglycerol and digalactosyldiacylglycerol – and these are essential to membrane structure organization and subsequent development of the etioplast.⁴⁴⁻⁴⁷ The mechanism of PLB assembly is still not fully understood and it is likely that several regulatory pathways are required to control PLB formation.^{39,41} Any impairment in the production of Pchl_{ide} is known to prevent PLB formation, which, in turn, is also thought to be coupled to the import pathway of LPOR into the plastid.^{41,48} The incorporation pathway of lipids into PLBs remains unclear but it is suggested that the interaction between LPOR and monogalactosyldiacylglycerol is essential for the assembly of the PLB.^{39,41,44-46}

PLBs contain a range of spectrally distinct forms of Pchl_{ide}, attributed to the formation of aggregates of different sizes with LPOR and NADPH.^{49,50} These aggregates account for approximately 95 % of total protein in the PLBs.³⁹ Diverse interactions of LPOR-Pchl_{ide} complexes with PLB lipids are thought also to influence the spectral properties of the pigment.^{44,50} Interactions with PLB lipids regulate LPOR activity in plants and might even be required to form efficient photoactive LPOR-Pchl_{ide}-NADPH complexes.^{44,45} Non-photoactive forms of Pchl_{ide}, representing unbound forms of the pigment, are also found in etioplasts but these are mainly restricted to the prothylakoids.^{43,50} Characteristic PLB structures

have been restored in a LPOR knockout strain of *Arabidopsis thaliana* by replacing the native plant LPOR with those from cyanobacteria.⁵¹ However, it should be noted that although the size and structure of PLBs were normal, there was a lower ratio of photoactive to non-photoactive Pchl_{id}. PLB-like ultrastructures also form in etiolated cyanobacteria by overexpressing LPOR in DPOR-deficient cells.⁵² It seems therefore that LPOR enzymes, irrespective of their origin, are sufficient to trigger PLB formation in photosynthetic organisms.

LPOR is the basis of the light-dependent switch between skotomorphogenesis and photomorphogenesis following irradiation of etioplasts with visible light. Illumination triggers profound changes in the physiological development of plants that are manifest in a reorganization and modification of plastid membranes.^{18-20,39,41-43}

Illumination results in rapid photoreduction of Pchl_{id} to form Chl_{id}, followed by conversion of LPOR-Chl_{id}-NADP⁺ aggregates into LPOR-Chl_{id}-NADPH complexes, which are proposed to play a photoprotective role in PLBs as they act as a strong energy quencher.³⁹ Photocatalysis ultimately leads to dispersion of PLBs as the LPOR-Chl_{id} complexes dissociate,^{9,39,41} aided by the presence of the galactolipid digalactosyldiacylglycerol (Figure 2).⁴⁷ Following dispersal of PLBs, chlorophyll levels increase and photosynthetic proteins are expressed as the etioplasts are transformed into mature chloroplasts to establish the photosynthetic apparatus.^{39,41,43}

Morphologically, the tubular structure of PLBs transforms into a linear system of parallel lamellae that develop into the photosynthetically active thylakoid membranes of the grana in a process that occurs in a number of stages over several hours / days.^{39,43} Initially, the dispersed PLBs flow outward into the stroma (1-2 hours) but until recently, it was unclear whether the PLB transforms into thylakoids continuously or through the formation of vesicles.^{39,41-43,53} Recent three dimensional reconstruction of the etioplast to chloroplast transformation show direct conversion of PLBs to flat slats and the appearance of the first grana as overlapping thylakoids (<8 hours).⁴³ During these early stages partial reformation of PLBs, connected to the developing thylakoids, is also possible due to the accumulation of photoactive LPOR-Pchl_{id}-NADPH ternary complexes.^{43,54} More complex grana structures with a helical character are slowly generated over 2-3 days, concomitant with the formation of ordered supercomplexes of light-harvesting complex II and photosystem II that are proposed to stabilize the structure of the grana stacks.^{43,55} What is clear is that

photocatalysis by LPOR is crucial for this entire process, triggering multiple events across a hierarchy of timescales. These include photon capture (sub-picoseconds), catalysis (micro – milliseconds), product release (millisecond – seconds), and dispersal of PLBs (minutes-hours). These timescales are extended further (hours-days) as the chlorophyll-binding proteins accumulate and mature chloroplasts form.

