LIL3, a Light-Harvesting Complex Protein, Links Terpenoid and Tetrapyrrole Biosynthesis in Arabidopsis thaliana

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The LIL3 protein of Arabidopsis (Arabidopsis thaliana) belongs to the light-harvesting complex (LHC) protein family, which also includes the light-harvesting chlorophyll-binding proteins of photosystems I and II, the early-light-inducible proteins, PsbS involved in nonphotochemical quenching, and the one-helix proteins and their cyanobacterial homologs designated high-light-inducible proteins. Each member of this family is characterized by one or two LHC transmembrane domains (referred to as the LHC motif) to which potential functions such as chlorophyll binding, protein interaction, and integration of interacting partners into the plastid membranes have been attributed. Initially, LIL3 was shown to interact with geranylgeranyl reductase (CHLP), an enzyme of terpene biosynthesis that supplies the hydrocarbon chain for chlorophyll and tocopherol. Here, we show another function of LIL3 binding to protochlorophyllide oxidoreductase (POR), which possesses four transmembrane domains (including two LHC motifs) and is involved in nonphotochemical quenching of chlorophyll biosynthesis. We suggest that LIL3 associates with POR and CHLP and thus contributes to the supply of the two metabolites, chlorophyllide and phytol pyrophosphate, required for the final step in chlorophyll synthesis.

The proteins of the light-harvesting complex (LHC) superfamily are characterized by up to four hydrophobic and α-helical membrane-spanning domains, one or two of which represent LHC motifs because they include potential chlorophyll (Chl)-binding sites (Klimmek et al., 2006). The most abundant members of the LHC protein family are the light-harvesting Chl-binding proteins (LHCPS) of PSI and PSII, each of which has three transmembrane motifs (Jansson, 1994; Montané and Kloppstech, 2000; Rochaix, 2014). The S subunit of PSII (PsbS), which possesses four transmembrane domains (including two LHC motifs), is involved in nonphotochemical quenching of PSII (Li et al., 2000; Niyogi et al., 2005). In addition, so-called light-harvesting-like (LIL) proteins share one or two LHC motifs with LHCPs, but they are more diverse in size and function. At least six different LIL proteins, with between one and three hydrophobic α-helical motifs, have been identified in Arabidopsis (Arabidopsis thaliana; Klimmek et al., 2006). The cyanobacterial high-light-inducible proteins (HLIPs) and their eukaryotic orthologs, the one-helix proteins (OHPs), each possess one LHC motif-containing transmembrane sequence (Dolganov et al., 1995; Jansson et al., 2000; Andersson et al., 2003). The so-called stress-enhanced proteins (SEPs, SEP2, and SEP3/LIL3) contain two transmembrane domains, one of which includes an LHC motif (Heddad and Adamska, 2000). As in the LHCPs, three membrane-spanning LHC domains are found in the early light-inducible proteins (ELIPs; Grimm and Kloppstech, 1987; Green et al., 1991). Interestingly, one of the two isoforms of ferrochelatase, ferrochelatase II, also possesses a C-terminal LHC motif (Sobotka et al., 2011). Removal of this motif does not affect its catalytic activity but results in dissociation of the ferrochelatase dimer and impairs Chl synthesis (Sobotka et al., 2011). Thus, the LHC motifs in these various members of the superfamily appear to have multiple functions.

Each member of the LHC superfamily is potentially capable of binding photosynthetic pigments and, consequently, may be affected by light absorption. However, Chl and/or carotenoid binding have not been...
demonstrated for all of the different groups. Although they are thought to be localized to thylakoid membranes, they seem to function in diverse processes in chloroplasts. LHCPs bind Chl a and b as well as xanthophylls and lutein, enhance the light-harvesting capacity and energy transfer toward the reaction center of the two photosystems by increasing their antenna size, and provide photoprotection against excessive light (Horton, 2012; Ruban et al., 2012; Pribil et al., 2014; Allahtverdidiyeva et al., 2015). The ELIPs bind Chl and carotenoids and are encoded by genes that are transiently induced during deetiolation and abiotic stress (Heddad and Adamska, 2000; Montané and Kloppstech, 2000). The homologous HLIPs and OHPs have been assigned multiple functions ranging from roles in responses to temperature stress and nutrient deficiency, to Chl metabolism, in transient pigment storage during the assembly of Chl-binding proteins of the antenna or core complexes, or during the repair of the photosynthetic complexes (Hernandez-Prieto et al., 2011; Yao et al., 2012; Komenda and Sobotka, 2016).

In Arabidopsis, two SEP3 isoforms (here designated LIL3) are encoded by nuclear genes (here termed LIL3.1 and LIL3.2). A molecular function in protein-protein interaction was assigned to LIL3 when LIL3 double mutants (lil3.1/lil3.2) were found to accumulate small amounts of geranylgeranylated Chl instead of wild-type amounts of phytylated Chl. Lack of LIL3 was shown to destabilize the geranylgeranyl reductase (CHLG) in plastid membranes, which is encoded by the nuclear gene CHLG (At1g74470; Tanaka et al., 2010). These authors concluded that the physical interaction of LIL3 with CHLG tethers the enzyme to the plastid membranes and prevents its rapid degradation. CHLG catalyzes the reduction of geranylgeranyl pyrophosphate before or after esterification with chlorophyllide (Chlide) and, thus, provides the hydrophilic alkyl chain found in Chls and in the vitamin E derivatives tocopherols and tocotrienols. LIL3 has been detected in protein fractions of etioplasts of barley (Hordeum vulgare), which also contain protochlorophyll (Reisinger et al., 2008). LIL3 proteins also are present in fractions of Arabidopsis chloroplasts that contain Chlde and geranylgeranyl pyrophosphate, which supports the findings mentioned above regarding LIL3’s involvement in late steps of terpenoid and Chl biosynthesis (Hey et al., 2015). Finally, the association of LIL3 with Chl has been demonstrated directly using a thermophoresis approach (Mork-Jansson et al., 2015).

The mature LIL3 isoforms can be dissected into three different structural domains. An extended water-soluble N-terminal globular domain (Fig. 1) is followed by the two transmembrane domains and a hydrophilic C-terminal segment that protrudes into the stroma of the plastid. In previous reports (Tanaka et al., 2010; Takahashi et al., 2014; Mork-Jansson et al., 2015a, 2015b), LIL3 was proposed to serve as a membrane anchor for other proteins and as a binding site for Chl. Specifically, it was proposed that LIL3 contributes to the formation of diverse protein complexes involved in Chl synthesis or to the assembly of Chls into Chl-binding proteins during their integration into the thylakoid membrane and subsequent assembly into photosynthetic protein complexes.

