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Light-harvesting antenna complexes in the moss *Physcomitrella patens*: implications for the evolutionary transition from green algae to land plants

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Plants have successfully adapted to a vast range of terrestrial environments during their evolution. To elucidate the evolutionary transition of light-harvesting antenna proteins from green algae to land plants, the moss *Physcomitrella patens* is ideally placed basally among land plants. Compared to the genomes of green algae and land plants, the *P. patens* genome codes for more diverse and redundant light-harvesting antenna proteins. It also encodes Lhcb9, which has characteristics not found in other light-harvesting antenna proteins. The unique complement of light-harvesting antenna proteins in *P. patens* appears to facilitate protein interactions that include those lost in both green algae and land plants with regard to stromal electron transport pathways and photoprotection mechanisms. This review will highlight unique characteristics of the *P. patens* light-harvesting antenna system and the resulting implications about the evolutionary transition during plant terrestrialization.

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Introduction

The extant terrestrial flora displays remarkably diverse characteristics and has succeeded in dominating incredibly varied environments. During the evolution of green plants, the transition from aquatic to terrestrial environments was a key process involving a series of changes conferring the ability to cope with terrestrial conditions including exposure to dry air, gravity, atmospheric CO_2, and light. To address fundamental questions related to plant terrestrialization, bryophytes (liverworts, mosses, and hornworts) are ideally positioned basally among the extant land plants. The moss *Physcomitrella patens* has been developed as a model organism [1], and its genome data have provided new knowledge about how the early land plant lineage would have facilitated the adaptation to terrestrial environment [2,3].

As photosynthesis is the major source of energy for photoautotrophic organisms, the change in light regime during plant terrestrialization must have been a substantial environmental stress [4]. Although the fundamental architecture of the photosynthetic reaction centers is highly conserved among photoautotrophic organisms, the light-harvesting antenna proteins are quite diverse, which is suggested to reflect the changes essential for adaptation to different environments [5]. Interestingly, *P. patens* possesses light-harvesting antenna proteins even more diverse than those found in either green algae or land plants [6]. *P. patens* also has its own unique light-harvesting antenna protein, Lhcb9 [7••]. Furthermore, the light energy dissipation mechanism, so-called non-photochemical quenching (NPQ), that is activated under excess light conditions is also operated by both green algal- and land plant-type proteins in *P. patens* [8]. Recent progress in elucidating the molecular mechanisms of NPQ induction in *P. patens* has been covered in detail elsewhere [9]. Here, we focus on what the unique characteristics of the *P. patens* light-harvesting antenna system imply about the evolutionary transition from green algae to land plants.

The light-harvesting antenna system is redundantly diversified in *P. patens*

Light-harvesting complex (LHC) proteins are a protein superfamily that bind chlorophylls (Chls) and carotenoids to facilitate the absorption of light energy and the transfer of excitation energy to the reaction centers of photosystems I (PSI) and II (PSII). LHC genes are generally classified into two groups, Lhca and Lhcb, which encode LHC proteins for PSI (LHCI) and PSII (LHCII), respectively [10]. In the green alga *Chlamydomonas reinhardtii* and the land plant *Arabidopsis thaliana*, there are at least 9 and 6 Lhca genes, respectively (Table 1). In addition, there are at least 12 and 15 Lhcb genes in *C. reinhardtii* and *A. thaliana*, respectively. LHCII proteins are further categorized into two groups, the major and minor

Table 1

Phenotype comparison among *C. reinhardtii*, *P. patens*, and *A. thaliana*

LHCI	*Lhca1*	*Lhca2*	*Lhca3*	*Lhca4*	*Lhca5*	*Lhca6*	*Lhca7*	*Lhca8*	*Lhca9*
C. reinhardtii	1	1	1	1	1	1	1	1	1
P. patens	3	5	4	0	1	0	0	0	0
A. thaliana	1	1	1	1	1	1	0	0	0

LHCII	*Lhcbm*	*Lhcb1*	*Lhcb2*	*Lhcb3*	*Lhcb4*	*Lhcb5*	*Lhcb6*	*Lhcb7*	*Lhcb8*	*Lhcb9*
C. reinhardtii	9	0	0	0	1	1	0	1	0	0
P. patens	14	0	0	1	2	4	2	1	0	2
A. thaliana	0	5	3	0	2	1	1	1	1	0

Number indicates the orthologous Lhca and Lhcb genes identified in *C. reinhardtii* genome (v5.5, DOE-JGI), *P. patens* genome (v3.3, DOE-JGI), and *A. thaliana* genome (The Arabidopsis Information Resource annotation release 10). Lhcb8 is previously known as Lhcb4.3 in *A. thaliana* [22].