Angiosperms can contain multiple isoforms of LPOR. These have different patterns of light and developmental regulation and allow the plant to optimize levels of chlorophyll production at different stages of the greening process.⁵⁶⁻⁵⁸ One isoform of LPOR is only expressed in etiolated seedlings or plants, whereas another isoform is thought to be necessary for maintaining chlorophyll synthesis throughout the entire life cycle of a plant. A third isoform has been identified in *Arabidopsis*.⁵⁶⁻⁵⁸ A recent phylogenetic analysis of LPOR sequences revealed that all LPORs diverged from a single common ancestor 1.36 billion years ago.⁵⁹ The LPOR gene has since been duplicated at least 10 times in angiosperms, leading to the appearance of two or more LPOR isoforms in several species. A new classification of LPOR enzymes was proposed based on their evolutionary origin and biochemical properties. Z-type LPORs are present in bacteria, whereas S-type and L-type isoforms are found in plants, but differ in their interactions with lipids.⁵⁹ Two of the isoforms, originally termed named LPORA and LPORB – have been shown to form high molecular weight, putative light-harvesting complexes in the PLBs of barley.²⁰ These complexes have been reconstituted *in vitro* using synthesized Pchlides (Pchlides *a* and Pchlides *b*) and might allow rapid chlorophyll synthesis under low light intensities as well as offering a photoprotective role during greening.^{20,60} This, however, remains a controversial hypothesis. Whether such complexes exist in plants has been hotly debated,⁶¹ and a similar LPOR light-harvesting complex does not form in *Arabidopsis* due to a different geometry of the LPOR proteins.⁶² A role for LPOR in regulating oxidative stress and vitamin E levels has also been suggested,^{63,64} extending further the physiological reach of LPOR.

Structural biology shines new light on LPOR function

The size, shape and architecture of PLBs is known to be determined by the structural properties of the LPOR protein. These include its ability to form oligomers,^{62,65-71} the

association of LPOR with the membrane^{71,72} and the interaction of the enzyme with lipids.^{44,59,71} The propensity of LPOR to form large, substrate-bound, PLB aggregates in the etioplast – and potentially during the greening process – was first observed over 30 years ago⁶⁵ and has generated much interest in understanding this process *in vitro*.^{62,66-71} LPORs from different organisms can adopt a variety of soluble oligomeric forms in the absence of substrates, ranging from monomers in cyanobacterial LPORs^{66,67,70} through to higher order oligomers in plant enzymes.^{62,68,69} In all cases, formation of a LPOR–Pchl_a–NADPH ternary complex leads to formation of larger multimeric complexes (Figure 3).⁶⁶⁻⁷¹ In plant LPORs, binding of Pchl_a is surmised to alter the oligomeric structure. This triggers further coupling of oligomers to form higher molecular weight aggregates.^{62,68,69,71} Recent cryoelectron microscopy studies show that these LPOR oligomers form helical filaments with the lipid bilayer, allowing efficient exchange of the Pchl_a substrate and Chl_a product from the membrane.⁷¹ Such LPOR filaments also directly shape the membranes into the characteristic curved tubules found in the PLB and may provide a source of lipids upon light-driven dissociation for the subsequent assembly of the thylakoids during biogenesis of the photosynthetic apparatus.⁷¹

