

Characterization of photosystem I antenna proteins in the prasinophyte *Ostreococcus tauri*

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ABSTRACT

Prasinophyceae are a broad class of early-branching eukaryotic green algae. These picophytoplankton are found ubiquitously throughout the ocean and contribute considerably to global carbon-fixation. *Ostreococcus tauri*, as the first sequenced prasinophyte, is a model species for studying the functional evolution of light-harvesting systems in photosynthetic eukaryotes. In this study we isolated and characterized *O. tauri* pigment–protein complexes. Two photosystem I (PSI) fractions were obtained by sucrose density gradient centrifugation in addition to free light-harvesting complex (LHC) fraction and photosystem II (PSII) core fractions. The smaller PSI fraction contains the PSI core proteins, LHCI, which are conserved in all green plants, Lhcp1, a prasinophyte-specific LHC protein, and the minor, monomeric LHCII proteins CP26 and CP29. The larger PSI fraction contained the same antenna proteins as the smaller, with the addition of Lhca6 and Lhcp2, and a 30% larger absorption cross-section. When *O. tauri* was grown under high-light conditions, only the smaller PSI fraction was present. The two PSI preparations were also found to be devoid of the far-red chlorophyll fluorescence (715–730 nm), a signature of PSI in oxygenic phototrophs. These unique features of *O. tauri* PSI may reflect primitive light-harvesting systems in green plants and their adaptation to marine ecosystems. Possible implications for the evolution of the LHC-superfamily in photosynthetic eukaryotes are discussed.

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1. Introduction

Two classes of light-harvesting systems exist in eukaryotic phototrophs: the soluble phycobilisome (PBS) complex, a photosystem II (PSII) antenna inherited from cyanobacteria, and the integral-membrane light-harvesting complex (LHC) proteins, which are not found in cyanobacteria. Contemporary red algae maintain PBS complexes as a PSII antenna, but PBS have not been found in green plants, which diverged from red algae very early in algal evolution [1]. However, eukaryotic algae developed LHC antenna before the red/green evolutionary split [2–4], evidenced by genes encoding LHC proteins in recently sequenced primitive algae [5–7].

As one of the earliest branches in the green algal lineages, the Prasinophyceae offer us a potential evolutionary link to the earliest green plants, photosynthetic machinery [8]. The most abundant

antenna proteins in prasinophytes and mesostigmatophyceae, Lhcp, belong to a unique LHC lineage that shares a distant relationship to both PSI-specific (LHCI) and PSII-specific (LHCII) antenna [9–11]; however, a long evolutionary distance between Lhcp and other LHC-family proteins currently hampers the resolution of their relationship to the greater LHC-superfamily. Unlike the specific binding-affinity to PSI in LHCI and PSII in LHCII, Lhcp were suspected to associate with both photosystems [11,12]. *Ostreococcus tauri*, the first sequenced prasinophyte [5], encodes two classes of Lhcp (Lhcp1 and Lhcp2) along with six LHCI-family proteins, annotated as Lhca1–6 (LHCI) and two minor monomeric LHCII-family proteins, annotated as CP26 and CP29, with the notable exception of major, trimeric LHCII proteins [10]. While Lhca2 was localized with PSI-rich fractions, the association of the other LHC proteins is currently unclear [11].

Here we present a thorough biochemical characterization of photosynthetic pigment–protein complexes in *O. tauri* to clarify the light-harvesting systems for PSI in this species. We discretely separated pigment–protein complexes and performed polypeptide profiling, pigment profiling, and spectroscopic analyses, which reveals a unique PSI antenna system in *O. tauri*. We discuss the possible implications of these results on our current understanding of LHC evolution.

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2. Materials and methods

2.1. Cell culture preparation

Cultures of *O. tauri* were prepared on K-medium (Sigma) adjusted to pH 8.0. Cultures were grown under 50 and 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiation at 20 °C using fluorescent bulbs. The growth rate of both cultures was approximately equal during the tested period.

2.2. Preparation of pigment–protein complexes

O. tauri pigment–protein complexes were isolated using methods adapted from Takahashi et al. [13] with the following changes: cells were suspended in 25 mM HEPES (pH 7.5) buffer containing 0.33 M sucrose and disrupted using a BioNeb nebulizer (Glas-Col, Terre Haute, IN) at 15 kg/cm² force (of N₂). Concentrated *O. tauri* thylakoids were solubilized in β -dodecylmaltoside (β -DM) (Sigma) at an 8:1 (mass:mass) β -DM to (Chl) ratio for ~30 min at 4 °C. Step-gradients containing 0.2, 0.4, 0.7, 1.0, and 1.3 M sucrose in 25 mM HEPES (pH 7.5) and 0.05% β -DM were centrifuged for 24 h at 152,000 g at 4 °C in a P40ST rotor (Hitachi, Japan). *Chlamydomonas reinhardtii* and spinach thylakoids were isolated as described previously [13,14].

