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LHCII is an antenna of both photosystems after long-term acclimation

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ABSTRACT

LHCII, the most abundant membrane protein on earth, is the major light-harvesting complex of plants. It is generally accepted that LHCII is associated with Photosystem II and only as a short-term response to overexcitation of PSII a subset moves to Photosystem I, triggered by its phosphorylation (state1 to state2 transition). However, here we show that in most natural light conditions LHCII serves as an antenna of both Photosystem I and Photosystem II and it is quantitatively demonstrated that this is required to achieve excitation balance between the two photosystems. This allows for acclimation to different light intensities simply by regulating the expression of LHCII genes only. It is demonstrated that indeed the amount of LHCII that is bound to both photosystems decreases when growth light intensity increases and vice versa. Finally, time-resolved fluorescence measurements on the photosynthetic thylakoid membranes show that LHCII is even a more efficient light harvester when associated with Photosystem I than with Photosystem II.

1. Introduction

Photosynthetic organisms harvest light via two large pigment-protein assemblies called photosystems I (PSI) and II (PSII) that work in series to transform light energy into chemical energy. For optimal performance the excitation pressure on the two photosystems needs to be balanced. However, the absorption spectra of PSI and PSII are different and as a result, variations in light quality drive both photosystems to a different extent, leading to imbalances. As a short term acclimation response, a process called state transitions redistributes the amount of excitations between PSI and PSII. When PSI is preferably excited (in far-red light), plants go to State 1 (St1), where all major antenna complexes (LHCIIs) are associated with PSII. When excitation pressure changes in favor of PSII, a mobile pool of LHCII moves and reversibly associates with PSI (St2) [1–7]. If the imbalance persists for longer times, plants adjust their PS stoichiometry [8–10].

In thylakoids of higher plants PSI and PSII are laterally segregated. PSII is mainly found in the appressed grana membranes, while PSI is restricted to the non-appressed grana end-membranes and margins and to the stroma lamellae [11]. The location of mobile LHCII is regulated by phosphorylation [12–16]. The LHCII kinase is under control of the interphotosystem electron carrier, plastoquinone [17]. When the plastoquinone pool is reduced, the kinase is activated and phosphorylated LHCII moves from PSII to PSI [17]. When the plastoquinone pool is oxidized, the LHCII kinase is inactivated, mobile LHCII is dephosporylated and moves back to PSII [18]. This process occurs in low light, while in high-light conditions the LHCII kinase is inactivated by the stromal electron carrier thioredoxin even if the plastoquinone pool is reduced [19,20].

PSII is composed of a core complex where the primary photochemistry takes place, and a peripheral antenna system, encoded by the *Lhcb1–6* genes [21]. The major antenna of PSII, LHCII, is a trimeric light-harvesting complex (Lhc) composed of a combination of the Lhcb1–3 gene products. The minor Lhcbs consists of three monomers, Lhcb4–6, also named CP29, CP26 and CP24. All Lhcbs coordinate chlorophylls (Chls) *a* and *b*, and several xanthophylls [22]. Lhcbs associate with dimeric PSII cores to form PSII supercomplexes. The largest PSII supercomplex observed in *Arabidopsis thaliana* contains one copy of each minor complex and two LHCII trimers per core (C), which are indicated as S (strongly bound) and M (moderately bound) forming the $C_2S_2M_2$ supercomplex. Depending on light growth conditions up to two more LHCII trimers per PSII core can be present in the thylakoids [23], but their interaction with the PSII supercomplex is rather weak, as the association does not survive even very mild solubilization [24].

At variance with PSII, it has been shown that the antenna size of PSI is not dependent on light growth conditions [25]. PSI is composed of a core and four Lhca antennas located at one side of the core [26,27]. In St2 the LHCII trimer binds on the opposite side interacting with the PsaA, -H, -K, -L subunits [4,28,29]. It remains to be determined how

Abbreviations: Chl, chlorophyll; DAS, decay associated spectra; EE, excitation energy; HL, high light; FR, far-red light; Lhc, Light-harvesting complex; LL, low light; LHCII, major light-harvesting complex of PSII; ML, moderate light; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; PSI, photosystem I; PSII, photosystem II; RC, reaction center; St1, State 1; St2, State 2

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fast LHCII is in providing excitation energy (EE) to PSI or PSII when associated to either of the photosystems in the thylakoid membrane. The EE transfer and primary photochemistry have to compete with the intrinsic excited state lifetime of the Lhcs. Therefore, the faster the EE transfer the higher the efficiency of the PS will be.

In this work we study the structural and functional organization of PSI and PSII in response to acclimation to different light intensities. The results lead to a new view on light acclimation of the photosynthetic apparatus in higher plants, challenging several existing dogmas.