The binding of Pchl_a to LPOR elicits structural change in the protein, which in turn induces LPOR dimer formation and subsequent oligomerization (Figure 3).^{66,70} Conformational changes in two highly conserved loop regions, which fold over the Pchl_a and form a ‘lid’ upon binding, are proposed to trigger oligomer formation.⁷⁰ The protein conformational changes linked to Pchl_a binding are rate-limiting in the overall LPOR photocatalytic cycle and also lead to a red-shift in the electronic absorption spectrum of Pchl_a.⁷³ One suggestion is that oligomerization allows neighboring Pchl_a molecules to interact, leading to higher reaction efficiency.^{68,69} This idea is supported by excited state energy transfer between neighboring pigment molecules in LPOR–Pchl_a complexes,⁷⁴ and between Pchl_a and chlorophyll binding proteins during assembly of the photosynthetic membranes assembly.⁷⁵ The helical array of Pchl_a molecules observed in the recent structure of the LPOR filaments suggest a distance of ~25 - 78 Å between adjacent Pchl_as, allowing efficient energy transfer upon illumination.⁷¹ Also, pre-illumination of the LPOR–Pchl_a–NADPH ternary complex with low levels of light has been reported to lead to

progressive protein rigidification and increased enzyme activity.^{76,77} This is an intriguing finding but molecular details of this process remain unclear.

Structural understanding of LPOR function has, for many years, been restricted to modelling studies.^{60,68,78,79} X-ray crystal structures of cyanobacterial LPORs in their free form, and in complex with NADPH, have recently been solved and these offer new insights into LPOR function (Figure 3).^{67,80} LPOR has a typical dinucleotide binding Rossmann-fold with a central β -sheet comprised of 8 β -strands surrounded by 6 α -helices.^{67,80} There are multiple flexible loops, one of which extends from the central β -sheet and becomes ordered on binding NADPH. This loop acts as a lid, covering the nicotinamide group of the coenzyme in the enzyme active site.⁶⁷ Other residues are also implicated in binding NADPH.⁶⁷ There is still no crystal structure of the LPOR–Pchl_a–NADPH complex from which to visualize how Pchl_a is bound.^{67,80} Molecular docking and dynamics simulations,⁶⁷ together with recent cryoelectron microscopy studies,⁷¹ have highlighted important and multiple interactions between residues in the active site and Pchl_a (Figure 3). Many of these interactions have been validated in functional–mutagenesis studies of photocatalysis and binding.^{67,78} Pchl_a polar functional groups include the keto, methylester and carboxylic acid groups, and these sit deep in a hydrophilic active site pocket where they form hydrogen bonding interactions with active site residues. The hydrophobic edge of Pchl_a is located in a hydrophobic region of the enzyme, which includes the aforementioned ‘lid’ regions that close over Pchl_a and are also crucial to optimally position the Pchl_a for efficient photocatalysis.^{46,70} Changes in the structure of these hydrophobic regions upon Pchl_a binding are also proposed to be important for association of the protein with the membrane.⁷¹ The spatial organization of the active site offers some insight into the structural rearrangements triggered by the binding of Pchl_a. These recent structural advances are leading to a more detailed understanding of how conformational changes in the LPOR–Pchl_a–NADPH complexes enable monomers of the complex to nucleate and initiate the formation of oligomers, as well as interact with the membranes of the PLB.^{67,70,71,80}

Determinants of photocatalysis identified from enzyme structures

Photocatalysis by the LPOR ‘master switch’ triggers chemical and structural change at molecular and cellular levels that result in changes in plant morphology over many hours / days (Figure 4). This cascade of events occurs over timescales that span an unprecedented 10^{15} - 10^{18} orders of magnitude. LPOR is one of only a few enzymes that are known to have a direct, natural requirement for light, the others being photosynthetic reaction centres, including the related chlorophyll f synthase,⁸¹ and the flavoenzymes DNA photolyase⁸² and fatty acid photodecarboxylase.⁸³ As a consequence, LPOR is an experimental system from which we can learn how to harness photocatalysis.^{19,21,84} There are unprecedented opportunities to advance mechanistic understanding of enzymes with photo-initiation. Because most enzyme reactions are thermally activated, experimental studies are often limited to slower timescales (milliseconds and longer), due to the necessity of bringing together, through rapid mixing, a substrate and enzyme. The limitations of mixing are side-stepped in photocatalysis because the enzyme-substrate complex can be pre-formed in the dark. Photocatalysis can then be initiated, typically using a laser pulse or LED, and the photocatalytic cycle studied over a wide range of timescales (femtoseconds to seconds, or longer) and across a wide range of temperatures, including cryogenic, to identify short-lived reaction intermediates.^{19,21} All this combines to make the chemistry of photocatalysis accessible in ways that are not possible with conventional diffusion-controlled enzymes.