In this context, it is interesting that the expression of the CHLG protein supplemented with the transmembrane domain of either LIL3 or ascorbate peroxidase is sufficient to anchor and stabilize CHLG in the absence of LIL3 (Takahashi et al., 2014). The authors concluded that CHLG function could be restored in lil3 mutants, although the oligomeric CHLG complexes observed in the presence of LIL3 might not be rescued. Thus, both the extent to which LIL3 is dispensable and the function of its LHC membrane domain remain open.

Chl is synthesized via the tetrapyrrole biosynthesis (TBS) pathway, which also provides other macroyclic end products, such as heme, phytochromobilin, and siroheme, in plants. These tetrapyrroles associate with various proteins and act either as redox cofactors or as chromophores in multiple cellular processes (Tanaka et al., 2010; Tanaka and Tanaka, 2011; Brzezowski et al., 2015). In the Mg branch of TBS, Mg porphyrin is converted into a chlorine ring structure by the reduction of protochlorophyllide (Pchlide) to Chlide a. In angiosperms, this reduction is catalyzed by the light-dependent protochlorophyllide oxidoreductase (POR). In Arabidopsis, three POR isoforms are encoded in PORA, PORB, and PORC genes. Chl a synthesis is terminated by the esterification of Chlide with phytyl pyrophosphate or geranylgeranyl pyrophosphate by the Chl synthase (CHLG; At3g51820). This reaction links TBS to the plastid-localized branch of the terpenoid synthesis pathway and results in the formation of a rather hydrophobic Chl, which can be attached to the pigment-binding proteins of PSI and PSII. The enzymes of the Mg branch of Chl synthesis and terpenoid synthesis also are found to be attached to plastid membranes. They are either indirectly associated with these membranes or have thylakoid membrane-spanning domains for integration into their lipidic surroundings (Tanaka and Tanaka, 2007; Tanaka et al., 2011). It remains unclear how the supply of substrate moieties to successive enzymes during the synthesis of Chls and tocopherols is coordinated.

TBS is subject to tight transcriptional and posttranslational control, which, among other things, prevents the accumulation of photoreactive tetrapyrrole intermediates and directs the appropriate amounts of metabolites into the branched TBS pathway. The unimpaired flow of metabolites is thought to be based on protein-protein interactions during the course of tetrapyrrole metabolism (Wang and Grimm, 2015). Ultimately, the dynamic formation of protein complexes is achieved by successive protein-protein interactions, which lead to assembly, disassembly, and reformation in alternative combinations (Zhang et al., 2015). This concept of multienzyme complexes is based on the need for smooth and balanced transfer of metabolites from one enzyme to the next without the release of potentially
toxic intermediates. The stability of macromolecular protein assemblies is facilitated through direct physical interactions among the proteins. This interplay is often supported by nonenzymatic chaperones and auxiliary factors (Tanaka et al., 2010; Albus et al., 2012; Mork-Jansson et al., 2015a).

In this work, we have extended our previous studies on the posttranslational organization of TBS with the aim of elucidating the function of LIL3 as an auxiliary factor in other steps in TBS, and we examined LIL3’s interactions with other proteins involved in Chl biosynthesis. We observed that lil3.1/lil3.2 double mutants not only contain reduced amounts of CHLP but also of POR and other proteins of TBS, implying that LIL3 has a broader role in the organization of the Chl biosynthesis pathway. Based on an analysis of the interactions of LIL3, an updated model for the function of LIL3 in the coordination of terpenoid and Chl synthesis is presented.

RESULTS
LIL3 Deficiency Destabilizes Chl-Synthesizing Enzymes

In 12-d-old lil3.1 and lil3.2 seedlings grown under short-day conditions (10-h-light/14-h-dark photoperiod, 100 μmol photons m⁻² s⁻¹), the Chl content was normal, but the Chl a/b ratio was increased to more than 5 in lil3.1 (Fig. 2A). Immunoblot analysis revealed that steady-state levels of several enzymes involved in

Figure 1. Alignment and domain structure of the Arabidopsis LIL3 proteins. Protein sequences of the two LIL3 proteins were aligned using Jalview 2.0 (Waterhouse et al., 2009). Numbers indicate amino acid positions, and protein domains are highlighted by different background shadings. The first transmembrane helix (TMH) carries a conserved LHC motif, which is indicated by a black line below the respective sequence.
Chl synthesis are lower in the *lil3.1/lil3.2* double mutant than in wild-type seedlings, while little or no change was detectable in either of the single mutants (Fig. 2B). Apart from CHLH, the content of PORA and PORB was strikingly reduced, while levels of PORC, CHLG, GUN4, CHL27, and LHCBI (light-harvesting Chl-binding proteins of PSII) were depressed to a lesser extent. GUN4 is a positive regulator of Chl synthesis, which stimulates Mg chelatase activity (Larkin et al., 2003; Peter and Grimm, 2009; Adhikari et al., 2011). CHL27 is an essential subunit of the Mg protoporphyrin monomethylester (MgPME) oxidative cyclase (the enzyme that precedes POR in the pathway) and has been proposed to contain the catalytic, substrate-binding domain (Tottey et al., 2003).