2.3. Separation of PSI core and LHCI

LHCI in PSI supercomplexes (A3 fractions) from sucrose density gradients were solubilized using Zwittergent 3–16 (Anatrace, Maumee, OH) as described previously [14]. *O. tauri* A3 samples were subsequently subjected to 1 h incubation and 3 liquid nitrogen freeze–thaw cycles. Following solubilization, samples were resolved on sucrose density gradients as described above.

2.4. SDS-PAGE, 2-dimensional electrophoresis, and immunoblotting

Pigment–protein complexes were analyzed by SDS-PAGE or 2-dimensional electrophoresis (2DE) as described previously [15] with samples normalized by Chl concentration. Polypeptide bands were stained using a silver stain MS kit (Wako, Osaka, Japan). Immunoblotting was performed as described previously [13]. Antibodies raised versus *O. tauri* CP26 and CP29 (1/5000 dilution, Agrisera, Vannas, Sweden) was used to detect CP26 in *O. tauri*. These antibodies were raised against a synthetic peptide corresponding to the *O. tauri* CP26 sequence, accession #AAY27549, and CP29 sequence, accession #AAY27543, respectively.

2.5. Mass spectrometry

In-gel trypsin digestion of polypeptides and subsequent mass spectral analysis (MS/MS) was performed as described previously [15]. Obtained MS/MS was analyzed with SEQUEST software (revision 3.3; Thermo Fisher Scientific, Waltham, MA) using a genomic protein database for *O. tauri* (<http://genome.jgi-psf.org/Ostta4/Ostta4.home.html>) with the following filters: number of top matches = 1, probability <0.001 and Sf-final score >0.85.

2.6. Gel filtration

Pigment–protein complexes from sucrose density gradient fractions were dialyzed in sucrose-free sample buffer containing 0.03% dodecyl- β -D-maltoside (β -DM). Samples containing ~1 mg Chl/ml were applied to a Superdex 200PC 3.2/30 column on a SMART system (GE Healthcare, Uppsala, Sweden). Chromatographic separation was performed in 25 mM HEPES buffer (pH 7.5) containing 0.03% β -DM at a flow rate of 25 $\mu\text{L/min}$ at 10 °C.

2.7. Spectral analysis

Absorbance measurements were conducted on a Hitachi U-3310 spectrophotometer (Tokyo, Japan). Low-temperature (77 K) fluorescence was measured on a Hitachi F-2500 fluorescence spectrophotometer (Tokyo, Japan).

2.8. Estimation of photosystem I antenna size

Light-induced P700 kinetics were measured as described previously [16,17] using a multifunctional kinetic spectrophotometer built in-house as described previously [18,19]. Purified photosystems (55 $\mu\text{g/mL}$ of Chl) were suspended in 25 mM HEPES buffer (pH 7.5) containing 5 mM sodium ascorbate and 200 nM methyl viologen. Actinic illumination by red LED (634 nm) was tested at 6.5, 26, 72, 184, and 432 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

2.9. High pressure liquid chromatography

HPLC analyses were performed using a 4.6 mm $\times$ 250 mm Zorbax Eclipse XDB-C18 column (Agilent, Santa Clara, CA). Gradients were run from 62% acetone (vs. water) to 75% acetone at 15 min, 90% at 25 min, 95% at 30 min, 100% at 32 min to 37 min before returning to 62% until 45 min. Pigment concentrations were calculated using commercial pigment standards (DHI, Hoersholm, Denmark).