	Stromal electron flow pathways				qE	
	PGR5/PGRL1	PSI-NDH	PSI-Cyt b_6f	Flv	LHCSR	PsbS
C. reinhardtii	Yes	No	Yes	Yes	Yes	Transient
P. patens	Yes	Partial	TBD	Yes	Yes	Yes
A. thaliana	Yes	Yes	TBD	No	No	Yes

Three protein complexes operating cyclic electron flow exist—PGR5/PGRL1 complex (PGR5/PGRL1), PSI-NDH complex (PSI-NDH), and PSI-Cyt b_6f complex (PSI-Cyt b_6f). There is also flavodiiron protein (Flv)-dependent pathway exists as an alternative pathway [31,35]. TBD, to be determined. The partial PSI-NDH complex is observed in *P. patens* (Partial) [18]. Energy-dependent non-photochemical quenching (qE) is operated by LHCSR and/or PsbS. PsbS is expressed transiently during the activation of qE in *C. reinhardtii* (Transient) [47••].

LHCIIs, which normally exist as trimers or monomers, respectively. In *A. thaliana*, *Lhcb1*, *Lhcb2*, and *Lhcb3* encode major LHCIIs, and *Lhcb4*, *Lhcb5*, and *Lhcb6* encode minor LHCIIs [10]. In green algae, the genes encoding major LHCIIs are designated as *Lhcbm* ("m" for major) and have relatively low amino acid sequence similarity to *A. thaliana* Lhcb1 (AtLhcb1; "At" for *A. thaliana*, hereafter), AtLhcb2, and AtLhcb3 [11,12].

The *P. patens* genome contains more LHC genes with greater redundancy compared to those of both the green alga *C. reinhardtii* and the land plant *A. thaliana* (Table 1). For example, the orthologs of AtLhca1 and AtLhca2 are encoded by 3 and 5 paralogous genes in *P. patens*, respectively (Figure 1). The homologs of AtLhca3 seem to have arisen prior to the divergence of green algal lineages [12], and both *C. reinhardtii* and *A. thaliana* have one *Lhca3* gene. However, the *P. patens* genome contains four paralogs for Lhca3. The Lhcbm proteins in *P. patens* clearly show more diversity and redundancy compared to those in the other species, and some of their amino acid sequences are almost identical even though the loci are different (the genes encoding major LHCII in *P. patens* are designated as *Lhcbm* as well [6]). There are at least four different isoforms of Lhcb5 in *P. patens*, although most other organisms contain only one [2], except for the recently sequenced plants soybean [13] and *Kalanchoe laxiflora* [v1.1, Department of Energy-The Joint Genome Institute (DOE-JGI), http://phytozome.jgi.doe.gov/], which is a model plant well-adapted to dry growth conditions. Not only LHC genes, but other genes, for example those related to housekeeping and metabolic pathways, are highly abundant in the *P. patens* genome, which is therefore suggested to arise from genome-wide duplication events in the ancestral lineage of mosses [3,14,15].

In addition to the diversified LHC proteins, the *P. patens* genome shows intriguing composition of the light-harvesting antenna system. For example, the ortholog of AtLhca4 is missing in *P. patens*, although it appears to be important for stabilizing the PSI-LHCI structure in *A. thaliana* [16], resulting in a blue-shift in the low-temperature fluorescence emission of the *P. patens* PSI-LHCI supercomplex [6]. *P. patens* contains an ortholog of AtLhca5, but not of AtLhca6, both of which are essential for the formation of the supercomplex comprising chloroplast type I NADH dehydrogenase (NDH) and PSI in *A. thaliana* [17], and in fact only a partial NDH-PSI supercomplex is assembled in *P. patens* [18]. Another bryophyte, the liverwort *Marchantia polymorpha*, lacks orthologs of both AtLhca5 and AtLhca6, and the NDH-PSI supercomplex has not been detected in that species [19]. The different PSI light-harvesting antenna system found in *P. patens*, in addition to some exceptional characteristics of PSI subunits (*e.g.*, subunit F is a primitive type, three different isoforms of subunit K are present, the cyanobacterial-type subunit M is present, and subunit N is missing [20]), may contribute to a unique set of protein–protein interactions involved with PSI assembly in the moss.