The reduced levels of enzymes of the TBS pathway in the *lil3.1/lil3.2* double mutant (Fig. 2B) could be due to changes in nuclear gene expression in response to LIL3 deficiency or altered plastid-derived retrograde signaling. Analysis of transcript levels indicated that *lil3.1* contains up to 20% of the wild-type amount of the LIL3.1 transcript. Using the available antibodies for the two LIL3 isoforms, a weak immunoreactive band was indeed detectable with the anti-LIL3.1 antibody in both *lil3.1* and the *lil3.1/lil3.2* double mutant (Fig. 2B). The top band in the PORC gel is caused by unspecific binding of the antibody. GUN4 is known to appear at least in two different sizes, as already described by Larkin et al. (2003). These observations are in agreement with previous suggestions that *lil3.1* is a knockdown mutant (Tanaka et al., 2010). In *lil3.2*, the LIL3.2 transcripts were not detectable (Fig. 2C). Thus, *lil3.2* is a null mutant. The anti-LIL3.2 antibody labels another weak band with a slightly different mobility from wild-type LIL3.2. This must represent a cross-reacting protein of unknown nature (Fig. 2B). Inactivation of either of the two LIL3 genes did not modify the expression of the other paralog. No significant changes in transcription levels of the other genes analyzed were found in either of the single *lil3* mutants, while some noteworthy alterations were noted in the double mutant (Fig. 2C; Supplemental Fig. S1, A–C). Specifically, the CHLH, LHCA1, and FC2 transcripts accumulated to levels at least 2-fold higher than in the controls. The CHLH gene encodes the Mg chelatase subunit H, and FC2 codes for the dominant ferrochelatase isomerase 2 in chloroplasts. In contrast, both *lil3.1* and the double mutant contained only 50% of the wild-type level of the FC1 transcript. But, insofar as antibodies were available for these studies, immunoblot analysis of the corresponding products of these genes indicated no changes in protein content. We conclude from these results that the reduced contents of POR and a few other proteins of Chl synthesis do not correlate with the levels of transcription of their corresponding genes and are due to posttranslational modifications. Moreover, these data point to partial inhibition of Pchlide reduction, which prompted us to take a closer look at plants deficient in both LIL3 isoforms.

**LIL3 Inactivation by Virus-Induced Gene Silencing**

Based on the normal Chl content of each of the single *lil3* mutants, we inferred that each LIL3 isoform can compensate partially for loss of the other. The severely reduced growth rate of the *lil3.1/lil3.2* double mutants precluded detailed biochemical analysis of chloroplast protein complexes and tetrapyrrole intermediates. Therefore, we made use of virus-induced gene silencing (VIGS) to inactivate LIL3 gene expression in 12-d-old Arabidopsis seedlings. One week after infection, developing leaves showed a uniform pale-green phenotype in comparison with control plants expressing VIGS constructs for GFP inactivation (Fig. 3A). LIL3 transcript levels were reduced to 40% (*LIL3.1*) and 10% (*LIL3.2*) of those in controls (Fig. 3B). As a result of gene inactivation, the total amount of LIL3 protein was strongly diminished (Fig. 3C). As in the case of the LIL3

**Figure 3.** Characterization of VIGS-LIL3.1/LIL3.2 plants. A, Phenotype of Arabidopsis plants with rosette leaves 3 weeks after infiltration with a *pTRV2-Lil3.1/Lil3.2* construct. Leaves of VIGS-LIL3 transformants are pale green compared with control leaves. B, Quantitative PCR (qPCR) analysis of VIGS-LIL3.1/LIL3.2 plants, confirming the down-regulation of both LIL3 genes. The ACT gene was used as a reference, and data were normalized to expression in VIGS-GFP control plants. C, Steady-state levels of selected proteins/enzymes involved in Chl biosynthesis. Similar amounts of total protein were fractionated by SDS-PAGE, transferred onto a membrane, and probed with specific antibodies.
T-DNA insertion mutants, CHLP, PORA, and PORB contents also were reduced drastically (below our detection limits), while CHLG content was similar to the control value.

Short-day-acclimated *lil3* mutants are already characterized by reduced rates of ALA synthesis (Tanaka et al., 2010). The drop in ALA synthesis and the low levels of CHLP and POR observed in our VIGS seedlings prompted us to determine their Chl content and the steady-state levels of Chl precursors (Fig. 4). The Chl content was reduced by approximately 50% as a result of VIGS-induced *LIL3* silencing (Fig. 4A).

Amounts of tocopherols, the phytyl chain-containing tocochromanols, also were depressed by 50%, and no tocotrienols were detectable (Fig. 4B), which is compatible with the loss of CHLP. Levels of Mg porphyrins and chlorins, as well as rates of ALA synthesis, also were strongly diminished (Fig. 4, C and D). Less than 20% of the Chl was phytylated in the *LIL3*-VIGS plants (Fig. 4, E and F). Conversely, these plants contained enhanced amounts of geranylgeranylated Chl *a* and *b* and of all intermediates formed during the three-step reduction of geranylgeranylated Chl to phytylated Chl (Fig. 4E). During the reduction step catalyzed by CHLP, three double bonds present in the geranylgeranyl moiety are reduced to single bonds, which lead to the formation of the phytol chain.

**LIL3-Mediated Protein-Protein Interactions**

The reduced content of POR in the *lil3.1/lil3.2* mutant and VIGS-*LIL3* plants can be explained if LIL3 acts to stabilize not only CHLP (Tanaka et al., 2010) but also POR. Bimolecular interaction between the two LIL3 isoforms and PORB, therefore, was explored using the bimolecular fluorescence complementation (BiFC) assay. Gene constructs encoding protein fusions of the target proteins to either of the two halves of YFP were transiently expressed in *Nicotiana benthamiana* and revealed plastid-localized interaction of the two transgenic proteins (Fig. 5A). BiFC assays with LIL3 and the two other isoforms PORA/B and PORC detected similar positive interactions within chloroplasts (Supplemental Fig. S2A). Moreover, a POR-GluTR interaction was found by the same approach. This interaction is consistent with the findings reported by Kauss et al. (2012), who proposed the formation of a protein complex consisting of CHL27, POR, FLU, and GluTR in chloroplasts of dark-grown plants. The POR reaction provides Chlide, one of the

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**Figure 4.** Levels of selected tetrapyrroles in VIGS-LIL3.1/LIL3.2 plants. After extraction of freeze-dried leaf samples with basic acetone, levels of Chls and other intermediates were quantified by HPLC using pure standards. A, Total Chl contents of VIGS-LIL3.1/LIL3.2 plants and Chl *a/b* ratio. B, Steady-state levels of tocopherol *a*. C, Steady-state levels of Chl precursors. MME, Mg protoporphyrin IX-monomethyl ester; MgP, Mg protoporphyrin IX. D, Rates of ALA synthesis, the rate-limiting step of Chl biosynthesis, in VIGS-LIL3.1/LIL3.2 plants. Measurement of ALA synthesis is accomplished by blocking the subsequent enzymatic step of ALA dehydratase and in vitro conversion of ALA into a pyrrole. E, Characterization of Chl *a* and *b* species by HPLC. Similar amounts of total Chl (1.5 μg) were separated on a Prontosil 200-3-C30 column. Geranyl and phytylated Chl species could be separated due to their different retention times. Identification of Chl *a* and *b* was performed based on their fluorescence properties (Chl *a*, excitation at 440 nm, emission at 660 nm; Chl *b*, excitation at 460 nm, emission at 660 nm). Numbers 1 to 4 indicate different Chl species, where 1 represents phytylated Chl and 2 to 4 represent geranylated Chls. Labels (a) and (b) indicate cross-detected Chl species (i.e. Chl *b*, which was detected with the Chl *a* detection parameters, and vice versa) due to the similar fluorescence emission properties. F, Amounts of phytylated Chl are given in percentages (representing elution peak 1). DW, Dry weight; FW, fresh weight.

