Acclimation of *Chlamydomonas reinhardtii* to Different Growth Irradiances

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**Background:** Photosynthetic organisms deal with different irradiance conditions.

**Results:** In *Chlamydomonas reinhardtii*, acclimation to different irradiances of growth involves modulation of photosynthetic protein content per cell. Growth in high light induces activation of photoprotective mechanisms.

**Conclusion:** Higher plants and green algae have evolved different acclimatizing mechanisms.

**Significance:** Investigation of light-dependent modulation of photosynthetic proteins is crucial for directing optimization of microalgal growth for biomass production.

We report on the changes the photosynthetic apparatus of *Chlamydomonas reinhardtii* undergoes upon acclimation to different light intensity. When grown in high light, cells had a faster growth rate and higher biomass production compared with low and control light conditions. However, cells acclimated to low light intensity are indeed able to produce more biomass per photon available as compared with high light-acclimated cells, which dissipate as heat a large part of light absorbed, thus reducing their photosynthetic efficiency. This dissipative state is strictly dependent on the accumulation of LhcSR3, a protein related to light-harvesting complexes, responsible for nonphotochemical quenching in microalgae. Other changes induced in the composition of the photosynthetic apparatus upon high light acclimation consist of an increase of carotenoid content on a chlorophyll basis, particularly zeaxanthin, and a major downregulation of light absorption capacity by decreasing the chlorophyll content per cell. Surprisingly, the antenna size of both photosystem I and II is not modulated by acclimation; rather, the regulation affects the PSI/PSII ratio. Major effects of the acclimation to low light consist of increased activity of state 1 and 2 transitions and increased contributions of cyclic electron flow.

Sunlight is the most abundant source of energy available on Earth, and it is used by photosynthetic organisms to fix inorganic carbon into organic molecules and produce biomass. Oxygenic photosynthesis occurs by the activity of two types of photosystems, photosystem I and II (PSI and PSII), in which reaction centers catalyze primary light-dependent charge separation events driving electron transport from H₂O to NADPH. Reaction centers are functionally connected with each other and to their electron donor/acceptor by redox transporters such as the cytochrome b₆/f complex, whereas their capacity for absorbing photons is increased by light-harvesting antenna complexes that bind most of the chlorophyll and carotenoid pigments. In addition, ATPase synthesizes ATP from the *trans*-thylakoid proton gradient generated by the photosynthetic electron transport. The diversity in the organization of photosynthesis in organisms, originated from common prokaryotic ancestor(s), is largely because of their different light-harvesting machinery, although the structure of reaction centers is much more conserved.