3. Results

3.1. Separation and identification of pigment–protein complexes

Fig. 1A shows *O. tauri* pigment–protein complexes separated by sucrose density gradient centrifugation yielding five bands (from lightest to heaviest): A0, A1, A2, A3, and A3/L. Polypeptides in all pigment–protein complexes were visualized by SDS-PAGE (Fig. 1B) and those in A3/L fractions were further separated by 2DE (Fig. 2). MS/MS identification of both SDS-PAGE bands (Fig. 1B) and 2DE spots (Fig. 2) are shown in Supplementary Table 1. SDS-PAGE reveals that A0, A1, and A2 bands are primarily formed by free pigments, free LHC proteins, and PSII core proteins, respectively (Fig. 1B). Both A3 and A3/L fractions contained PSI subunits, Lhca1–5 (spots 9, 6, 5, 8, and 7, respectively, in Fig. 2), CP26, CP29, and Lhcp1, but the most abundant LHC protein in *O. tauri* Lhcp2 (Spot 1) and an unnamed sixth Lhca-type protein (accession number CAL53128, hereafter called Lhca6, Spot 4) were only detected in the green–brown A3/L fraction (Figs. 1B and 2). The newly identified Lhca6 was homologous to Lhca2 in *Mesostigma viride* and *C. reinhardtii*, as well as Lhca6 from the other sequenced *Ostreococcus* species *O. lucimarinus* [20]. Trace amounts of CP29 as well as Lhcp2 detected in the A2 fraction were most likely due to tailing from the previous fractions, which contained the highest concentrations of these bands.

3.2. Light-harvesting systems for PSI

To assess the stability of Lhcp binding in the PSI-antenna supercomplex, *O. tauri* thylakoids were subjected to sucrose density gradient centrifugation in the presence of 250 mM NaCl, which yielded only fractions A0–A3 (Supplementary Fig. 1A). SDS-PAGE analysis shows that A3 in NaCl-containing gradients is identical to the original A3 (data not shown), indicating that the association of Lhcp2 and Lhca6 (but not Lhca1–5, CP26, CP29, and Lhcp1) with PSI were susceptible to ionic disruption. A similar result is observed in standard gradients containing thylakoids from high-light grown (HL) *O. tauri* cultures (500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Fig. 1A); in contrast to cultures grown under normal growth-light (50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), where the Chl content of the two PSI fractions is approximately equal, the A3/L band is barely detectable in HL cultures. This result suggests two possibilities: 1) the

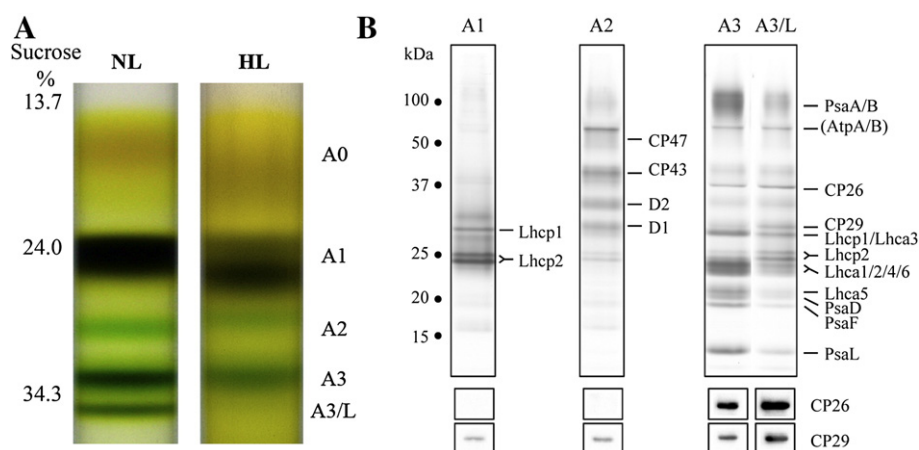


Fig. 1. Composition of *Ostreococcus tauri* thylakoids. A) Sucrose density gradient centrifugation results from cultures grown at 50 (NL) and 500 (HL) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ irradiance. B) SDS-PAGE separation of proteins from sucrose density gradient fractions. Labeled proteins were identified by MS/MS (Supplementary Table 1). In the lower panel are the results of an immunoblot versus CP26/CP29 antibodies. All proteins were identified from MS/MS of trypsin-digested fragments as identified in Supplementary Table 1.

proportion of A3/L supercomplexes is decreased under HL or 2) the stability of A3/L supercomplexes is weaker under HL. Both possibilities imply that *O. tauri* can modify its antenna system to accommodate different light conditions. A similar mode of light-acclimation in diatoms suggests that this technique may be an important marine survival mechanism [21].