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two substrates of CHLG. We made several attempts to uncover LIL3-CHLG interactions using BIFC and yeast two-hybrid assays but were unable to demonstrate any stable interaction (Supplemental Fig. S2).

The LIL3-POR interaction was confirmed using additional biochemical strategies. The split-ubiquitin-mediated two-hybrid approach in yeast allows one to analyze interactions among membrane-bound proteins. Interaction between all members of either protein family, LIL3 and POR, was demonstrated, independent of the combination of the respective fusion proteins considered (Supplemental Fig. S2B). Heterodimer formation of POR isoforms was verified by the BIFC method (Supplemental Fig. S2) and is consistent with the reported tendency of POR to oligomerize (Szenzenstein et al., 2010; Yuan et al., 2012).

Additional confirmation of the POR-LIL3 interaction was achieved in pull-down experiments (Fig. 5B). Recombinant His-tagged LIL3 was incubated with DDM-solubilized chloroplast extracts. After intensive washing, the bound proteins were eluted from the affinity column. In contrast to an eluate from the control assay with recombinant RibA1 as the bait, a plastid-localized bifunctional protein in riboflavin biosynthesis (Hiltunen et al., 2012), PorA/B and PORC were immunologically identified among the chloroplast proteins that bound specifically to LIL3 (Fig. 5B).

Dissection of LIL3 Domains and Interaction of POR with Specific Domains

To identify the LIL3 domain that interacts with the POR isoforms (Supplemental Fig. S2B), the two LIL3 variants were dissected into the N-terminal water-soluble half (LIL3 glob) and the membrane-spanning domain (LIL3 H1H2) with and without the C-terminal segment (LIL3 H1H2+C and LIL3 H1H2, respectively). Gene constructs were designed for the expression of these peptides and for parallel expression of recombinant POR paralogs in yeast. Experiments revealed an interaction of the N-terminal globular LIL3 peptide with all three POR isoforms, while the membrane domains and the C terminus together were insufficient to mediate POR binding. In contrast, all three LIL3 peptide variants failed to interact with CHLG in yeast two-hybrid assays (Supplemental Fig. S2B).

Stability of LIL3 in PORB-Deficient Lines

As LIL3.1 and LIL3.2 deficiency correlates with reduced contents of POR (Fig. 2B) and CHLP (Tanaka et al., 2010), we asked whether POR deficiency also might affect LIL3 expression or accumulation. An Arabidopsis CHLP knockout line is not available, but we selected four allelic PORB knockout mutants (Fig. 6A), which carry their T-DNA insertions at different sites in the PORB gene (Supplemental Fig. S3A). In Arabidopsis, three isoforms of POR (PORA–PORC) exist, of which PORB is the dominant isoform in green tissue (Armstrong et al., 1995). The allelic porB mutants displayed slightly retarded growth rates and developed fewer leaves than wild-type seedlings over the same growth period. The mutants accumulated either non-PORB transcripts or far fewer than the control line (Supplemental Fig. S3B). The presence of the T-DNA insertions in the coding regions precludes the detection
of PORB on immunoblots (Fig. 6B). Thus, the immuno-reactive bands that are still detectable in the porB extracts either represent PORA or result from unspecific binding of unknown proteins to anti-PORA/B antibodies. Moreover, in the porB mutants, chlorophyll contents and chlorophyll a/b ratios were similar to those of the wild type (Fig. 6, A and C), while the steady-state levels of Mg Proto and MgPME were lower. Interestingly, the levels of Pchlide were 2-fold higher in at least two of the four porB mutants, and in three mutants, the Chlide contents were reduced by as much as 75% relative to the wild-type level (Fig. 6E). These changes point to a defect in the enzymatic reduction of Pchlide, as a consequence of the lack of PORB and the inability of other POR isoforms to compensate for its loss. Notably, the rate of ALA synthesis is hardly affected at all, indicating that regulatory feedback at the level of POR is not detectably disturbed (Fig. 6D).

Comparative immunoblot analysis of proteins required for chlorophyll synthesis revealed several interesting differences between porB mutants and wild-type seedlings. Interestingly, the content of the two LIL3 isoforms, CHLP and CHLG, was higher in porB than in the wild type. These proteins belong to the late steps of chlorophyll synthesis. Quantification of the immunoblot signals of LIL3.1 blots revealed an up to 40% increase in the steady-state level in the porB lines compared with the wild type (Fig. 6B). We also examined whether the lack of PORB expression modulates the expression of LIL3 and genes involved in tetrapyrrole biosynthesis, but the analyzed genes showed unaltered expression levels (Supplemental Fig. S3B). This indicates that the modified protein contents of TBS enzymes are not explained by a different nuclear gene expression in the porB mutants but rather by post-translational effects.

Loss of LIL3 Impairs Photosynthetic Performance and the Accumulation of Photosynthetic Complexes in the Thylakoid Membrane

As mentioned above, LIL3-VIGS plants showed 50% reduced chlorophyll contents compared with control plants (Fig. 4A). Interestingly, the amounts of the major
LHC proteins Lhca1 and Lhcb1 appeared to be unaltered and slightly reduced, respectively (Fig. 7A). Thus, we analyzed the protein complexes of the thylakoid membrane using blue native (BN)-PAGE to obtain a more detailed view of the effects of LIL3 deficiency. Extracts with similar amounts of chlorophyll were solubilized with 1% DDM and separated on native gels (Fig. 7B). We found that silencing of both LIL3 isoforms drastically changes the composition of the protein complexes found in the thylakoid membrane (Fig. 7B). PSII-LHCII supercomplexes were almost absent, whereas PSI monomers and the PSII dimer were clearly reduced compared with the control. In contrast, monomeric LHCs accumulated. Separation of the protein complexes in the second dimension revealed a substantial loss of PSII core subunits (Fig. 7C), whereas the amount of subunits of the other complexes remained unchanged.