2. Materials and methods

2.1. Plant material

A.thaliana (Col) WT plants were grown at 100 μE/m^2/s, 70% humidity, 22 °C, and 8 h of daylight (Plant Climatics Percival Growth Chamber, Model AR-36L, Germany). After four weeks, moderate light plants (ML) were grown an additional 2.5 weeks at this light intensity, while low light (LL) and high light (HL) plants were transferred to 20 μE/m^2/s and 800 μE/m^2/s (for LL and HL Growth Chamber Models were AR-36L and SE 1100), respectively, for an additional 4 weeks. Sunlight plants were grown in the green house under fluctuating natural light for six weeks (Supplementary Fig. S1A, in April and May, The Netherlands), or under controlled conditions (100 μE/m^2/s, 70% humidity, and 14 h of daylight) for four weeks and in the greenhouse for an additional 12 days (Supplementary Fig. S1B, in June, The Netherlands). Only leaves fully exposed to the light were used for measurements.

2.2. Plant treatment and thylakoid isolation

Leaves were harvested and directly transferred to ice/water after the plants were treated with different light conditions: 6–7 h of daylight at the light intensity to which the plants were acclimated (LL, ML, HL), overnight dark adaptation, 50 min of 20 μE/m^2/s 450 nm LED light (low light) + far red light (FR) in Fytoscope FS130 (Photon Systems Instruments, Brno, Czech Republic), or 50 min of 20 μE/m^2/s white light after overnight dark adaptation. Thylakoid membranes were prepared according to [24] with the addition of 10 mM of NaF to all buffers, to inhibit phosphatase activity. The membrane was resuspended in 20 mM Hepes pH 7.5, 0.4 M sorbitol, 15 mM NaCl, 5 mM $MgCl_2$ and 10 mM NaF, quickly frozen in N_2 (l) and stored at 193 K until use.

2.3. Pigment composition, absorption and fluorescence spectroscopy

Absorption spectra were recorded on a Cary 4000 UV–vis spectrophotometer (Varian, Palo Alto, CA). For pigment analysis the absorption spectra of the 80% acetone extract with the spectra of the individual pigments was used, as described before [30]. The electrochromic carotenoid band shift of intact leaves, after a saturating light pulse, was measured with the JTS-10 (BioLogic, France) and used to estimate the PSI/PSII ratio (See also M&M in SI). Fluorescence spectra were recorded on a Fluorolog 3.22 spectrofluorimeter (HORIBA Jobin Yvon, Longjumeau,France). 77 K emission spectra were measured in 66% w/v glycerol, 10 mM Hepes pH 7.5, 5 mM $MgCl_2$, 15 mM NaCl and 10 mM NaF. Time correlated single photon counting (TCSPC) was performed at 283 K for PSI and PSI-LHCII, and at ambient temperature for thylakoids with a homebuilt setup, as described previously [31]. Excitation was performed with a light pulse at 475 nm and a repetition rate of 3.8 MHz. Other experimental settings were as in [32] for PSI and PSI-LHCII and as in [33] for thylakoids. The steady-state fluorescence emission spectra were used to calibrate the decay associated spectra (DAS). The average lifetime was calculated as $<\tau> = \Sigma A_i{}^*\tau_i/\Sigma A_i$, with A_i the amplitude (thylakoids) or the area under the DAS (PSI and PSI-LHCII) and τ_i the lifetime of the i^{th} component.

2.4. Polyacrylamide gel electrophoresis (PAGE)

Denaturing PAGE was performed with the Tris–Tricine system [34] at a 14.5% acrylamide concentration. Clear native PAGE was performed as in [35], with the 25BTH20G buffer and an acrylamide/bisacrylamide ratio of 32:1 in both stacking (3.5%) and resolving (4 to 14%) gel. The final Chl concentration was 0.5 mg/ml and the final detergent concentration was 1% digitonine/0.1% α-DDM, as in [36]. The cathode buffer was supplemented with 0.02% α-DDM and 0.05% sodium deoxycholate. Pro-Q Diamond Phosphoprotein Gel Stain (Molecular Probes) was used as described in the user manual, except that the samples were not desalted and delipidated. Immunoblot analysis was performed as in [37], with CP43 AS11 1787, Lhcb1 LHCII Type 1 AS09-522, Lhcb2 AS01-003 and Lhcb3 AS01-002 antibodies from Agrisera (Sweden).

2.5. LHCII per PSII in thylakoid membranes

The number of LHCII complexes per PSII core was evaluated by SDS-PAGE (14.5% Tris–Tricine; [34] and colorimetric detection. After Coomassie Brilliant Blue staining (fix: 50% methanol, 10% acetic acid; stain: 10% acetic acid, 0.025% Coomassie; destain: 10% acetic acid), gels were digitized with a Fujifilm LAS 300 scanner, and the optical density integrated on the area of the band was quantified using the GEL-PRO Analyzer (Media Cybernetics). The number of Lhcb1,2 per PSII core was quantified as in [38], while the level of Lhcb3 per core was assumed to be 1, based on the presence of one Lhcb3 per trimer M [39,40].