Along with the Lhcbm genes encoding LHCII proteins, *P. patens* also has an ortholog of AtLhcb3, while

Figure 1
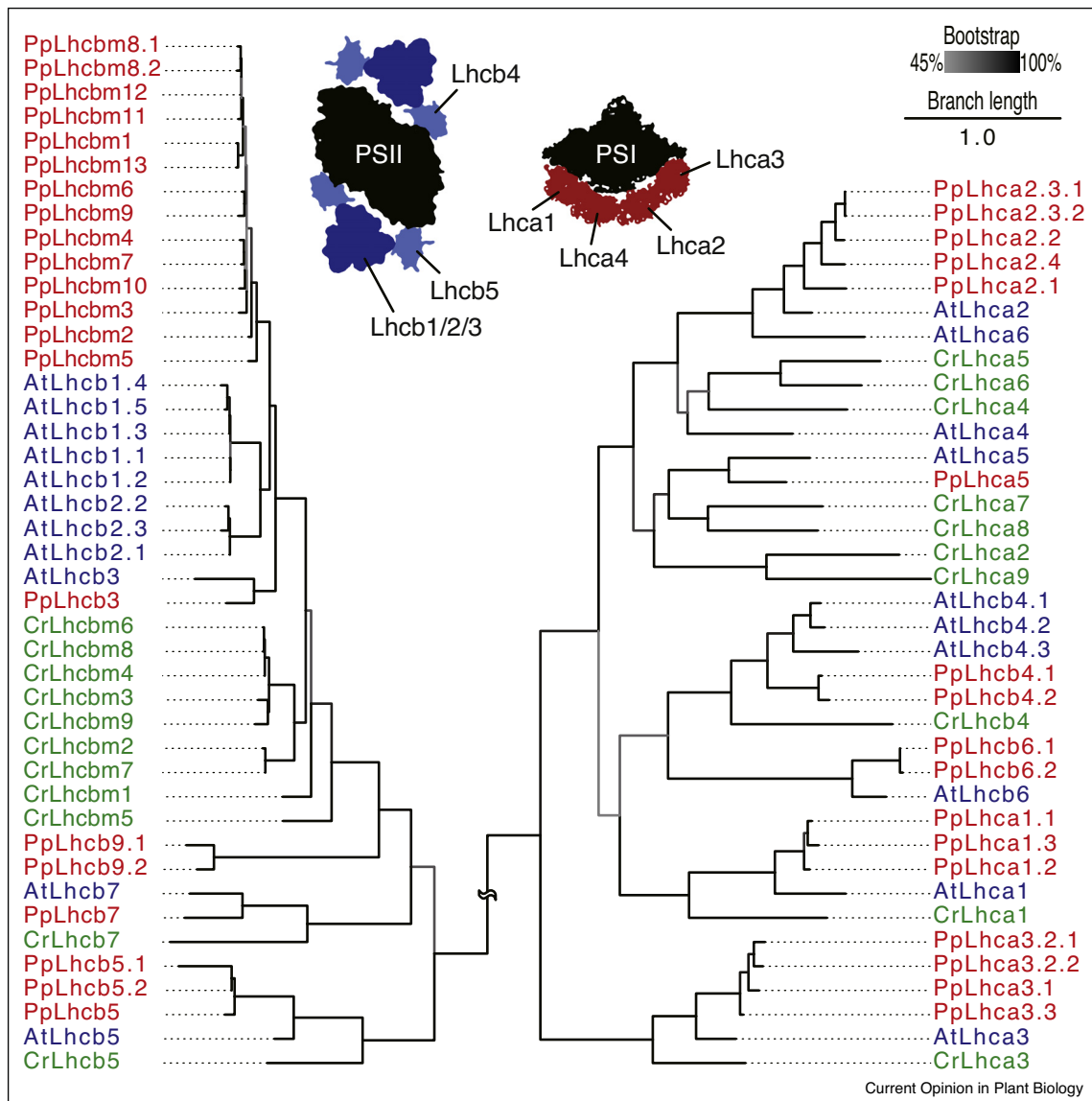
Phylogenetic diversity of LHC proteins. The analysis includes amino acid sequences from LHC proteins found in *A. thaliana* (At), *P. patens* (Pp), and *C. reinhardtii* (Cr). The sequences for *A. thaliana* were retrieved from The Arabidopsis Information Resource, and those for *P. patens* and *C. reinhardtii* were retrieved from JGI (v3.3 and v5.5, respectively). The positions of Lhca and Lhcb proteins in PSI and PSII supercomplexes, respectively, are indicated as examples of those found in land plants. The structures for PSII-LHCII and PSI-LHCI are drawn according to Protein Data Bank (3WU2, 2BHW, 3PL9, 3JCU, and 4XK8).