We further analyzed the photosynthetic performance of these plants using room temperature Chl fluorescence spectroscopy (Fig. 7D). LIL3-VIGS plants clearly showed a high-Chl fluorescence phenotype (Meurer et al., 1996), and, as a result of the elevated basal fluorescence, the maximum photochemical efficiency of PSII in the dark-adapted state (Fv/Fm) decreased from 0.85 ± 0.005 to 0.624 ± 0.049. In addition, the operating efficiency of PSII decreased from 0.791 ± 0.006 to 0.508 ± 0.044, whereas nonphotochemical quenching more than doubled from 0.054 ± 0.011 to 0.133 ± 0.024 in LIL3-silencing plants compared with wild-type plants. To assess the relative photosynthetic activity of PSII and PSI, we performed 77K spectroscopy (Fig. 7E). These experiments revealed that PSI also was severely impaired, as is clearly indicated by the strong decrease in the PSI emission peak around 730 nm.

**LIL3.1 Shows High Affinity for Pchlide and Other Tetrapyrroles**

Its membership of the LHC protein family characterizes LIL3 as a potential Chl-binding protein. LIL3 has a typical LHC-binding motif (Fig. 1), which, in principle, should enable it to bind tetrapyrroles. To complement the results relating to the binding of LIL3 to enzymes as well as regulatory and auxiliary factors of late Chl synthesis, we explored its binding capacity for the substrates and products of POR (Fig. 8A). Purified recombinant LIL3.1 (Fig. 8B) was incubated with several tetrapyrrole intermediates and Chls. The binding constants were determined by monitoring the quenching of intrinsic Trp fluorescence (Fig. 8C). Upon addition of the ligands, fluorescence quenching increased with increasing concentrations of the tetrapyrrole metabolites. LIL3.1 bound free Chl and intermediates in almost equimolar concentrations. Data analysis was performed assuming one specific binding site, as fitting of the data with equations representing two binding sites resulted in anomalously high affinities for the second site. Moreover, in particular for Pchlide and Chls, high levels of nonspecific binding were observed, which were taken into account during curve fitting of all ligands (Table I). The lowest K_D was determined for LIL3.1-Pchlide binding (251 ± 7 nm). Affinities for Chl and the other analyzed Chl precursors were somewhat lower. A K_D of about 350 nm for Chl fits well with a

![Figure 7](https://example.com/image7.png)

**Figure 7.** Photosynthetic characterization of VIGS-LIL3.1/LIL3.2 plants. A, Steady-state levels of the major light-harvesting proteins Lhca1/Lhcb1. Similar amounts of total leaf protein were fractionated by SDS-PAGE, transferred onto a membrane, and probed with specific antibodies. Two independent biological samples for VIGS-LIL3.1/LIL3.2 are shown. B, BN-PAGE of thylakoids isolated from VIGS plants. Equal amounts of thylakoids (equivalent to 10 μg of Chl) were solubilized with 1% DDM and fractionated on native gels. C, Separation of thylakoid membrane proteins into the second dimension. Lanes from BN-PAGE were denatured and placed on top of SDS-PAGE gels. D, Room temperature Chl fluorescence analysis of LIL3.1/LIL3.2 VIGS plants. E, The 77K Chl spectroscopy of LIL3.1/LIL3.2 VIGS plants. Data were normalized to the fluorescence at the PSI emission peak at 688 nm.
previously reported value of 230 nM obtained using microscale thermophoresis (Mork-Jansson et al., 2015b). The specificity of LIL3.1’s affinity for tetrapyrroles was confirmed when binding assays with FMN yielded a $K_D$ value of 7,600 nM and complete quenching of Trp fluorescence (data not shown). Interestingly, the specific quenching of the intrinsic Trp fluorescence did not exceed 40% when tetrapyrroles were used as ligands. We suspected that quenching might occur only at specific Trp residues and performed a structure prediction for LIL3.1 using the Phyre2 server (Kelley et al., 2015). LIL3.1 possesses nine Trp residues, of which three are clustered near the beginning of the transmembrane helix 1 (Trp-161, Trp-162, and Trp-164), which harbors the LHC motif (Supplemental Fig. S4). With one possible exception (Trp-112), the other Trp residues are localized in the N-terminal globular domain or in the C-terminal region, respectively. We propose that fluorescence quenching occurs only at these four Trp residues, which are localized nearest to the proposed binding site (i.e. LHC motif). This would explain the incomplete fluorescence quenching and confirms the specificity of tetrapyrrole binding to the LHC motif.

DISCUSSION

LIL3 Stabilizes POR

Previous observations of the metabolic phenotype of lil3.1/lil3.2 double mutants revealed that LIL3 is required specifically for the reduction of geranylgeranyl pyrophosphate to phytyl pyrophosphate. LIL3 serves to stabilize CHLP, an enzyme that is involved in modifying the hydrophobic alkyl chain of the geranylgeranyl moiety by reducing the three double bonds to single bonds in preparation for the subsequent synthesis of Chls and tocopherols. As two LIL3 isoforms are present in Arabidopsis, we asked whether other enzymes of Chl biosynthesis also might depend on LIL3. As shown here, the abundance of several enzymes that mediate late steps in Chl biosynthesis is indeed decreased in lil3 double mutants and VIGS-LIL3 plants (Figs. 2A and 3C). In particular, the POR content was reduced markedly. Comparison of the expression levels of transcripts coding for enzymes of Chl biosynthesis with the steady-state amounts of their products confirms that these effects are due to decreases in protein stability (Fig. 2, B and C; Supplemental Fig. S1). In particular, the POR content was reduced markedly. Comparison of the expression levels of transcripts coding for enzymes of Chl biosynthesis with the steady-state amounts of their products confirms that these effects are due to decreases in protein stability (Fig. 2, B and C; Supplemental Fig. S1). These findings suggest that both LIL3 isoforms might interact with the enzymes themselves.