2.6. Electrochromic shift (ECS)

Kinetic measurements were performed using the JTS-10 Joliot type spectrometer on intact dark adapted leaves [41] (BioLogic, France). Saturating flash was provided by a xenon flashlamp (3 μs full width halve maximum), measuring flashes were provided with a white LED source passed by an interference filter (520 nm/546 nm). PSI and PSII charge separation capacity was calculated from changes in the amplitude of the fast phase of the ECS signal at 520 nm (corrected for signal at 546 nm) upon excitation with a saturating flash, before and after infiltration of the leaves with PSII inhibitors (200 μM DCMU and 1 mM hydroxylamine).

2.7. Purification of PSI-LHCII complex

For solubilization, 150 μg Chls of St2 thylakoid membranes were solubilized by adding an equal volume of a solution containing 1% digitonine-0.2% α-DDM to have a final Chl concentration of 0.5 mg/ml, vortexing for a few seconds and incubating 20 min on ice. The solubilized samples were fractionated by ultracentrifugation on a sucrose gradient containing (obtained by freezing and thawing a solution of 0.6 M sucrose, 0.02% digitonin and 10 mM Hepes, pH 7.5) in a SW41 rotor, for 17 h at 41000 rpm and 4 °C, after removing the sucrose the samples were loaded on a second gradient to further purify the complex. PSI was obtained in the same way from St1 membranes. See [36] for further details.

3. Results

3.1. Is LHCII associated with PSI after long term acclimation?

LHCII is commonly defined as the major antenna complex of PSII. Only in response to "PSII light" (peaking around 480 nm or 650 nm) or low light, a fraction (around 15% in land plants) is thought to move to PSI [42]. However, even though the kinase is supposed to be inhibited in high light [19,20], the high-light acclimated plants of Tikkanen et al. showed several St2 characteristics [43]. Here we investigated if LHCII is associated with PSI after long term acclimation to

different light intensities: low light (LL, 20 μE/m^2/s), moderate light (ML, 100 μE/m^2/s) or high light (HL, 800 μE/m^2/s). Plants were harvested either after several hours of white daylight (in the following indicated as "growth light" plants) or after 50 min exposure to low light + far red (FR) light. The phosphorylation state of the thylakoid proteins was evaluated with Diamond Pro-Q Phosphostain and the presence of PSI-LHCII supercomplexes with clear native PAGE. Note that it is essential to use digitonin to solubilise the thylakoid membranes, because the PSI-LHCII supercomplexes dissociate in all other common detergents [4,28,35,36]. Fig. 1 shows that growth light, compared to FR light, led to: increased level of PSI-LHCII supercomplexes, increased Lhcb1,2 phosphorylation and enhanced 735 nm (PSI 77 K fluorescence) emission.

The identity of the PSI and PSI-LHCII band in the native gel was confirmed by a second dimension (2D) denaturing gel (Fig. S1). To study the protein composition of the PSI and PSI-LHCII complexes in more detail, the bands were excised and eluted from the native gel and analyzed by denaturating PAGE and western blotting (Fig. 2). Lhcb1 and 2 but not Lhcb3 were detected in the PSI-LHCII fraction. CP43 was also not detected in the PSI-LHCII fraction, thus excluding the possibility of PSII contamination. The absence of Lhcb3 in the PSI-LHCII supercomplex is in agreement with the results of [44] showing that this polypeptide is not present in St2 stroma membranes, and with the recent results of [36] which show the absence of Lhcb3 in PSI-LHCII supercomplexes purified by sucrose density ultra-centrifugation. Furthermore, Pro-Q stain revealed that the LHCII polypeptides associated with PSI are phosphorylated, in agreement with the model that LHCII moves to PSI when phosphorylated [13,15,16,42]. The fraction of PSI complexes containing LHCII was estimated from the green native gel, taking into account that PSI-LHCII has a 28% larger absorption cross section compared to PSI (see Fig. S2A). It was found that a large fraction of PSI is present as PSI-LHCII in growth light (65% LL; 54% ML; 40% for HL). It can be concluded that in acclimated plants LHCII serves as antenna for PSI under all light intensities. This state resembles St2, however we avoid this term because St2 is usually defined as a short term acclimation state induced by "PSII light", while our experiments show that plants are also in this state after long term acclimation to white light.

To verify if the results obtained under controlled growing chamber conditions are also relevant for natural conditions we studied plants grown in sunlight, with fluctuating light intensity. Thylakoids were isolated from plants harvested at midday directly or after 50 min of LL + FR treatment. Also in the sun plants LHCII was associated with PSI, although the level was with 32% somewhat lower than found in controlled light conditions (Fig. 1).