C. reinhardtii does not, suggesting its acquisition during plant terrestrialization [6] (however, Lhcb3 appears to have been lost during the divergence of Pinaceae and Gnetales [21]). The orthologs of three minor LHCII proteins (Lhcb4, Lhcb5, and Lhcb6) in *A. thaliana* are also found in *P. patens* [6]. It appears that none of the *P. patens* Lhcb4 (PpLhcb4; "Pp" for *P. patens*, hereafter) isoforms are related to AtLhcb4.3 [6], which is found only in dicots and is now classified as Lhcb8 due to its distinct function from AtLhcb4.1 and AtLhcb4.2 [22]. Further, as the ortholog of AtLhcb6 has not been found in green

algae so far [11,12], the presence of an AtLhcb6 ortholog in *P. patens* suggests its uniqueness to land plants, although it is absent in Pinaceae and Gnetales, similar to the case of Lhcb3 [21]. In addition to major and minor LHCIIs, the ortholog of AtLhcb7, which is rarely expressed [22], is present in *P. patens* [6].

It is of interest that *P. patens* has maintained a more diversified set of LHC proteins than has *A. thaliana*, which has also adapted to nonaquatic conditions. Given its lack of anatomical adaptation to terrestrial conditions,

with a simplified morphology and poikilohydry, the highly diversified LHC genes in *P. patens* might confer the robustness of the light-harvesting antenna system required for the unique physiological conditions encountered during the terrestrialization of their ancestral lineage.

Lhcb9 is a unique Lhcb protein found in *P. patens*

Intriguingly, a novel Lhcb gene *Lhcb9* is found in *P. patens* [6]. According to Phytozome v.11.0, a Lhcb9-like protein appears to be present in the bog moss *Sphagnum fallax* (v0.5, DOE-JGI), but no ortholog of Lhcb9 is found in other sequenced taxa so far. Based on its sequence, Lhcb9 appears to belong to the group of LHCII proteins and most likely exists as a monomer [6,7••]. However, one of the unique features of Lhcb9 is that it binds Chl pigments that show fluorescence emission longer than ~680 nm, the typical fluorescence emission maximum of LHCII [7••]. Analyses using the reconstituted Lhcb9 complex suggest that an asparagine, instead of a histidine, serves as the central ligand for Chl 603, which is a characteristic observed in AtLhca3 and AtLhca4 [23] but not in Lhcb proteins, and is responsible for the red-shifted fluorescence emission [7••]. Although the degree of red-shift of Chl 603 is smaller than that of 'red Chls' observed in the PSI light-harvesting antenna system [24], Lhcb9 is the only Lhcb protein showing this feature.