Pchl_a is both the substrate and the light-sensing chromophore for LPOR photocatalysis. It has excited state properties that allow it to capture sunlight and initiate reduction of the C17–C18 double bond when bound in the active site. Time-resolved spectroscopy has been used to investigate these excited state properties, and studies have shown that Pchl_a is highly reactive.⁸⁵⁻⁹² Excitation brings about charge separation across Pchl_a.⁸⁵⁻⁹¹ This process is highly dependent on solvent polarity^{85,86,90} and is attributed to a number of polar groups attached to Pchl_a, including an electron-withdrawing carbonyl group on ring E.⁹¹ The excited state of Pchl_a is quenched through solvation of this intramolecular charge-transfer species,⁸⁵⁻⁹¹ and a triplet state is then formed on the nanosecond timescale with a quantum yield of approximately 30 %.⁸⁹⁻⁹² Excited state charge separation across Pchl_a has also been confirmed computationally, and these calculations suggest that this leads to site-specific solvation of photoexcited Pchl_a through strengthening of

H-bonding interactions.^{91,93} Photochemical studies of the mechanism in the ternary enzyme-substrate complex are challenging^{74,76} but it appears that the dipolar character of excited state Pchl_a is also important in LPOR photocatalysis (Figure 4).⁹⁴ Structural modelling of the LPOR–Pchl_a–NADPH complex has highlighted how the LPOR active site stabilizes excited state charge separation in Pchl_a by strengthening an extensive hydrogen bonding network between active site residues and Pchl_a (Figure 3). These excited state interactions generate an electron-deficient site at the C17-C18 region, activating it for subsequent reduction.^{67,94}

Reduction of the C17-C18 bond of Pchl_a has been studied using a combination of time-resolved spectroscopy^{78,94-101} and cryogenic spectroscopy techniques with purified LPOR protein.^{97,98,102-107} Crucially, the mechanism of photocatalysis is identical *in vivo* as the same reaction intermediates are also observed in low temperature analyses of plants.^{18,54,108} After initial excitation of the Pchl_a substrate, a hydride equivalent is transferred – sequentially as 2 electrons and a proton – from NADPH to the C17 position of Pchl_a.^{95,105} This is followed by proton transfer to C18, either directly from an active site tyrosine residue, or mediated by solvent (Figure 4).^{95,97} Both H-transfer reactions proceed by quantum mechanical tunnelling.⁹⁵ In biology, enzymatic hydride transfers are generally concerted, involving hydride anion transfer in a single reaction step. This is not so, however, in LPOR which represents – to the best of our knowledge – the only known example of stepwise hydride transfer in biology.^{98,101} Resolution of fast sequential reaction steps highlights the benefits of using short laser pulses to initiate enzyme reactions – a clear advantage of working with photocatalysts to study enzyme mechanisms.¹⁰¹

The chemistry of ‘hydride’ transfer is conserved across many LPOR enzymes, from plants, to algae and cyanobacteria. Hydride transfer leads to the formation of a deprotonated Chl_a anion, which has no detectable fluorescence and has a characteristic broad absorbance band at 696 nm.^{95,99,100,102,104,107,108} Hydride transfer occurs well below 200 K in studies with many LPOR enzymes. This indicates that hydride transfer progresses at temperatures lower than the ‘glass transition’ temperature of proteins, below which most protein motions, other than bond vibrations, are frozen.^{102,105-108} Hydride transfer is not influenced by bulk solvent viscosity. This further implies that large-scale protein motions are not involved in, or required for, stepwise ‘hydride’ transfer.^{96,99,100} Structural models of the LPOR–

Pchlide–NADPH complex have confirmed that the *pro-S* hydrogen of NADPH⁹⁵ is transferred, consistent with experimental findings.⁶⁷