Three alternative methods established that LIL3 interacts directly with POR isoforms. (1) BiFC assays confirmed the plastid-localized interaction (Fig. 5A; Supplemental Fig. S2A). (2) Pull-down experiments revealed selective purification of POR proteins from the plastid extracts when immobilized LIL3 was used as the bait (Fig. 5B). (3) Bimolecular interaction also was demonstrated in the yeast two-hybrid system
Based on these data, we conclude that the stability of CHLP and POR depends on LIL3 expression and that its interaction with these proteins mediates their selective localization at the plastid membranes. These results also are consistent with the stabilization of transgenic CHLP variants, which were supplemented with the transmembrane domain of LIL3 (Takahashi et al., 2014). These transformants showed complementation of the lil3.1/lil3.2 double mutant.

**Functional Role of LIL3 in Chl Synthesis**

Interestingly, CHLP and POR share a common function in that they catalyze the formation of the two substrates required for the subsequent enzymatic step implemented by Chl synthase. The fact that LIL3 associates with both enzymes strongly suggests that it facilitates the coordination of their action in the membrane, so that their products are simultaneously made available to Chl synthase. Therefore, we also tested whether LIL3 interacts with CHLG (Supplemental Fig. S2B). However, BiFC assays, yeast two-hybrid experiments, and pull-down experiments all failed to provide evidence for stable physical contact between CHLG and LIL3. Thus, a direct involvement of LIL3 in the final step in the synthesis of Chl a could not be demonstrated.

A previous study of CHLG assembly into complexes with HLIPs in cyanobacteria (Chidgey et al., 2014) also argues against a direct LIL3-CHLG interaction. The authors of that report presented evidence for the formation of a complex containing HLIPs and CHLG, together with multiple chaperones and auxiliary factors, such as YCF39, in close proximity to the site of the final steps of cyano bacterial Chl synthesis and assembly of photosynthetic Chl-binding proteins of PSII (Chidgey et al., 2014). Based on these results in cyanobacteria, it cannot be excluded that the channeling of pigments and the supply and integration of photosynthetic proteins in the thylakoid membrane require the additional assistance of proteins of the HLIP/OHP group.

It should be noted here that (like all other representatives of the two-helix proteins of the LHC family) LIL3 is absent in cyanobacteria. The current model implies that LIL3 facilitates the supply of substrates to the plant CHLG by interacting with CHLP and POR (Fig. 9). We have demonstrated here that LIL3 makes a substantial contribution to the stability of POR, as well as CHLP, and thus helps to ensure a balanced flow of metabolites for Chl synthesis. However, our current knowledge of the organization of late Chl biosynthesis remains fragmentary, and further studies on plant LIL3 and OHPs and their involvement in Chl synthesis and Chl supply for photosynthetic protein complexes are needed. It is expected that the control of Chl synthesis and the biogenesis of Chl-binding proteins are more complex in higher plants. Chl synthesis serves for more Chl-binding proteins in plants than in cyanobacteria, as the light-harvesting proteins also are assembled with Chl. The integration of nucleus- and plastid-encoded proteins...

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**Table 1. Binding constants for LIL3.1 pigment binding obtained by Trp fluorescence quenching analysis**

Quenching analysis was performed assuming one specific binding site plus unspecific binding ($F = \frac{F_{max} + N_s \cdot x}{K_D + x + N_s}$, where $x$ represents the ligand concentration). The dissociation constant ($K_D$) represents the ligand concentration at which half of the binding sites are occupied, and the term $N_s$ is a proportionality constant representing the slope of the linear nonspecific binding.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>KD [nM]</th>
<th>Nonspecific Binding [$N_s$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protoporphyrin IX</td>
<td>397 ± 15</td>
<td>0.0120 ± 0.0009</td>
</tr>
<tr>
<td>Mg protoporphyrin IX</td>
<td>624 ± 30</td>
<td>0.0042 ± 0.0013</td>
</tr>
<tr>
<td>Mg protoporphyrin IX-monomethylester</td>
<td>615 ± 19</td>
<td>0.0088 ± 0.0009</td>
</tr>
<tr>
<td>Protoporphyrin IX-monomethyl ester</td>
<td>251 ± 7</td>
<td>0.0345 ± 0.0006</td>
</tr>
<tr>
<td>Chl a</td>
<td>359 ± 26</td>
<td>0.0284 ± 0.0018</td>
</tr>
<tr>
<td>Chl b</td>
<td>342 ± 25</td>
<td>0.0393 ± 0.0020</td>
</tr>
</tbody>
</table>

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**Figure 9.** Working model for LIL3 function in the regulation of Chl biosynthesis. In the current working model, LIL3 acts as a scaffold/membrane anchor protein not only for CHLP but also for POR. Whereas CHLP provides phytol pyrophosphate, POR produces Chlide. Together, they provide the substrates for the reaction catalyzed by CHLG. By stabilizing CHLP as well as POR, LIL3 also may help to direct the flow of intermediates through the final steps of tetrapyrrole and terpenoid biosynthesis toward Chl biosynthesis, as it would allow the synchronous accumulation of precursors for the final Chl assembly. In addition, its relatively high affinity for Pchlide also would permit LIL3 to supply the POR protein with its substrate.
Chl-binding proteins into thylakoids during the simultaneous association with pigments and cofactors probably requires the combined action of LIL3 and OHP isoforms, apart from other auxiliary factors. To what extent ELIPs and other representatives of the LIL family contribute to these processes is an open question (Hutin et al., 2003; Tzvetkova-Chevolleau et al., 2007; Hayami et al., 2015).

**LIL3 and Its Tetrapyrrole-Binding Property**

It remains a challenging task to assign further functions to LIL3, apart from its contribution to protein stability in Chl biosynthesis (Tanaka et al., 2010) and the assembly of proteins into the photosynthetic complexes (Lohscheider et al., 2015; Mork-Jansson et al., 2015a). Functions of LIL3 in Chl and precursor transfer require at least the physical interaction of LIL3 with other proteins. Here, we show that Chl precursors bind to LIL3.1 (Fig. 8). It is particularly noteworthy that Pchlide, a substrate for its binding partner POR, binds to LIL3 with a higher affinity than any other tetrapyrrole tested. The $K_d$ values of LIL3 for metabolites of Chl synthesis are in agreement with previous values reported by Mork-Jansson et al. (2015b), while Takahashi et al. (2014) placed more emphasis on the membrane-anchoring function of LIL3 for its interacting protein partner. Further specific binding properties of LIL3 for tetrapyrroles will be explored in the future, including the identification of the specific amino acid residues involved. Moreover, it will be worthwhile to find out whether LIL3 also binds carotenoids, which would enable the quenching of transferred energy from light-absorbing tetrapyrroles. Taken together, our data support a role of LIL3 in PSII assembly. Based on our results, we favor a model that further studies of LIL3 and other members of the LIL family will be required before the roles of LIL3 in the biogenesis of photosynthetic complexes and in other enzymatic steps in Chl biosynthesis can be defined more precisely.