Two studies have shown contradictory results regarding the association of Lhcb9 with PSII [7••], as might be expected for a protein related to Lhcb family members, or with PSI [25••], which may arise from the different growth conditions used, leading to the different localization of Lhcb9. The exact localization of Lhcb9 is thus unclear without further analyses. One study found that the association of Lhcb9 with PSI results in the presence of two different sizes of PSI-LHCI supercomplexes in *P. patens* thylakoid membranes [25••]. Interestingly, the smaller and larger PSI-LHCI supercomplexes have similar sizes as PSI-LHCI supercomplexes in *A. thaliana* and *C. reinhardtii*, respectively (Figure 2). In the *A. thaliana* PSI-LHCI supercomplex, four LHCI proteins are aligned laterally into a crescent shape (the so-called LHCI belt) and positioned at the PsaG/F/J/K side of the PSI core [16,26]. By contrast, the *C. reinhardtii* PSI-LHCI supercomplex harbors up to nine LHCI proteins (CrLhca1-9; 'Cr' for *C. reinhardtii*, hereafter), forming a double crescent shape located at the PsaG/F/J/K side of the PSI core [27]. In a *P. patens* Lhcb9 knockout line, the larger PSI-LHCI supercomplex is completely missing (Figure 2), suggesting that Lhcb9 could function as a linker between the smaller PSI-LHCI supercomplex and the additional antenna that forms the larger supercomplex [25••]. Although the structural organization of this larger PSI-LHCI supercomplex in *P. patens* still needs to be resolved, having the two different sizes of PSI-LHCI

Figure 2


Current Opinion in Plant Biology
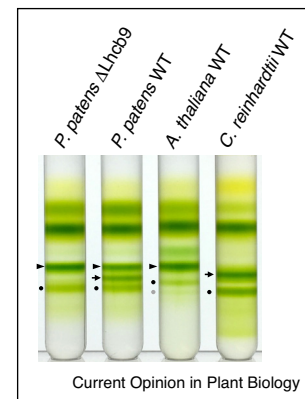
Density gradient analysis of solubilized thylakoid membrane proteins. Thylakoid membranes (100 μg Chl at 0.5 mg Chl/mL) isolated from *P. patens* WT and *Lhcb9* knockout mutant (ΔLhcb9), *A. thaliana* Col-0 (WT), and *C. reinhardtii* 4A⁺ (WT) were solubilized with 1% (wt/vol) *n*-dodecyl-α-D-maltoside and subjected to maltose density gradient centrifugation [0.1–1.3 M maltose with 25 mM MES-NaOH (pH 6.5) and 0.03% (wt/vol) *n*-dodecyl-α-D-maltoside] at 154 300 × *g* (SW 41 Ti rotor, Beckman Coulter) for 24 h at 4°C. Arrowheads and arrows indicate the bands corresponding to the smaller and larger PSI-LHCI supercomplexes, respectively. Dots indicate the bands corresponding to PSII-LHCII supercomplexes.

supercomplexes that correspond to the ones in *A. thaliana* and *C. reinhardtii* might reflect the evolutionary demand for the transition during plant terrestrialization.

Another curious feature about Lhcb9 is that its most similar protein outside of mosses is Lhcbm5 from *C. reinhardtii* [25••] (the moss *S. fallax* contains the most similar Lhcb9). All other Lhca and Lhcb proteins in *P. patens* are most similar to the ones in *A. thaliana* (except for PpLhcb7, which is more similar to the one in *Populus trichocarpa*). Only Lhcb9 appears to be distantly related to land plant LHC proteins. Phylogenetic analyses also suggest that Lhcb9 was derived prior to the divergence from the green algal LHC (*i.e.*, is orthologous to CrLhcbm5), while all the other LHC proteins appear to have evolved after that [25••] (Figure 3). The liverwort *M. polymorpha* does not seem to contain the ortholog of Lhcb9 (v3.1, DOE-JGI), highlighting the uniqueness of this protein even within the bryophyte lineage. One possible explanation for this inconsistency is that the ancestral LHC protein for Lhcb9 was introduced to *P. patens* genome by horizontal gene transfer (HGT) during the evolution. Intriguingly, the most similar LHC to Lhcb9, CrLhcbm5 is also found to be associated with the PSI-LHCI supercomplex in *C. reinhardtii* [28]. The shared ability to bind with PSI might be derived from their common ancestral LHC protein present in the earlier green algal lineage, which evolved into CrLhcbm5 in the succeeding *C. reinhardtii*, while the one horizontally transferred to the early moss lineage evolved into Lhcb9