Antenna complexes, besides harvesting photons, have an important role in photoprotection, which is an essential function because production of toxic reactive oxygen species (ROS) and consequent photoinhibition is an unavoidable consequence of oxygenic photosynthesis at all light intensities. In addition to species-specific differences in the molecular organization of the light-harvesting apparatus, acclimatized processes have been developed to cope with changes of irradiance in different environmental niches and/or different atmospheric conditions. Short term acclimatizing mechanisms respond to fast light intensity changes during the day length, due for example to transient clouds. Long term adaptation responds to shading by competing species, low/high irradiance in specific environments resulting in limitation in photosynthesis or oversaturating light intensity. Physiologic consequences range from metabolic energy shortage to absorption of excess excitation energy that leads to production of ROS. Responses to these environmental cues have been studied in higher plants, which have evolved a number of mechanisms including modulation of the stoichiometry and composition of peripheral light-harvesting antenna subunits (Lhcb) serving as photosystem II (PSII) antenna (5–7). In *Arabidopsis thaliana* PSII antenna system two monomeric (Lhcb4 and Lhcb6) and a trimeric subunit...
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(LHCI-S) are constitutively accumulated and bound to the PSII core to form C252 supercomplexes, whereas additional subunits located more peripherally, namely the monomeric Lhcb6 (CP24) and trimeric LHClI-M and LHClI-L, are accumulated with decreasing irradiance (6, 8). Higher plant PSI is also made of a core complex plus LHC antenna moieties, but its size is constitutive, whereas modulation is restricted to stoichiometric changes with respect to PSII to match plastoquinone reduction rate. Because the absorption spectra of PSI is somehow red-shifted with respect to PSII, changes in light quality may lead to an imbalance of PSII versus PSI excitation, leading to a decrease in light use efficiency, particularly under low light conditions. This can be corrected through a reversible transfer of LHClI-L from PSII to PSI in a mechanism named “state transitions” (7, 9) that are promoted by reversible phosphorylation of LHClI subunits by the STN7 thylakoid kinase (10), a mechanism inhibited in high light. The activity of the dark phase of photosynthesis is also modulated by light intensity; ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) accumulates with increasing light being a sink for ATP and reducing power accumulated in HL (6). Even HL-acclimated plants can experience excess illumination and respond by activating non-photochemical quenching (NPQ), by which energy dissipation into heat is catalyzed, preventing ROS formation. Accumulation of the carotenoid zeaxanthin upon excess light from pre-existing violaxanthin increases NPQ (11) and ROS scavenging (12). Strong illumination conditions was also reported to induce alternative pathways of electron transport, in particular cyclic electron flow of electrons around PSI (CEF) and the reduction of oxygen to water, mediated by the plastid alternative oxidase PTOX (13–17). Information available on acclimation behavior of green algae is somehow contrasting: Dunaliella salina and Dunaliella viridis were reported to undergo progressive reduction of the antenna size of both photosystems with increasing irradiance (18–20) similar to the case of the diatom Skeletonema costatum (21). In contrast, Dunaliella tertiolecta was reported to undergo changes in the stoichiometry of PSI versus PSII rather than modulation of their antenna sizes (21), suggesting that acclimation mechanisms in microalgae are species-specific. Chlamydomonas is the model system for green microalgae (10, 22–27), and yet its acclimation behavior to light intensity is unclear. Previous work showed that HL induced a decrease in the transcription and translation of Lhc proteins (28) suggesting modulation of antenna size. Besides its importance as a model system for molecular analysis of photosynthesis, Chlamydomonas reinhardtii is widely used in biofuel research as a converter of solar energy into biomass within photobioreactors where algae are grown at high density, thus producing a steep gradient of light intensity with excess light on the surface (leading to energy loss by thermal dissipation and photoinhibition) and limiting light below (limiting photosynthesis and producing energy loss by respiration). Engineering of light-harvesting apparatus has been proposed as a strategy for improving light use efficiency in producing biomass (20, 29–30, 32); moreover, knowledge of the biogenesis and acclimatizing response of photosystems becomes of crucial importance for planning genetic strategies. In particular, acclimatized modulation of antenna size implies compensation of genetically induced modifications by overexpression of other subunits (33). In this work, we analyzed in detail the acclimatized response of C. reinhardtii cells to light intensity and observed that modulation of the relative abundance of Lhc proteins is restricted to minor component(s) of the antenna system, whereas bulk antenna subunits are constitutively expressed. These results support the possibility of a genetically manipulated light-harvesting apparatus of C. reinhardtii for increased light use efficiency in photobioreactors.

EXPERIMENTAL PROCEDURES

C. reinhardtii Growth—C. reinhardtii cell wall-deficient mutants (cw15) were grown photoautotrophically in flasks in minimal medium under stirring and continuous light at different irradiances for at least 10 generations. The conditions used are as follows: control light (CL), 25 °C, 60 μmol m<sup>−2</sup> s<sup>−1</sup>; low light (LL), 21 °C, 20 μmol m<sup>−2</sup> s<sup>−1</sup>; high light (HL), 21 °C, 400 μmol m<sup>−2</sup> s<sup>−1</sup>.