To analyze the organization of PSI-specific LHCI proteins, A3 supercomplexes from *O. tauri* as well as *C. reinhardtii* and spinach were solubilized with zwitterionic detergent, which disrupts the tight association between PSI core and LHCI (Supplementary Fig. 1B) [14,22], and loaded onto sucrose density gradients. In *C. reinhardtii*, five bands were formed, containing (from lightest to heaviest): free pigments, LHCI-680, LHCI-705, PSI core complex, and PSI-LHCI supercomplex (Supplementary Fig. 1B) as in ref. [22], while in spinach, the two LHCI complex bands are replaced by a single, diffuse LHCI band (Supplementary Fig. 1B) as in ref. [14]. Under the same conditions [14], however, *O. tauri* A3 was unchanged (data not shown). Harsher solubilization using 1 h incubation and 3 freeze-thaw cycles ultimately disassembled A3 supercomplex, where three bands contained (from lightest to heaviest): LHCI monomers, PSI core complex, and residual A3 supercomplex (Supplementary Fig. 1B). Our experimental conditions did not yield dimeric LHCI antenna, which are present in higher plants [14].

3.3. Protein complex mass estimation

The sizes of A1 (primarily Lhcp2), A2 (PSII core complex), A3 (PSI-Lhca1/5-Lhcp1-CP26/CP29 supercomplex), A3/L (PSI-Lhca1/6-Lhcp1/2-CP26/CP29 supercomplex), PSI core complex, and free LHCI proteins were estimated using gel filtration (Fig. 3). LHCI samples from zwitterionic solubilization migrated at 60.3 min (50–

60 kDa) (Fig. 3B), consistent with monomeric Lhca polypeptides. Lhcp2 proteins in A1, on the other hand, elute at 46.4 min (275–300 kDa). This is nearly double the size of major LHCI trimers from *C. reinhardtii* (Fig. 3B), which measures approximately 158 kDa, indicating a hexameric (or higher) organization.

PSI core from zwitterionic solubilization migrated at 44.51 min (~ 340 kDa) (Fig. 3A), versus the A3 fraction at 40.3 min (~ 540 kDa). The estimated MW of A3 is comparable to the 600 kDa pea PSI-LHCI supercomplex [23]. Protein complexes in the A3 fraction are approximately 200 kDa larger than the PSI core. This 200 kDa difference accommodates the inclusion of Lhca1–5, CP26/CP29, and

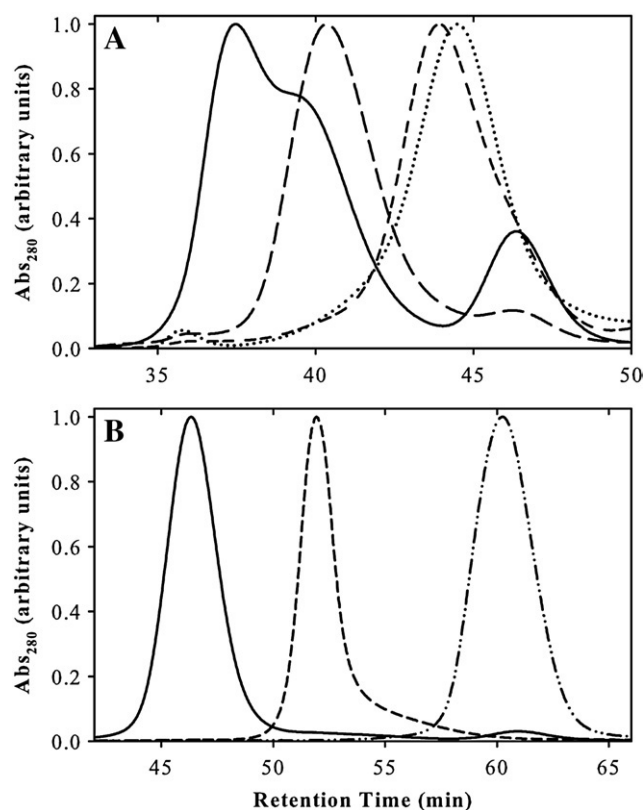


Fig. 3. Gel filtration profiles of *O. tauri* pigment-protein complexes. Fractions isolated from sucrose density gradients were separated via gel filtration. A) Photosystem curves: solid – A3/L, long dash – A3, dotted – A2, dash/dash – PSI core. B) Antenna curves: solid – A1, short dash – *C. reinhardtii* trimeric LHCI, dash/dot-dot – LHCI. Curves represent absorbance at 280 nm.