To get a complete picture we also checked the state of our plants upon short term acclimation to different light intensities. In agreement with previous results, St2 characteristics are observed in ML and HL plants after short term low light treatment (Fig. S3A) [13,15,45–47], while 30 min of high light (non-photochemical quenching (NPQ) condition) led to the dissociation of LHCII from PSI (Fig. S3B).

It should thus be concluded that in contrast to what it is generally believed under a wide range of light intensities, including sunlight, LHCII is associated with PSI, while in shade-light (FR) or sudden high-light LHCII moves to PSII.

3.2. Excitation pressure on PSI and PSII

Recently, we have shown that the PS excitation balance can be estimated based on the absorption spectra of PSI, PSII and Lhcs, taking into account PS composition and stoichiometry [10] and we applied this method here to characterize the ML grown plants. The PSI/PSII ratio was estimated from the electrochromic carotenoid shift after a single turnover saturating flash [48], and the number of LHCII trimers per PSII core was evaluated with SDS-PAGE. A PSI/PSII ratio of 0.75 and 2.4 LHCII trimers per PSII core were obtained. Based on the pigment composition of the individual thylakoid complexes, a Chl *a*/*b* ratio of 3.33 [10,22] is expected, in excellent agreement with the measured value (Fig. 1). These numbers were used to calculate the relative absorption spectra of PSI and PSII in St1, thus considering that all LHCII complexes are associated with PSII (Fig. S2B). The

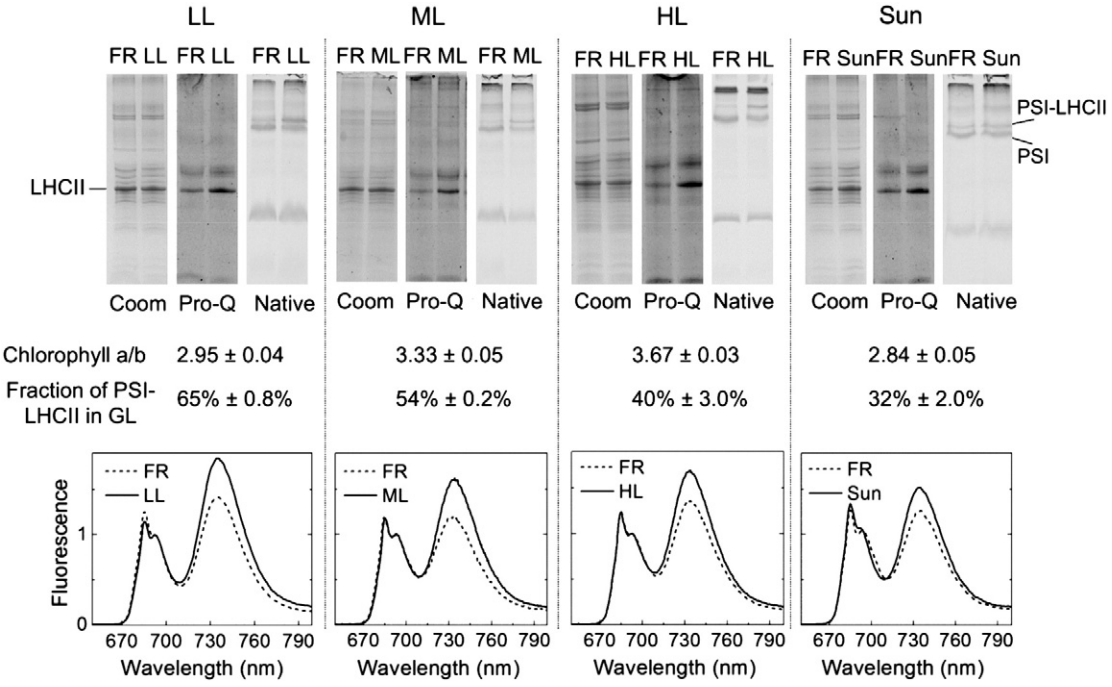

Fig. 1. Analysis of the transition state of plants grown in different light intensity. Thylakoid membranes were isolated after 50 min of low light + far red light (FR) or 6–7 h of growth light intensity from LL, ML, HL and sunlight acclimated plants. Top: Coomassie blue (Coom) and Diamond Pro-Q Phospho (Pro-Q) stained SDS-PAGE gel and clear native (native) gels are shown. Middle: Chlorophyll a/b ratio of the thylakoids, and fraction of PSI with LHCII associated in the growth light (data are presented as mean ± SD, n ≥ 3). Bottom: 77 K fluorescence emission spectra of thylakoids, after excitation at 484 nm, normalized at the maximum in the 690–700 nm PSII core emission region.