Protonation of the C18 atom of the hydride-reduced Pchlide leads to formation of the final Chlide product (Figure 4).^{95,97} Proton transfer is different across LPOR enzymes from cyanobacteria compared to plants.^{99,100,107} In cyanobacterial LPORs, proton transfer occurs above 200 K and is coupled to solvent dynamics, suggesting involvement of a complex, extended network of protein motions that accompany proton transfer on the microsecond timescale.^{96,99,100,103,106} In plant LPORs, proton transfer is faster, can occur below the ‘glass transition’ temperature, and is not dependent on an extended solvent-coupled dynamic network.^{99,100,107} This implies that the proton donor (presumed to be an active site tyrosine) is positioned optimally in plant LPORs, close to C18 of Pchlide, whereas extended solvent-coupled dynamics are needed to optimize donor-acceptor proton transfer distances in cyanobacterial enzymes.^{99,100,107} An equilibrium donor-acceptor distance of about 5 Å is observed between the active site tyrosine (the presumed donor) and C18 of Pchlide in structural models of cyanobacterial LPOR, although this may decrease following hydride transfer.⁶⁷ A potential and extensive proton-relay pathway from solvent to this tyrosine residue is observed, comprising a water network coordinated by several active site residues.^{67,80} However, as noted from the recent cryoelectron microscopy structure of plant LPOR it is possible that an alternative proton donor may exist.⁷¹ Following proton transfer to C18, a series of ordered product release and substrate binding events, then complete the catalytic cycle.^{104,109} In plants, these steps lead to a blue shift in the Chlide absorbance spectrum as the LPOR-Chlide complexes breakdown and the Chlide is esterified to produce chlorophyll,⁵⁴ causing dispersal of the PLBs (Figure 4).^{39,41-43} Significant conformational changes in the protein are proposed to accompany Chlide release, which also disrupt interactions with the lipid bilayer and lead to the disassembly of the LPOR filaments.⁷¹

Outlook and future perspectives

Chemical and molecular level understanding of photocatalysis by LPOR, and implications for the dissolution of PLBs, is beginning to emerge. What is clear is that photocatalysis is the ‘master switch’ for plant photomorphogenesis, and that this leads

to maturation of the chloroplast correlated with chlorophyll pigment supply (Figure 4). However, important questions remain. In terms of chlorophyll biosynthesis it is still not fully understood why some photosynthetic organisms have retained two separate enzymes for this step and it is possible that DPOR might be involved in developmental functions that have yet to be established.²⁵ The function of LPOR in anoxygenic phototrophic bacteria is also still an open question and it will be interesting to ascertain if they show the same structural and biochemical properties as their plant and cyanobacterial counterparts.²⁹⁻³¹ It will also be important to build on recent pioneering advances in understanding how LPOR interacts with other enzymes and regulatory components to control the levels of chlorophyll production and coordinate this with the assembly of the chlorophyll-binding proteins required for the photosynthetic apparatus.⁷

Despite a number of recent breakthroughs about the role of PLBs in the greening process we still do not fully understand how PLBs are formed and what their function is in the development of the chloroplast.³⁹⁻⁵³ PLB-like structures can form in cyanobacteria and this offers an attractive opportunity to study their formation in an amenable model system.⁵² It has been suggested that PLBs may play an important role in the storage of lipids for the formation of thylakoids but the pathway for delivering galactolipids to the PLBs remains poorly understood.³⁹ It is also unclear how LPOR interacts with the lipid membranes to form highly organized networks in PLBs, although new hypotheses for this process are beginning to emerge with the recent advances in cryoelectron microscopy.⁷¹ Models for how Pchl^a binding drives oligomer formation to promote PLB formation are also now being developed and involve protein structural reorganization.^{67,70,71,80} Further molecular insights into LPOR oligomerization are now likely to be developed and will be important for a complete understanding of how PLBs are formed. Developments in 3D reconstruction techniques will also be crucial for deriving mechanisms for the disassembly of the PLBs and subsequent formation of the photosynthetic apparatus.