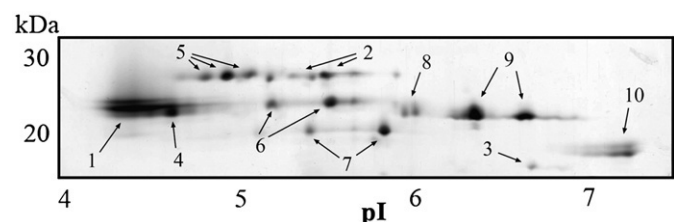


Fig. 2. Two-dimensional separation of proteins in A3/L. Shown is the silver-stained region between 18–40 kDa and pH 4–7 containing all antenna proteins. Labeled spots represent the following peptides: 1 – Lhcp2; 2 – Lhcp1; 3 – CP29; 4 – Lhca6; 5 – Lhca3; 6 – Lhca2; 7 – Lhca5; 8 – Lhca4; 9 – Lhca1; 10 – PsaF. All proteins were identified from MS/MS of trypsin-digested fragments as identified in Supplementary Table 1.

Lhcp1, which were previously found to associate with A3 (Figs. 1B and 2), though their stoichiometry is not yet known.

The dominant component of A3/L is the PSI-Lhca[1–6]–Lhcp1/2–CP26/CP29 supercomplex, migrating at 37.5 min (~750 kDa), followed by an A3-like shoulder at ~40 min (Fig. 3A). The >200 kDa difference between the A3 and A3/L supercomplexes suggests the addition of multiple copies of Lhcp2 proteins as well as Lhca6. The presence of multiple peaks indicates that A3/L fraction is heterogeneous. The secondary A3/L peak at 46.4 min was identical to the A1 peak, suggesting these extra Lhca6 and Lhcp2 could form a single unit of ~275 kDa.

3.4. Spectral analysis of pigment–protein complexes

77 K fluorescence emission from isolated PSI core shows a peak at 683 nm, with a significantly smaller yield than the other complexes, but no peak in the far-red region (between 710 and 735 nm) beyond the vibrational band of the 680 nm Q_y transition (Fig. 4, Table 1, and Supplementary Fig. 2). The lack of PSI-associated red-Chl has thus far been observed only in the cyanobacterium *Gloeobacter violaceus* [24] and some diatoms [25], but never been reported in any green plant. While the function of red-Chl in PSI core and its antenna is not yet fully understood, they are thought to help concentrate trapped energy in the PSI core or help mitigate excess energy in LHCI [26]. The lack of red-Chl in *O. tauri* suggests red-Chl is not essential in its marine habitat.

Absorbance spectra of A3 contain a higher level of carotenoid/Chl *b* peaks between 450 and 500 nm compared with those of PSI core (Fig. 4 and Table 1). These peaks are even more pronounced in A3/L (Fig. 4 and Table 1). This suggests that the relative proportion of LHC-type proteins increases progressively from PSI core to A3 to A3/L.

LHCI polypeptides dissociated from PSI absorb significantly in the ~475 nm Chl *b*/peraxanthin (Px) region (Fig. 4 and Table 1). However, 77 K 470 nm excitation of LHCI yields emission primarily in the Chl *b* range at 658 nm, indicating that LHCI polypeptides dissociated from PSI are unstable, causing the bulk of the Chl *b*/Px pool in LHCI to become energetically unlinked from Chl *a* [27,28].

Table 1
Spectral data from results shown in Fig. 4.

	Absorbance	Excitation peaks	Emission peaks
A1	436, 466, 645, 672	437, 469	681
LHCI	438, 466, 669	416, 440 (439, 469)	(658, 679) 680
A2	419, 436, 673	418, 436, 467	689
PSI Core	419, 439, 677	415, 438	683
A3	437, 467, 677	418, 437, 467	682
A3/L	437, 467, 677	438, 467	682

3.5. Estimation of PSI antenna size

The functional antenna size of both PSI-antenna supercomplexes (A3 and A3/L) was estimated using the oxidation rate of the primary PSI electron donor, P700 (Fig. 5 and Supplementary Fig. 3). Maximum P700 oxidation was ~30% lower in A3/L as compared to A3 (Supplementary Fig. 3A and B) due to the relatively lower number of reaction centers per Chl present in the antenna-rich A3/L sample. The initial slope of ΔA_{700} was the same in both samples (Supplementary Fig. 4), indicating that the efficiency of excitation transfer from the antenna was the same in A3 and A3/L. The initial rate of P700 oxidation is more than 30% greater in A3/L than in A3 (Fig. 5). This suggests that the antenna system in A3/L (discussed above) increases its absorption cross-section by at least 30% over A3.