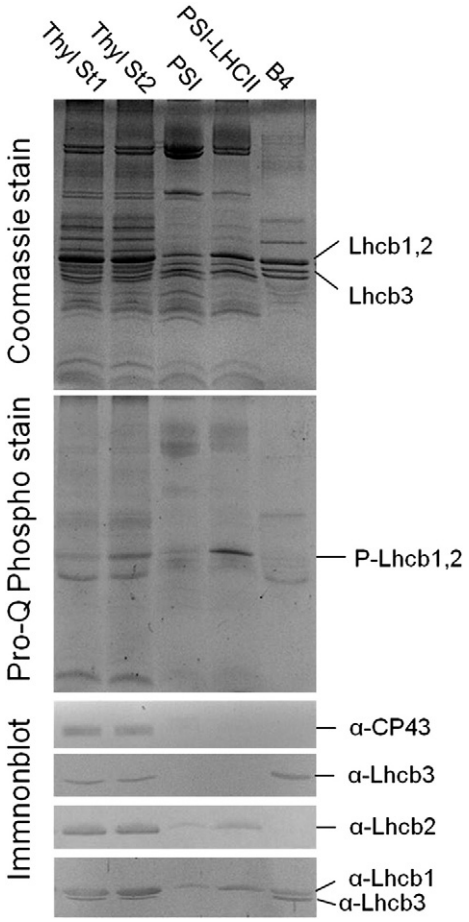

Fig. 2. Analysis of the LHCII protein composition in PSI-LHCII. Thylakoids isolated from plants in St1 or St2 were solubilized with 1% digitonin/0.1% α-DDM and run on a clear native PAGE (see Fig. 3). PSI-LHCII and PSI were excised from the gel, eluted and run on SDS-PAGE. Thylakoids in St1 and St2, as well as the B4 supercomplex (trimer-M-CP29-CP24) purified by sucrose gradient ultracentrifugation were loaded as reference. The total protein content was visualized by Coomassie Blue staining (top), the phosphorylated proteins were stained with Pro-Q Diamond Phosphoprotein Stain (middle) and specific proteins were identified with immunoblot analysis (bottom). The presence of small amounts of Lhcb1 and Lhcb2, but not of Lhcb3 in the PSI lane indicates that the sample is somewhat contaminated with PSI-LHCII.

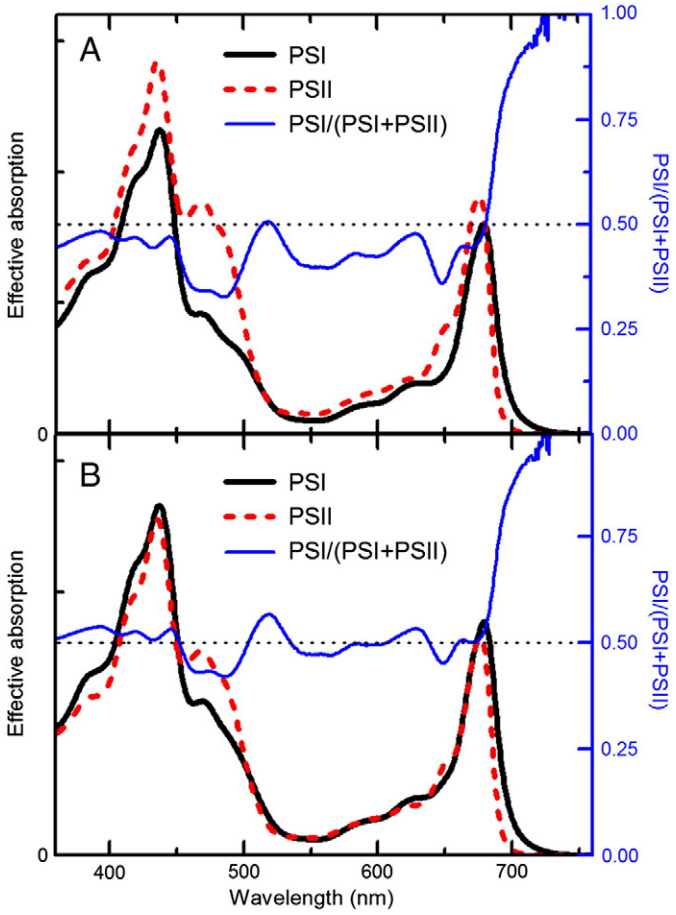

Fig. 3. Absorption spectra of PSI and PSII. Wavelength dependence of relative PSI and PSII absorption was estimated based on the PSI/PSII stoichiometry, PS composition and PS efficiency in ML plants assuming that all LHCII is associated with PSII (A), or part of LHCII is associated with PSI (B) as found to be the case under growth light.