Figure 3
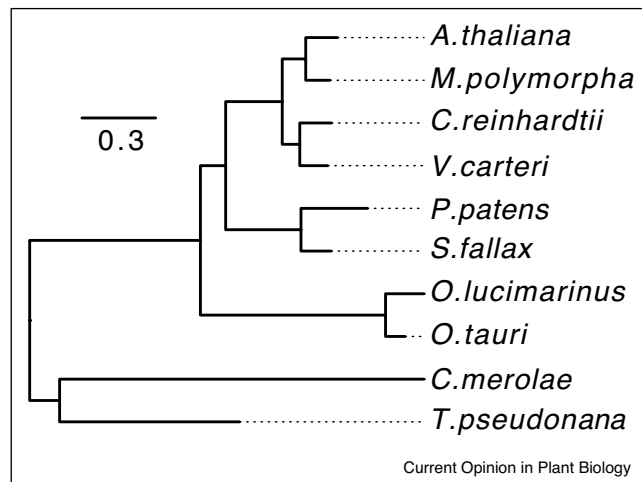
Phylogenetic tree of Lhcb9 and the most closely related LHC proteins of other photosynthetic eukaryotes. Lhcb9 was used as query sequence for BLAST searches against the genomes of 9 organisms [land plants, *Arabidopsis thaliana* (Lhcb2.1); bryophytes, *Marchantia polymorpha* (Mapoly0026s0025), *Physcomitrella patens* (Lhcb9.1), and *Sphagnum fallax* (Sphfalx0043s0013); chlorophytes, *Chlamydomonas reinhardtii* (Lhcbm5) and *Volvox carteri* (Vocar.0014s0016); prasinophytes, *Ostreococcus lucimarinus* (XP_001420273) and *O. tauri* (XP_003081908); red algae, *Cyanidioschyzon merolae* (CMQ142C); and diatoms, *Thalassiosira pseudonana* (XP_002295184); In parentheses, the names of LHC protein are indicated, whereas the locus names are provided in case that no specific LHC name is annotated.]. The scale bar indicates the expected fraction of amino acids changed.

in *P. patens*. As HGT is essential for the transition during plant terrestrialization [29], it is hypothesized that the Lhcb9-mediated, larger PSI-LHCI supercomplex might have promoted survival under certain environmental conditions for the ancestral lineage of mosses [25[••]].

Multiple chloroplast stromal electron flow pathways operate in *P. patens*

LHCII proteins not only transfer light energy to photosystems but dissipate the energy as heat to prevent photodamage under excess light conditions via the energy-dependent type of NPQ, or qE (reviewed in Ref. [30]). The molecular details of the qE mechanism are still debated, but the development of the transmembrane pH gradient across thylakoid membrane (ΔpH) is known to be the trigger. The ΔpH is generated by several factors related to linear and cyclic electron flow in different, alternative pathways (reviewed in Ref. [31]). In linear electron flow, water oxidation by PSII and the proton translocation by cytochrome b_6f (Q cycle) generate ΔpH. In cyclic electron flow, the electrons generated at the stromal side of PSI are transferred via ferredoxin back to the PGR5/PGRL1 complex in thylakoid membranes, which reduces plastoquinone, inducing the Q cycle and a buildup of ΔpH, in both *C. reinhardtii* [32] and *A.*

thaliana [33,34]. *A. thaliana* has an alternative pathway that is dependent on PSI-NDH complex (reviewed in Ref. [35]). In *C. reinhardtii*, the formation of a supercomplex including PSI-LHCI and cytochrome b_6f, which also involves the association of ferredoxin-NADPH oxidoreductase, PGRL1, Ca^{2+} sensor protein, and anaerobic response 1 protein, is shown to mediate cyclic electron flow [36–38].

In *P. patens*, PGR5/PGRL1-dependent cyclic electron flow appears to operate, based on analyses using the *pgrl1* knockout mutant [39[•]]. Interestingly, it is also suggested that PGRL1 is required for survival under high light and anoxic conditions in *C. reinhardtii* and *P. patens* and thus might have been essential for plant terrestrialization [39[•]]. The NDH complex is absent in green algae and some gymnosperms but present in *P. patens*. The association of the NDH complex with the PSI-LHCI supercomplex enhances cyclic electron transport in *A. thaliana*, although only the partial supercomplex formation is observed in *P. patens*, which might be due to the lack of PpLhca6 [18]. Alternatively, ΔpH can be developed through photoreduction of oxygen in the chloroplast stroma via flavodiiron (Flv) proteins, which are found in cyanobacteria, green algae, mosses, and gymnosperms but are absent from flowering plants [40]. It has been shown that Flv proteins in *P. patens* play a substantial role as electron sinks, which prevents photodamage in PSI, and are important for survival under fluctuating light conditions [41[•]]. Although Flv proteins were lost in flowering plants during evolution, PpFlv proteins are functional when expressed in *A. thaliana*, which leaves us with the question of why they were lost [42]. *P. patens* thus operates multiple ways of electron transport and generating ΔpH, which must have been physiologically essential for the evolution of moss lineage and might reflect an evolutionary demand during the transition to terrestrial environments.