3.6. Pigment analysis by HPLC

The Chl *b* to Chl *a* ratio of A1, where Lhcp2 is predominant, was found to be 0.736 in the present study (Supplementary Table 2), which is lower than previously reported values (1.0 in [29] and 1.3 in [11]). Normalization using two Px, which is proposed to fill the “cross-brace” position of lutein in higher plants [30], yields Lhcp2 polypeptides binding 2 Px, 1 dihydrolutein, 5 Chl *b* and 6 Chl *a* in addition to 1–2 Mg-2,4-divinyl-phaeoporphyrin a_5 monomethyl ester previously found to bind to Lhcp [30]. This is in good agreement with estimations from *M. squamata*, which bind 6 Chl *b* and 6 Chl *a* and 2 Px [29], as well

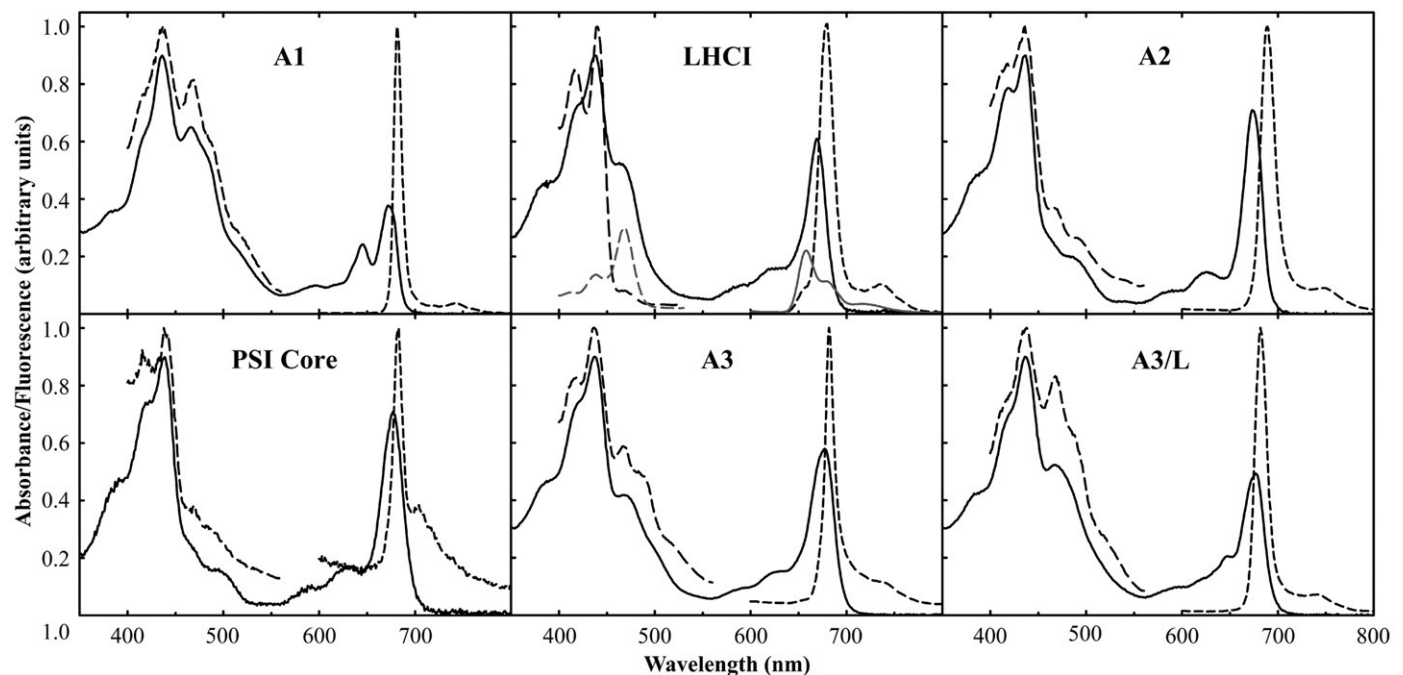


Fig. 4. Spectroscopic properties of *O. tauri* photosystems. Solid black lines represent absorbance spectra of fractions isolated from sucrose density gradients. 77 K fluorescence emission spectra (short dashes) were monitored at 440 nm excitation. 77 K fluorescence excitation spectra (long dashes) were monitored at 681 nm emission. LHCI 77 K fluorescence emission and excitation spectra, monitored at 470 nm (solid gray) and 655 nm (dashed gray) respectively, are shown to scale with the other fluorescent signals.