effective absorption spectra (Fig. 3) were then obtained by correcting for the PSI efficiency of 98% (see below) and the PSII efficiency of 83% [49]. In St1 conditions the effective absorption of PSII is larger in almost the entire photosynthetically active radiation (PAR) range; only above 681 nm the PSI absorption is stronger (Fig. 3A). In moderate growth light it was found that 54% of the PSI complexes is associated with an LHCII trimer (Fig. 1), meaning that PSII has 0.4 LHCII trimers less per PSII core (based on the PSI/PSII ratio of 0.75). These numbers were used to calculate the effective absorption of PSI (including PSI-LHCI) and PSII in growth light. Clearly, the excitation pressure for white light is far better balanced between PSI and PSII (Fig. 3B) when part of LHCII is bound to PSI. The total effective absorption of the PAR (360–750 nm) in growth light is 1.04 times higher for PSI than for PSII, whereas in St1 the effective PSI absorption is only 80% of that of PSII (for the growth light spectrum these values are 98% and 74%, respectively (Fig. S2C, D)). This result shows that in white light the PSI antenna size needs to be increased by LHCII to balance the excitation between the two photosystems.

3.3. Excitation energy transfer from LHCII to PSI

In order to study the excitation energy transfer from LHCII to PSI, isolated PSI and PSI-LHCII were measured with TCSPC using 475 nm excitation pulses; at this wavelength a relative large fraction (35%, Fig. S2A) of the excitations is created on LHCII in PSI-LHCII. The fluorescence decay associated spectra (DAS) of PSI and PSI-LHCII are given in Fig. 4. The decay of PSI occurs with lifetimes of 24 ps, 79 ps and a minor contribution of 0.20 ns. For PSI-LHCII the decay lifetimes are only slightly longer, 33 ps, 96 ps and 0.24 ns. The energy transfer component from blue to red Chls often observed in PSI [32,50] was not resolved here due to the lower time resolution of the TCSPC as discussed in details in [32]. However, the decay components of PSI have very similar spectra and lifetimes as observed previously [32]. The two ns components with extremely small amplitude (<1%) probably arise from uncoupled antennas and/or chlorophylls and are further not considered. The main difference between the PSI and PSI-LHCII DAS is the stronger contribution of the two fastest components in the LHCII emission region (680 nm). This demonstrates that LHCII decays on this short sub-100 ps time scale. The average fluorescence lifetime of PSI and PSI-LHCII (calculated over the complete spectral range) are 66 ps and 73 ps, respectively. For a system of well-connected Chls one would expect that the average trapping time scales approximately linearly with the number of pigments (see e.g. [51]). The trapping time of 73 ps that is obtained for PSI-LHCII (coordinating 1.16 as many Chls *a* as PSI) is very close to 76 ps, i.e. 1.16 times 66 ps (average lifetime of PSI) showing that there is no bottleneck for energy transfer from LHCII to PSI. This result supports the findings of Galka et al. [36].

The quantum efficiency (Φ_{PSI}) of PSI in the presence and absence of LHCII can be calculated according to $\Phi_{PSI} = 100\% * ((k_{total} - k_{loss})/k_{total})$ where k_{total} is 1/(average lifetime of PSI) and $k_{loss} = 1/$(average lifetime of PSI in the absence of charge separation). The loss rate k_{loss} is estimated

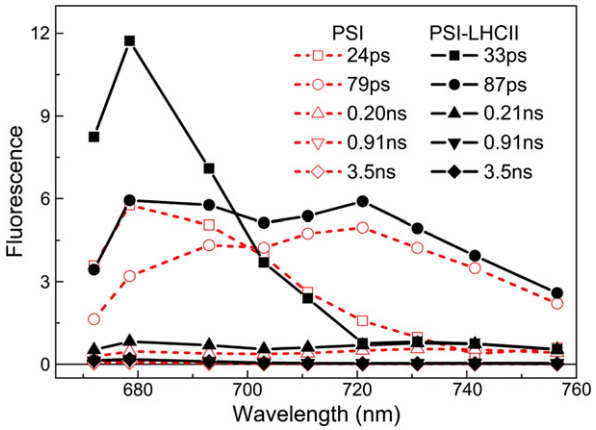

Fig. 4. Excitation energy trapping in PSI and PSI-LHCII. DAS of PSI (grey) and PSI-LHCII (black). Total area under the DAS of PSI-LHCII is normalized to 1.35 the value of PSI.

to be 0.3/ns based on the lifetimes of LHCI and LHCII, which are 2.5 ns [52] and 4 ns e.g. [53], respectively. The average PSI lifetime of 66 ps means that the total decay rate is 15.2/ns and $\Phi_{PSI} = 100\% * ((15.2 - 0.3)/15.2) = 98.0\%$. The average lifetime of PSI-LHCII is 73 ps, leading to $\Phi_{PSI\text{-}LCII}$ of 97.8%. Thus the association of LHCII with PSI, which increases the total number of Chls from 168 to 210 leads to a loss of quantum efficiency as small as 0.2%!