Chlorophyll Fluorescence and Photosynthetic Function—Chlorophyll fluorescence was measured at room temperature on whole acclimated cells at a concentration of 2 × 10<sup>6</sup> with a PAM-101 fluorometer with a saturating light at 4500 μE and actinic light at different intensities. Before measurements, cells were dark-adapted under stirring for at least 60 min at room temperature. The parameters F<sub>v</sub>/F<sub>0</sub>, NPQ, and photochemical quenching (qP) were calculated, respectively, as (F<sub>m</sub> − F<sub>0</sub>)/F<sub>0</sub>, (F<sub>0</sub> − F<sub>v</sub>)/(F<sub>m</sub> − F<sub>v</sub>) and (F<sub>m</sub>′ − F<sub>v</sub>)/(F<sub>m</sub>′ − F<sub>0</sub>) (34). Photosynthetic O<sub>2</sub> production was measured at different actinic light densities in a Clark-type oxygen electrode (Hansatech) on whole cells at 26 °C under vigorous stirring samples as described previously (35). When needed, 3 mM n-propyl gallate was added before O<sub>2</sub> evolution measurement to inhibit PTOX enzymatic activity (36). PSI/PSII ratio, trans-thylakoid proton-motive force (PMF), ΔpH formation, and variation in the electric field (ΔΨ) upon illumination with different light intensities were monitored in the different acclimated cells measuring the carotenoid electro-chronic shift (ECS) with a Joliot-type spectrophotometer (Bio-Logic SAS JTS-10) as described previously (37, 38). Maximal state transition induction was measured in the different acclimated cells as described previously (39). PSI functional antenna size was measured following the kinetics of PSI fluorescence emission in cells treated with 1 × 10<sup>−5</sup> M 3-(3,4-dichlorophenyl)-1,1-dimethyleurea (DCMU) as described; PSI antenna size is inversely proportional to the time required for reaching 2/3 of the maximum fluorescence emission (40).

Thylakoid Isolation—Thylakoid membranes isolated from whole cells are described in Ref. 41.

P700 Oxidation Measurements—PSI reaction center (P700) oxidation kinetics were measured using a Joliot-type spectrophotometer (Bio-Logic SAS JTS-10) as described previously (42) on dark-adapted cells for at least 60 min or cells light-treated at different light intensities for 30 min. Measurements were performed by using actinic light at different intensities as described under “Results.” When needed, 1 × 10<sup>−5</sup> M DCMU was added to dark-adapted cells to block electron transport from PSII to PSI and to determine the influence of CEF as described previously (24). PSI functional antenna size was esti-
imated by measuring the kinetics of P700 oxidation in dark-adapted isolated thylakoids treated with $1 \times 10^{-5}$ M DCMU and 250 mM methyl viologen using a very low actinic light intensity (12 $\mu$mol m$^{-2}$ s$^{-1}$); PSI antenna size is inversely estimated by the time required for reaching $\frac{2}{3}$ of the maximum P700 oxidation.

Spectroscopy and Pigment Analysis—Pigments were extracted from acclimated cells in 80% acetone and analyzed by a combined approach of absorption spectroscopy and HPLC analysis as described previously (6). Low temperature emission fluorescence spectra were performed at 77 K by using a Fluoromax-3 (HORIBA Jobin Yvon) fluorometer on whole cells that were dark-adapted or light-treated for at least 2 h, pelleted, and dissolved in 80% glycerol, 20 mM MgCl and Hepes, pH 7.8. Fluorescence emission spectra were performed with 24- and 3-nm bandwidths for excitation and emission respectively.

SDS-PAGE Analysis, Immunoblot Assays, and Western Blotting Quantifications—Thylakoid membrane or protein extracts were obtained from whole cells was analyzed with SDS-PAGE as described previously (43, 44) in the case of LHCl analysis. Immunoblot assays with antibodies against different polypeptides were performed as described previously (45). To avoid any deviation between different immunoblots, samples were compared only when loaded in the same gel, as described previously (6).