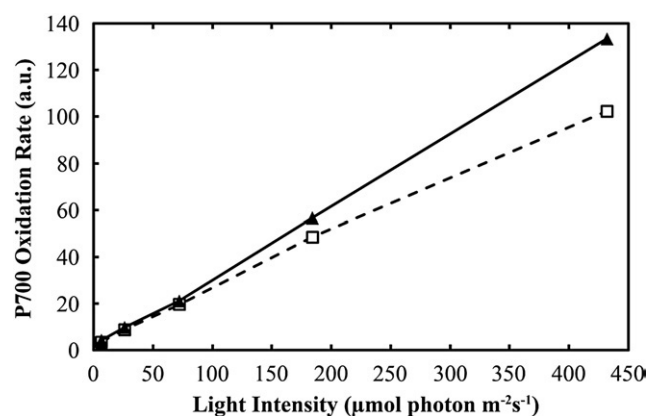


Fig. 5. Photosystem I functional antenna size. The rate of P700 oxidation was measured under increasing illumination (6.5, 26, 72, 184, 432 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in purified A3 (dashed line) and A3/L (solid line). The slope of A3/L was approximately 31% higher than that for A3.

as plant major trimeric LHCII, at 6 Chl *b* and 8 Chl *a* [31], suggesting Lhcp2 takes the role of major LHCII-type antenna in *O. tauri*.

LHCI obtained from zwitterionic detergent solubilization, which include 6 different Lhca polypeptides (Fig. 2), bind nearly half as much Px per Chl *a* (0.195) compared to Lhcp2, suggesting that only one Px is bound per Lhca polypeptide along with a single violaxanthin (Vx), which is present in a similar ratio to Chl *a* (0.139). Based on this assumption, the average Lhca binds 1 Px, 1 Vx, ~ 2 Chl *b*, and 6–7 Chl *a*. This Chl ratio and total pigment count agree with similarly isolated Lhca in plants [32].

The high concentration of Px and Chl *b* in both A3 and A3/L indicates that complexes in these fractions bind Lhcp antenna proteins (Supplementary Table 2), which is in agreement with the spectroscopic studies (Fig. 4). PSI core, on the other hand, contains nearly ten times less Px (vs. Chl *a*) and nearly four times less Chl *b* (vs. Chl *a*) than A3. The differences between Px/Chl *a* and Chl *b/a* ratios calculated between A3/L and A3 (0.275 and 0.702, respectively) are similar to Px/Chl *a* ratios calculated for Lhcp2 in A1 (0.325 and 0.736) in Supplementary Table 2. This data confirms SDS-PAGE results showing that the addition of Lhcp2 is the primary difference between A3 and A3/L.

4. Discussion

The unique organization of light-harvesting systems in *O. tauri* improves our understanding of the evolutionary differences between light-harvesting systems in green plants and their cyanobacterial ancestors. *O. tauri* binds all of its LHC-type antenna proteins, including prasinophyte-specific Lhcp proteins, LHCI, and minor LHCII, to PSI, while the antenna for PSI in Streptophyta is limited to LHCI. In the chlorophyte *C. reinhardtii*, on the other hand, PSI conditionally binds minor LHCII in addition to LHCI [13]. It is thus tempting to speculate that the prasinophytic light-harvesting systems reflect traits of ancestral green plants. Similarities between the strong PSI-LHC association found in *O. tauri* and that in red algae and diatoms [3,33–35] suggest that the ancestor to all modern algal lineages contained PSI-specific LHC antenna proteins, as PSII-absorbance was supplemented by PBS (Fig. 6). As green algal and land plant LHC-networks evolved to be more photosystem-specific, their PSI antenna system was probably reduced in favor of PSII absorption.