3.4. Excitation energy transfer from mobile LHCII to PSII

As it is not possible to purify PSII supercomplexes with the mobile LHCII, excitation energy transfer times in PSII were measured directly in the membrane. The fluorescence kinetics of intact FR and growth light thylakoids of ML plants were measured after excitation at 475 nm at 680 nm, 700 nm and 720 nm and fitted globally with four lifetimes (Table 1). PSI and PSI-LHCII contribute to the fastest (87 ps) component (Table 1) while PSII contributes to all four components [33]. A detailed description of the attribution of the individual lifetimes in the membrane is presented in [33]. Whereas for PSII supercomplexes with 2 trimers per reaction center (RC) and PSII membranes, with a slightly higher amount of LHCII trimers [54,55], no decay component is observed with a lifetime of ~0.54 ns, such a lifetime is present for thylakoid membranes with ~4 trimers per RC [33]. Thus this slow component is arising in the presence of relatively loosely bound LHCII trimers outside the supercomplexes (in the following indicated as "extra" LHCII). The 2.6 ns component stems from a small fraction of closed PSII RCs and/or uncoupled Chls. Upon relocating of LHCII from PSII (FR light) to PSI (growth light) the relative amplitude of the 86 ps increases at the expense of the 0.26 ns and 0.54 ns components. This can be explained by a decreased contribution of the "extra" LHCIIs to the "slow" PSII fluorescence decay, and an increased contribution to the 87 ps component by PSI-LHCII. This gives rise to a shorter average fluorescence lifetime of the thylakoids when LHCII is associated with PSI (Table 1). Taking together the data show that the mobile LHCII transfers its excitation energy slowly when associated to PSII (FR light) and fast when associated to PSI (growth light), leading to a decrease of the average lifetime in the latter case.

Table 1
Results of global fitting of fluorescence decay of ML thylakoids. Plants were harvested after treatment with 50 min of FR + low intensity blue light (FR) or after 6–7 h of growth light. The contributions (A_i) of the different lifetimes (τ_i) to the fluorescence decay, and the average fluorescence lifetime $<\tau> = \Sigma A_i * \tau_i$ are given.

	FR			Growth light		
	680 nm	700 nm	720 nm	680 nm	700 nm	720 nm
87 ps	40.1%	55.1%	62.8%	47.8%	59.5%	67.8%
0.26 ns	50.3%	37.3%	29.7%	46.1%	35.4%	27.5%
0.54 ns	9.2%	7.3%	7.3%	5.8%	4.9%	4.5%
2.6 ns	0.3%	0.3%	0.2%	0.3%	0.2%	0.2%
<τ>	215 ps	184 ps	171 ps	192 ps	169 ps	154 ps

4. Discussion

4.1. LHCII is part of the antenna of PSI under all growth conditions

LHCII is commonly defined as the major antenna complex of PSII. Only in response to short term exposure to PSII light or low light a fraction of LHCII (around 15% in land plants) is thought to move to PSI [42]. Our data show a different picture: in all growth conditions (LL, ML, HL, sunlight), a large part of the PSI population is complemented with one LHCII trimer, which acts as a functional PSI antenna with high excitation-transfer efficiency. The data actually show that LHCII transfers excitation energy faster and more efficiently to the reaction center of PSI than to that of PSII, when associated with either complex. This LHCII largely contributes to the "slow" 0.54 ns fluorescence decay component when it acts as a PSII antenna (Table 1), while it contributes to the fast sub-100 ps component when it is an antenna of PSI (Fig. 4).

The reason for favoring LHCII as PSI antenna becomes clear looking at the calculated absorption balance between the two photosystems which shows that if all LHCII is associated with PSII this PS would be overexcited at all wavelengths below 681 nm, while an almost perfect 1:1 ratio is obtained when the PSI antenna size is enlarged by LHCII as was found to be the case under growth light.

It is also shown that in agreement with previous results the transition to St1 is a short term response and it is induced by FR light and by sudden HL (NPQ conditions) when LHCII probably needs to move back to the grana to get quenched [19,47,56].

4.2. Short term vs. long term acclimation

It is generally believed that the redistribution of excitation energy between the two photosystems is maintained in the short term by state transitions and in the long term by photosystem stoichiometry adjustment [57–60]. In laboratory conditions St1 is induced by "PSI light" (far-red) and St2 by "PSII light" or low light. Changes in PSI/PSII stoichiometry are indeed observed upon long term acclimation to "PSII light" or "PSI light" [8–10]. However, while enrichment in far-red light mimics the light condition under a canopy, "PSII light" does not occur in Nature. Low light mimics far better the natural conditions, but the long term acclimation to low light leads to an increase of the antenna size of PSII [25,61], which is exactly the opposite of the effect produced by a St1 to St2 transition. This apparent contradiction has been pointed out before [19] but is still waiting for an explanation.