**Physiological Consequences of LIL3 Deficiency on the Assembly of Photosynthetic Complexes**

To assess the effects of a relative lack of LIL3 on the assembly and repair of photosynthetic complexes (Lohscheider et al., 2015; Mork-Jansson et al., 2015a), we performed BN-PAGE to examine photosystem-LHC complex formation. It was obvious that the amounts of stable supercomplexes are diminished as a result of LIL3 deficiency (Fig. 7B). However, the levels of LHCII (Figs. 2B and 7A) do not correlate directly with the decreased accumulation of PSII-LHCII supercomplexes. Indeed, in the relative absence of LIL3, the loss of PSII-LHCII supercomplexes is associated with an elevated accumulation of LHC monomers (Fig. 7B). LIL3 function was linked previously with the association of peripheral antennae with PSII core complexes (Reisinger et al., 2008; Lohscheider et al., 2015), but we currently see fewer indications for a specific function of LIL3 in PSII assembly. Based on our results, we favor a role of LIL3 in the management of the metabolic pathway. The binding of tetrapyrrole intermediates to LIL3 and its interaction with enzymes involved in Chl and terpenoid synthesis might reduce the risk of phototoxicity by freely diffusing light-absorbing tetrapyrroles and enhanced photosensitivity of Chl during exposure to high-intensity light. We propose that the organization of Chl metabolism implies the action of auxiliary factors, such as LIL3, which contribute to the fine-tuned channeling of tetrapyrrole intermediates. It is suggested that further studies of LIL3 and other members of the LIL family will be required before the roles of LIL3 in the biogenesis of photosynthetic complexes and in other enzymatic steps in Chl biosynthesis can be defined more precisely.

**Altered Levels of Chl Synthesis Proteins in PORB-Deficient Mutants**

Since the stability of CHLG and POR depends on LIL3 expression, we examined whether the converse also is true. We selected the porB mutants because they represent the isofrom that is constantly expressed in darkness and light, while PORC is light-dependently expressed and PORA is expressed only in etiolated tissue (Masuda et al., 2003). Interestingly, porB mutants actually express higher levels of LIL3 than the wild type. In addition, it is remarkable that the content of some enzymes of late Chl synthesis, such as CHLG and CHLH, also was elevated (Fig. 6B).

In conclusion, we report a more far-reaching role for LIL3 in tetrapyrrole metabolite binding and interaction with POR. This mutual interaction of LIL3 with enzymes associated in potential protein complexes (Fig. 9) in photosynthetic organisms is proposed to be crucial for the maintenance and productivity of the metabolic pathway.

**MATERIALS AND METHODS**

**Plant Material and Growth Conditions**

Arabidopsis plants (Arabidopsis thaliana ecotypes Columbia and Nossen) were grown on soil in a growth chamber (100 μmol photons m$^{-2}$ s$^{-1}$, 8-h photoperiod, 20°C, and 70% relative humidity). The single mutant T-DNA insertions line lil3.1 (DS13-3953) and lil3.2 (Ds13-0193) and the double mutant lil3.1/lil3.2 (DS13-3953 × Ds13-0193) were kindly provided by R. Tanaka. Seeds of the porB mutant (SALK_001829, SALK_043887, SALK_060191, and SAIL 429_A06) were obtained from the Nottingham Arabidopsis Stock Centre. Confirmation of the homozygous T-DNA insertion lines was performed by PCR using the primers listed in Supplemental Table S1.

**VIGS Assay**

Arabidopsis plants (ecotype Columbia) applied for the VIGS assay were grown on soil in a growth chamber (100 μmol photons m$^{-2}$ s$^{-1}$, 16-h photoperiod, 20°C, and 70% relative humidity). The plasmids pTRV1 and pTRV2, based on Tobacco rattle virus, were used as described by Liu et al. (2002). LIL3.1 and LIL3.2 cDNAs were amplified with specific primers (listed in...
Supplemental Table S1) from Arabidopsis total cDNAs generated by reverse transcription-PCR and cloned into pTRV2. As the negative control, a pTRV2-GFP vector was used. Infiltration of Agrobacterium tumefaciens cells transformed with the pTRV plasmids was done as described previously (Burch-Smith et al., 2006).

Pigment Extraction and HPLC

Leaf material for pigment extraction was harvested 2 to 3 hours after the transition from dark to light and ground in liquid nitrogen. Subsequently, the pigments were extracted from the powder with ice-cold acetone (0.2% NH4OH, 9:1), with or without prior freeze drying, separated by HPLC, and quantified using pure standards.

ALA Synthesis Rate

Rates of ALA synthesis were measured as described by Mauzerall and Granick (1956). Briefly, detached leaves or leaf discs were incubated in 40 mM levulinic acid in 50 mM Tris-HCL, pH 7.2, for 3 to 4 hours under growth light conditions. Afterward, the leaf material was ground in liquid nitrogen and resuspended in 50 mM KPO4 buffer, pH 6.8. After centrifugation, the supernatant was mixed 5:1 with ethyl acetocetate, boiled for 10 minutes, and mixed 1:1 with modified Ehrlich reagent. The ALA concentration was determined photometrically and compared with a standard as described.

Preparation of Total Leaf Protein, SDS-PAGE, and Immunoblot Analyses

Total leaf proteins were extracted from leaf material ground in liquid nitrogen by resuspending the powder in an equal volume of protein extraction buffer (35 mM Na2CO3, 5 mM DTT, 350 mM Suc, 2% SDS, and 2 mM EDTA) and incubating at 70°C for 10 minutes. After precipitation of 25% TCA and resuspension in 100 mM L of 0.1% NaOH, the protein concentration was determined with the BCA reagent (Thermo Fisher). Equal amounts of protein were fractionated by SDS-PAGE, transferred onto nitrocellulose membranes, and probed with specific antibodies. For signal detection, Clarity Western ECL Blotting Substrate (Bio-Rad) was used.