Based on the presented results, an evolutionary model of LHC affinity in photosynthetic eukaryotes is proposed (Fig. 6). Soon after the envelopment of a PBS-containing cyanobacterium, algae developed 3-helix LHC-type antenna to supplement PSI absorption (either before or after the transition from the cyanobacterial PSI trimer to monomeric plant PSI). In PBS-containing red algae these 3-helix LHC

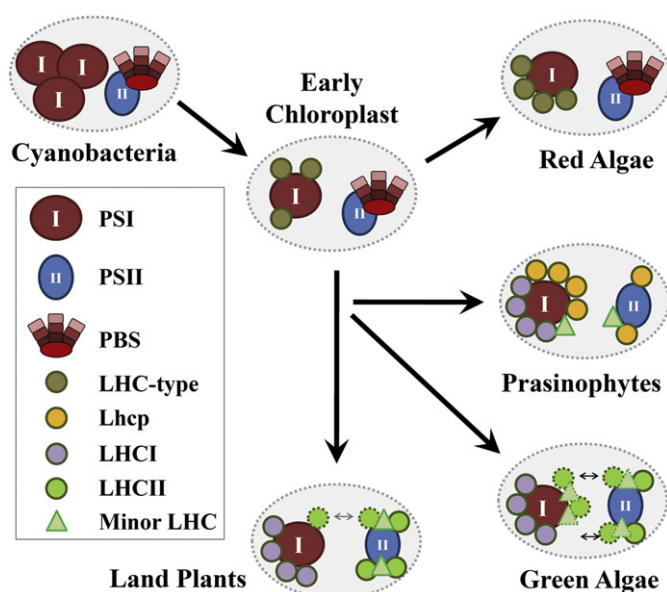


Fig. 6. Evolutionary model of LHC affinity in photosynthetic eukaryotes. Photosynthetic pigment–protein complexes within the thylakoid (large dotted oval) are separated by vast evolutionary space (approximated by black arrows). Accessory antenna proteins (small circles, triangles, and the large PBS) preferentially associate with either photosystem (blue and red ovals). Antenna known to selectively migrate between photosystems are shown with dotted outlines and corresponding arrows. The locations of antenna along a reaction center do not indicate structural orientation.

remained primarily associated with PSI. In prasinophytes, PBS were lost and the specialized PSI-specific LHCI emerged as well as Lhcp, which also associates with PSI. In green algae, LHCII antenna emerged to associate primarily with PSII, but migrate between both photosystems during state transitions. In land plants the roles of LHCI and LHCII became more specialized, with less transfer of LHCII between photosystems.

Six distinct LHCI polypeptides were found to form strong bonds with PSI in the A3/L supercomplex (Fig. 2). Like the PSI antenna in red algae [34], and LHCI in *C. reinhardtii* [22,36], *O. tauri* LHCI does not bind far-red Chl (~ 730 nm) (Fig. 4). This suggests that the evolution toward strong red-Chl fluorescence (>730 nm) may be limited to the streptophyte lineage. Although mutational analysis revealed that a His to Asn conversion in the Chl-binding site *a5* was vital for LHCI red-Chl formation in *Arabidopsis* [37] and *C. reinhardtii* [38], Lhca5 and Lhca6 in *O. tauri* also carry an Asn at *a5*. Thus *a5* does not appear to function as a red-Chl site at least in *O. tauri*. The characterization of LHCI in other primitive algae, especially the earliest branching streptophyte *Mesostigma viride* (in which all but Lhca3 have Asn at *a5* [10]), could help pinpoint the origin of red-Chl in LHCI.

The association between PSI and CP26/CP29 in prasinophytes challenges our current understanding of the role of these minor LHCII proteins. Minor LHCII, which link major LHCII trimers to PSII core in green plants [39] are implicated as mobile antenna between the two photosystems during state transitions in *C. reinhardtii* [15,40,41]. In *O. tauri* CP26 and CP29 are not necessarily mobile, rather they stably associate with both photosystems, which is reflected in a lack of detectable state transitions in this species (Swingley et al. unpublished data). Consequently, state transitions are strongest in Chlorophyceae, where CP26 and CP29 can be selectively bound/unbound to PSI or PSII.

Lhcp2, as the most abundant antenna proteins in *O. tauri*, appear to serve the same functional role as major, trimeric LHCII in other green plants. Intriguingly, while Lhcp2 proteins in *O. tauri* lack the entire trimer motif identified to be crucial in LHCII trimer formation [42], they appear to form multimeric complexes of up to 6 Lhcp proteins (Fig. 3B).

The formation of multimeric LHC complexes in both prasinophytes and diatoms [43] is likely due to the independent development of dynamic light-acclimation systems to cope with their common marine environment. Like diatoms [21], high-light grown *O. tauri* only produce the small antenna PSI supercomplex (A3) without significantly reducing the total pool of LHCs (Fig. 1A). Eliminating the need to constantly adapt LHC-gene expression to rapidly-shifting light conditions likely provides a competitive advantage to these two ubiquitous classes of marine organisms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbabi.2010.04.017.

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