RESULTS

Growth Rate upon Irradiance Changes—C. reinhardtii cells from the cw15 strain were grown in 15-ml liquid cultures at three different light intensities: 20 $\mu$E m$^{-2}$ s$^{-1}$ (LL), 60 $\mu$E m$^{-2}$ s$^{-1}$ (CL), and 400 $\mu$E m$^{-2}$ s$^{-1}$ (HL). Effects of light stress or light depletion were followed by measuring changes of PSI quantum yield ($Fv/Fm$) in the different conditions. $Fv/Fm$ was slightly lower in LL (0.66 ± 0.02) and HL (0.65 ± 0.03)-grown cells at the second generation upon transfer from control light, where the $Fv/Fm$ is generally 0.75 ± 0.02. In the following generations, the PSI efficiency recovered to a level similar to CL in LL but not in HL. HL cells indeed showed lower $Fv/Fm$ (0.65 ± 0.02) compared with CL (0.75 ± 0.01) and LL (0.73 ± 0.03) even after 10 generations. This result suggests that LL and HL cells are both acclimated to irradiance conditions, but acclimation to HL leads to a sustained reduction in $Fv/Fm$. The growth curve of LL, CL, and HL cells is reported in Fig. 1. HL cells grow slightly faster than CL, although LL grew substantially slower.

The generation time in the different conditions is reported in Table 1, being 36, 26, and 20 h for LL, CL, and HL cells, respectively. Biomass production in the different growth conditions was evaluated by measuring the average dry weight per cell per day of a culture in exponential phase (Table 1), resulting in slightly higher values for HL as compared with CL, although the value was substantially lower for LL cells. The photosynthetic light use efficiency for the different cultures was calculated dividing the dry weight production by the light intensity of growth (PAR); photosynthetic efficiency values are similar for CL and LL (4.94 $\times 10^{-4}$ and 5.23 $\times 10^{-4}$ g liter$^{-1}$ day$^{-1}$ PAR$^{-1}$, respectively) but strongly reduced in HL (8.64 $\times 10^{-5}$ g liter$^{-1}$ d$^{-1}$ PAR$^{-1}$).

Pigment Analysis—Acclimation to different irradiance conditions is well known to induce variations in pigment content and relative composition in higher plants. To verify if this was also the case in C. reinhardtii, pigments were extracted in 80% acetone and analyzed by a combined approach of HPLC and fitting of absorption spectra of acetone extracts with chlorophylls and carotenoids absorption forms (6). In Table 2, the pigment composition of the different acclimated cells is reported. Chlorophyll and carotenoid content per cell is inversely proportional to irradiance during growth, being strongly reduced in HL, suggesting a general reduction in the cell content in photosynthetic pigment proteins. Moreover, a higher amount of carotenoids per chlorophyll was observed in HL conditions resulting in a higher content in lutein and de-epoxidiized xanthophylls (anteraxanthin and zeaxanthin). Other xanthophylls, like violaxanthin and lorioxanthin, are instead enriched in LL and CL, compared with HL. No changes were observed in the Chl $a/b$ ratio in all conditions, within the error of the measurement. Because Chl $b$ is a component of light-harvesting antenna systems of both PSI and PSII, this result suggests that acclimation to different light conditions.

TABLE 1

| Sample | Generation time (h) | Dry weight/day (g liter$^{-1}$ day$^{-1}$) | PAR (µmol m$^{-2}$ s$^{-1}$) | PE (g liter$^{-1}$ day$^{-1}$ PAR$^{-1}$) |
|--------|---------------------|------------------------------------------|-----------------------------|----------------------------------------|
| LL     | 36 ± 2              | 1.83F-02 ± 3.79E-03                      | 35                          | 5.23E-04 ± 1.08E-04                    |
| CL     | 26 ± 1.5            | 2.97E-02 ± 7.44E-03                      | 60                          | 4.94E-04 ± 1.24E-04                    |
| HL     | 20 ± 1              | 3.45E-02 ± 4.69E-03                      | 400                         | 8.63E-05 ± 1.17E-05                    |
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**TABLE 2**