Thylakoid Extraction and BN-PAGE

Thylakoid membranes were extracted as described previously (Jarvi et al., 2011), except that leaves were homogenized in buffer containing 450 mM sorbitol, 20 mM Tricine-KOH (pH 8.4), 10 mM EDTA, and 0.1% BSA. Finally, thylakoids were resuspended in 25BTH20G buffer (25 mM Bis-Tris, pH 7, and 20% glycerol) and stored at ~80°C. BN-PAGE was performed as described earlier (Peng et al., 2008).

Gene Expression Analyses

Total RNAs were extracted from leaf material using Trisure (Bioline) following the manufacturer’s instructions. Subsequently, 1 µg aliquots of RNA were digested with DNase, and cDNA synthesis was performed with the RevertAid RT Enzyme (Thermo) according to the manufacturer’s instructions. Gene expression was analyzed with SensiMixSYBR (Bioline) using the CFX 96 real-time system (Bio-Rad) with the primers listed in Supplemental Table S2. ACT2 (At3g18780) was used as the reference gene. Data analysis was performed according to the ΔΔCt method (Pfaffl, 2001).

Expression and Purification of Recombinant LIL3.1 Protein

For the expression of recombinant LIL3.1 protein, an Escherichia coli-optimized cDNA sequence was ordered from Integrated DNA Technologies and cloned into the pET28a vector (Novagen) modified with an N-terminal TEV cleavage site. Protein expression in the E. coli BL21-pRIL strain (DE3; Stratagene) was performed at 20°C for 3 hours and was induced routinely with 0.25 mM isopropyl-β-D-thiogalactoside at an OD600 of 0.6 to 0.8. Purification of recombinant 6xHis-tagged proteins was performed using a 50% (v/v) suspension of Ni-NTA agarose (Thermo Scientific) according to the manufacturer’s protocol. Proteins were concentrated with Amicon Ultra-15 (molecular mass cutoff, 30 kDa) centrifugal filter units (Millipore).

Pull-Down Assay

Purified 6xHis-LIL3.1 protein (100 µg) was immobilized on Ni-NTA agarose (100 µL of a 50% [v/v] suspension of Ni-NTA agarose [Thermo Scientific] in equilibration buffer) for 60 minutes at 4°C. Total chloroplast proteins (100 mg of Chl) solubilized in 1% (w/v) DDM were incubated with immobilized 6xHis-LIL3.1 protein overnight at 4°C. The empty agarose and agarose bound to 6xHis-RibA1 were used as negative controls. The agarose was washed extensively four times with phosphate buffer containing 150 mM NaCl. Bound proteins were eluted with elution buffer containing 250 mM imidazole and 150 mM NaCl and analyzed by immunoblotting.

In planta Interaction Studies by BiFC

LIL3.1 and POR cDNAs were amplified from total cDNAs with primers carrying attB sites and cloned into the G1 and G3 vectors (pDEST-GW-VYNE/-VYCE) via pDON207 using the Gateway system (Invitrogen) according to the manufacturer’s instructions. A. tumefaciens (GV2260) cells transformed with the G1/G3 vectors were resuspended in transformation buffer to an OD of 0.2, mixed in the desired combinations, and infiltrated into Nicotiana benthamiana leaves. Plants were kept in the dark overnight and subsequently incubated at 120 µE PAR for 2 d. YFP fluorescence was recorded on a confocal laser-scanning microscope (CLSM TCS SP2 AOBS; Leica).

Yeast Two-Hybrid Assays

cDNA sequences of all genes analyzed were cloned either by restriction site cloning via pET2.1 into pDHB1MC2S (baits) or by Gateway cloning via pDONR221 into met25pXGATE (pNub; preys). The yeast strain L40ccA was cotransformed with bait and prey constructs in all variations. Transformed clones were selected on agar plates with synthetic dextrose (SD) medium lacking Leu and Trp, and liquid overnight cultures of these selected colonies were grown in 3 mL of SD medium lacking Leu and Trp. OD600 was measured and adjusted to 1. For the selection of positive interaction, the cultures were spotted on agar plates containing SD medium lacking His, Leu, Trp, and uracil, supplemented with 10 mM 3-amino-1,2,4-triazole, and incubated for 3 to 4 d at 30°C.

Chl Fluorescence Spectroscopy

Leaf material from Arabidopsis plants was homogenized in LHC buffer (0.4 M sorbitol in 50 mM Tricine, pH 7.8) and mixed with 1 volume of 80% glycerol. The suspension was filled into a capillary, frozen in liquid nitrogen, before its Chl fluorescence was measured with a fluorescence spectrophotometer (Hitachi F-7000). Pulse amplitude-modulated room temperature fluorescence was measured with an FMS2 Portable Pulse Modulated Chlorophyll Fluorometer (Hansatech). In general, a protocol described by Meurer et al. (1996) was followed, except that the actinic light was adjusted to growth light conditions (100 µmol photons m−2 s−1).

Trp Fluorescence Quenching

An aliquot of the 6xHis-tagged LIL3.1 protein (360 µL) was diluted in quenching buffer (50 mM Tricine, pH 7.9, 300 mM glycerol, and 1 mM DTT), mixed with increasing amounts of various porphyrins (final concentrations, 0–8 µM), dissolved in DMSO, and incubated for 5 minutes. Trp fluorescence was measured with a fluorescence spectrophotometer (Hitachi F-7000) after excitation at 280 nm.

Data Analysis

Generally, experiments were performed three times, and a representative result is shown. Data were analyzed using Microsoft Excel except for the curve fitting of the Trp fluorescence quenching data, for which SigmaPlot 11 (Systat Software) was used.

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. qPCR analysis of tetrapyrrole biosynthesis genes in il3 single and double mutants.
LITERATURE CITED


Montané MH, Kloppstech K (2000) The family of light-harvesting-related proteins (LHs, ELs, HLPs): was the harvesting of light their primary function? Trends Biochem Sci 25: 1–8


Kauss D, Bischof S, Steiner S, Apel K, Meskauskiene R (2012) FLU, a negative feedback regulator of tetrapyrrole biosynthesis, is physically linked to the final steps of the Mg2+-branch of this pathway. FEBS Lett 586: 211–216


Montané MH, Kloppstech K (2000) The family of light-harvesting-related proteins (LHs, ELs, HLPs): was the harvesting of light their primary function? Trends Biochem Sci 25: 1–8


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