Our data show that LHCII is also associated with PSI after long term acclimation. This allows for the simultaneous adjustment of the PSI and PSII antenna size by regulating the level of Lhcb1 and Lhcb2 only, as previously hypothesized [25]. This also explains the puzzling observation that the amount of Lhca (PSI antenna) is constant under all light conditions, while that of Lhcb1 and Lhcb2 varies, increasing in LL as compared to ML and decreasing in HL conditions [25]. Our data show that the amount of LHCII associated with PSI changes in the same way: in LL grown plants 65% of the PSI is in the PSI-LHCII form, while this is 54% in ML and 40% in HL grown plants, demonstrating that the modulation of LHCII affects the antenna size of both photosystems and explaining why there is no need for the modulation of the level of Lhca complexes in different light conditions.

4.3. Summary

The picture that emerges from our data is that LHCII is a very efficient antenna for PSI, independent of the light intensity (Fig. 5). Only when stress is induced by a sudden profound increase of light intensity,

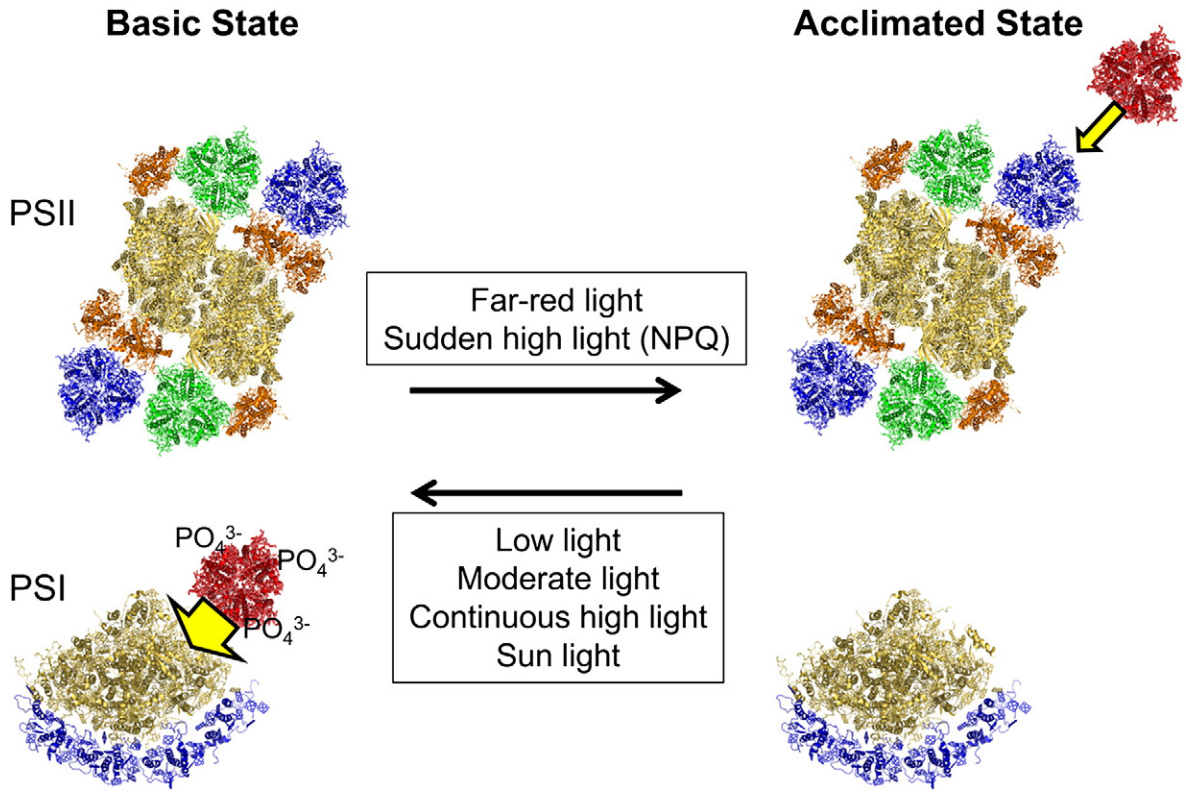

Fig. 5. Summary. PSII model is taken from [51] and PSI from [62]. The PSI and PSII cores are depicted in light orange, in PSII trimer M in blue, trimer S in green and the minor antenna, CP24, CP26 and CP29, in orange, and in PSI the Lhca antenna in blue. The mobile LHCII complex is depicted in red. The thickness of the yellow arrow represents the rate of excitation energy transfer to PSI or PSII.

or under strong over-excitation of PSI with far-red light, the LHCII antenna moves to PSII to get quenched or to increase the PSII antenna size.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbabio.2012.12.009.

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