Pigments analysis of the acclimated cells

| Condition | Chlorophyll a (μg)/cell | Carotenoids (μg)/cell | Chl a/Car ratio | Chl a | Chl b | Car | Neo | Loro | Viola | Antera | Lute | Zea | B-car |
|-----------|--------------------------|-----------------------|-----------------|-------|-------|-----|-----|------|-------|--------|------|-----|------|
| LL        | 2.91                     | 1.05                  | 2.71            | 100.00|       | 68.85| 31.15| 23.98| 4.37  | 5.25   | 3.42 | 0.07| 3.48 |
| S.D.      | 0.51                     | 0.18                  | 0.01            | 1.31  | 0.59 | 0.03 | 0.11| 0.09 | 0.06  | 0.01  | 0.02 | 0.01| 0.28 |
| CL        | 2.37                     | 0.96                  | 2.61            | 100.00|       | 69.02| 30.98| 27.09| 4.64  | 4.18   | 3.32 | 0.70| 4.32 |
| S.D.      | 0.58                     | 0.24                  | 0.01            | 2.52  | 1.13 | 0.03 | 0.15| 0.18 | 0.40  | 0.30  | 0.34 | 0.25| 0.45 |
| HL        | 1.06                     | 0.63                  | 1.53            | 100.00|       | 69.73| 30.27| 39.49| 5.59  | 1.09   | 3.02 | 1.57| 6.31 |
| S.D.      | 0.42                     | 0.25                  | 0.01            | 1.41  | 0.61 | 0.18 | 0.23| 0.63 | 0.13  | 0.23  | 0.66 | 0.48| 0.38 |

Chlorophyll a and b (Chl a, b) and carotenoid (Car) amount per cell, Chl a/b ratio, and Chl/Car were measured by fitting analysis of the absorption spectra of pigments extracted in acetone. Qualitative distribution of carotenoids was analyzed by HPLC. Neo is neoxanthin; Loro is loroxanthin; Viola is violaxanthin; Antera is anteraxanthin; Lute is lutein; Zea is zeaxanthin; B-car is β-carotene.

Acclimation does not strongly influence photosynthetic antenna sizes in C. reinhardtii.

**Acclimation-dependent Changes in Composition of Photosynthetic Light Phase and Dark Phase Machineries**—To further evaluate the effects of light acclimation, we analyzed the protein composition of chloroplasts. We first focused on thylakoid membranes containing the protein complexes responsible for the light phase. We used specific antibodies to measure the relative amount of different photosynthetic subunits in the thylakoids purified from cells grown at different conditions.

As for the PSII core complex, we chose the following subunits: D1, one of the subunits responsible for binding the reaction center of PSII; CP43, one of the inner antenna proteins of the PSII core complex, and the 33-kDa subunit of the oxygen evolving complex. In the case of PSI core, we chose the PsaA subunit, as this protein involved P700 binding. Finally, we analyzed by Western blotting the amount of cytochrome f, and the amount of the δ subunit of chloroplastic ATPase complex as markers for the Cyt b₆f and chloroplastic ATPase accumulation. The relative abundance of these subunits in thylakoid membranes is reported in Fig. 2B, upon normalization to chlorophyll content. The D1 level is similar in all three conditions, showing that the amount of PSII is similar in all the acclimated cells on a chlorophyll basis, consistent with the content in the oxygen evolving complex polypeptides and CP43 (data not shown).

The level of the PsaA subunit was instead reduced in HL compared with LL and CL cells, suggesting a reduction of PSI/PSII ratio in this condition (Fig. 2C). The δ/ATPase ratio was confirmed by ECS spectroscopic measurement (38), obtaining absolute ratios of 2.02 for LL and CL and 1.38 for HL compared with LL and CL cells, suggesting a reduction of PSII/PSI ratio.