Crystal structures^{67,80} are now being used to interpret a large body of biophysical information within a structural context.^{73,74,76-78,85-109} Atomic level insight into how excited state chemistry, bond making / breaking and the dynamics of photocatalysis are controlled by protein structure is within reach. Continued work in these areas will furnish a more complete understanding of how biology harnesses light energy to

derive catalytic power, to biosynthesize chlorophyll and to install the ‘master switch’ of early plant development. Ultimately, photoactive crystals of the LPOR–Pchl_a–NADPH complex may give access to time-resolved visualization of early phases of the photocatalytic cycle. Spatial and temporal resolution of photochemistry using X-FEL methods¹¹⁰ would add eye-watering details to atomic level cinematics of photocatalysis. Coupling this information to macromolecular depictions of PLB disassembly – visualized, for example, by photo-initiated cryoelectron microscopy, mass spectrometry and X-ray scattering – would bridge the crucial knowledge gap of how the photocatalysis marshals chloroplast maturation. These are exciting times to shine a light on the many roles of LPOR.

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Author contributions

D.J.H. and N.S.S. conceived and led on the writing of the manuscript. S.Z., A.T., L.O.J., S.J.O.H. and S.H. contributed to the writing of specific sections, critical reading of the manuscript and reviewing of appropriate references. S.Z., D.J.H and A.T. prepared figures.

Competing interests

The authors declare no competing interests

Figure legends

Figure 1. Overview of chlorophyll biosynthetic pathway. The enzymatic steps involved in the synthesis of chlorophyll starting from 5-aminolevulinic acid. The steps up to the formation of protoporphyrin IX are also common to heme biosynthesis and involve following enzymes: ALAD, aminolevulinic acid dehydratase; PBGD, porphobilinogen deaminase; UROS, uroporphyrinogen III synthase; UROD, uroporphyrinogen III decarboxylase; CPOX, coproporphyrinogen oxidase; PPOX, protoporphyrinogen IX oxidase. Insertion of Mg^{2+} by Mg chelatase leads to synthesis of chlorophyll. The penultimate step, which involves the reduction of protochlorophyllide, can be catalyzed by two different enzymes, a light-dependent and a light-independent protochlorophyllide oxidoreductase.

Figure 2. The role of LPOR in chloroplast development. The steps involved in the conversion of the proplast to the mature chloroplast *via* the etioplast are shown. Galactolipids, anionic lipids that are characteristic for plastids, are important for prolamellar bodies (PLBs) formation and during later greening stages. LPOR and Pchl_{id}e build up on the lipid bilayer to form the PLBs within the etioplast. Illumination triggers LPOR photocatalysis, leading to chlorophyll accumulation and the change from skotomorphogenesis to photomorphogenesis. PLBs disintegrate and chlorophyll-containing thylakoids are formed. These then fuse together to form the grana and stroma of the mature chloroplast as the photosynthetic complexes are generated. Timescales for each process are estimated from recent 3D reconstruction studies.⁴³

Figure 3. Overview of the structural organization of LPOR. The crystal structures of apo-LPOR and a LPOR-NADPH binary complex solved for cyanobacterial LPORs.^{67,80} A structural model of the LPOR–Pchl_{id}e–NADPH ternary complex highlights conformational change on Pchl_{id}e binding and active site residues that interact with the Pchl_{id}e molecule.⁶⁷ Structural changes induced by Pchl_{id}e binding lead to dimerization of LPOR–Pchl_{id}e–NADPH ternary complexes⁶⁶ and further interactions lead to larger oligomers required for PLB formation.

Figure 4: Timeline of chemical and structural changes triggered by illumination of LPOR–Pchl_{id}e–NADPH complexes. The initial absorption of light by bound Pchl_{id}e induces chemical and structural change at the molecular, cellular and whole organism levels. The Pchl_{id}e excited state leads to LPOR photocatalysis, involving

sequential hydride and proton transfer steps, to form Chlide and subsequent conversion to chlorophyll. At the cellular level, PLBs disintegrate to form thylakoids in the mature chloroplast as the plant begins to develop.

Figure 1.

Figure 2.

Figure 3.

Figure 4.