Mechanisms for excess light energy dissipation are independently induced by LHCSR and PsbS in *P. patens*

In plants, the buildup of ΔpH activates the thylakoid lumenal enzyme violaxanthin deepoxidase, converting violaxanthin to zeaxanthin, accumulation of which is essential for the induction of qE [43]. In *C. reinhardtii*, high light stress-related LHC (LHCSR [44]; originally known as LI818) protein is protonated on its lumenal side upon buildup of ΔpH [45], which enhances the induction of qE within the PSII-LHCII-LHCSR supercomplex [46]. PSII subunit S (PsbS) was recently shown to be expressed transiently during the activation of qE and stabilize the accumulation of LHCSR, implying a pivotal role for PsbS in qE induction in *C. reinhardtii* [47[••]]. In land plants, PsbS plays a key role in the induction of qE by sensing ΔpH [48] and reorganizing LHCII around PSII, while LHCSR was lost during the evolution of land plants (reviewed in Ref. [49]).

Interestingly, LHCSR is conserved in *P. patens* and *S. fallax* but appears to be lost in the liverwort *M. polymorpha* (v3.1) and the lycophyte *Selaginella moellendorffii* (v1.0, DOE-JGI). In *P. patens*, LHCSR is independently functional in addition to PsbS to induce qE [8,50,51]. Unlike its orthologs in *C. reinhardtii* [45], the qE activity of PpLHCSR is largely reliant on the accumulation of zeaxanthin [52•]. The two PpLHCSR isoforms, LHCSR1 and LHCSR2, are more responsible for quenching under high light or low temperature, respectively [53]. Also, unlike CrPsbS [47], PpPsbS is expressed under normal conditions and involved in the induction of qE [8]. Intriguingly, transient expression measurements of NPQ in *Nicotiana benthamiana* show that PpPsbS is able to induce faster and greater quenching than AtPsbS and CrPsbS [54]. A similar approach revealed an exceptional feature of PpLHCSR1—it lacks Chl *b*, which is usually required by LHC proteins for folding [55]. Surprisingly, the localizations of PpLHCSR and PpPsbS appear to be exclusive of each other in *P. patens* thylakoid membranes, where the former is found mostly in grana margin and stroma lamellae, and the latter is localized only in grana [56••]. It has been suggested that PpLHCSR can associate with both PSI and PSII to activate qE under excess light conditions [56••], while PpPsbS interacts with the moderately-bound major LHCII in the PSII supercomplex [57•], as similarly suggested in *A. thaliana* [30]. The dual mechanisms of PpLHCSR and PpPsbS to induce qE clearly imply the requirement for conquering newly experienced light environments during plant terrestrialization.

Concluding remarks

After at least 450 million years [58], the mosses have flourished in almost all terrestrial environments. Being poikilohydric, the characteristics that mosses derived from green algal-lineage genes might have been effective for acclimating to semiaquatic conditions, while the whole-genome duplication and HGT might have led them to acquire the characteristics required for survival in terrestrial conditions. Here, we have discussed only the unique characteristics pertaining to the light-harvesting antenna system in *P. patens*. Together with knowledge about other physiological features and mechanisms, which have also been substantially investigated using this moss, we have an avenue to a more complete understanding of how the light-harvesting antenna system evolved strategically during plant terrestrialization. Further, comparative genomics and phylogenomics analyses using other organisms positioned evolutionarily between green algae and land plants, such as freshwater green algae (charophytes) and liverworts, in relation to the paleophysiological aspects of each species should provide fascinating insights into the evolutionary dynamics of light-harvesting antenna systems.

Acknowledgments

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• of special interest
•• of outstanding interest

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