**Biochemical Analysis of Lhc Protein Content**—Acclimation to different light conditions in higher plants is well known to imply changes in the level of accumulation of PSII peripheral light-harvesting proteins, whereas PSI core complex maintains a fixed stoichiometry with its LHCI moiety. To monitor photo-system stoichiometry with their respective antenna moieties in C. reinhardtii, we used specific antibodies to analyze the accumulation of different Lhcb and LHCI subunits by Western blotting (Fig. 4). In particular, we investigated the presence and abundance of the following PSII and PSI antenna proteins, for which specific antibodies were available (44, 46): the PSII...
antenna complexes LHCII, CP26, CP29 (Fig. 4) and the nine LHCI subunits Lhca1–9 (Fig. 5). It should be noted that our antibody directed to LHCII trimers is not very specific and can detect most Lhcbm subunits with high efficiency, enabling the use of it as a general probe for Lhcbm proteins. In the case of LHCI proteins, we used the antibodies described in Ref. 44, in particular the specific antibodies p22.1, p14, and p15.1 for Lhca1, Lhca4, and Lhca5, respectively, and the antibodies p17.2, p13, p18.1, and p15 recognizing Lhca2, Lhca3, Lhca6, and Lhca7, respectively, but also other LHCI or Lhcb subunits at different molecular weights, as described previously (44).

Finally, we used the antibodies p18 and p22.1, recognizing Lhca8 and Lhca9, but also giving a faint cross-reaction with co-migrating Lhca5 and Lhca1 subunits (44). Density of the dye bands obtained from the immunological reaction was quantified and plotted versus chlorophyll or cell concentration (Figs. 4 and 5). The level of Lhcb and LHCI proteins per PSII and PSI can be calculated from data obtained by Western blot analysis, upon normalization to content of reaction center subunits D1 and PsaA, markers for accumulation of PSII and PSI, respectively. A general decrease of Lhc proteins per cells was evidenced in HL conditions, both in the case of PSII and PSI antenna proteins, in agreement with the reduction of photosynthetic reaction centers and chlorophyll per cells. On a chlorophyll basis or upon normalization to D1 content, PSII antenna proteins are instead accumulated to a similar level in all conditions, with a slight decrease in the case of CP26 and LHCII in HL, although within the error range (Fig. 4). LHCI proteins accumulation showed a dynamic behavior; most subunits, namely Lhca1–4, -6, -7, and -9, showed a decreased level of accumulation per chlorophyll in HL compared with CL and LL, with the exceptions of Lhca5 and Lhca8 (it is worth noting that antibody directed against Lhca8 partially also recognized the co-migrating subunit Lhca5). A slight increase of Lhca3 per PSI reaction center (PsaA level) was observed in LL conditions (Fig. 6), although the ratios LHCII/PsaA were essentially unchanged for the other subunits in LL compared with CL. In HL an increase of Lhca5 and Lhca8 and a decrease of Lhca4 and, to a greater extent, of Lhca7 were detected. It should be noted, however, that the average LHCII/PsaA ratio was similar in all light conditions, suggesting that acclimation induced changes in the relative composition of LHCI subunits, although the overall number of LHCI subunits per PSI core complex remained constant.

Functional Antenna Size of PSI and PSII—Western blot analysis enabled us to determine the stoichiometry of Lhc proteins per reaction center; however, these results based on the protein level cannot substitute for measurements of the functional antenna size of both photosystems. In fact, different Lhc protein family members might have different numbers of chromophores bound (2, 29, 47, 48) or do not bind pigments at all (49, 50). We thus complemented stoichiometric analysis with the measurement of functional antenna size on intact cells. This
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was performed by measuring the kinetics of PSI and PSII reaction center oxidation in limiting light in the presence of inhibitors of electron transport. In these conditions, the rate of P700 and P680 oxidation is inversely proportional to the functional antenna size of PSI and PSII, respectively. Fig. 6A shows the kinetics of fluorescence emission of PSII in dark-adapted cells treated with DCMU. In all conditions, CL, LL, and HL, the kinetics of PSII fluorescence emission was essentially the same, suggesting a similar functional antenna size. In Fig. 6B, the kinetics of P700 oxidation in dark-adapted cells treated with DCMU and methylviologen as electron acceptor are reported. Again, the rate of P700 oxidation is similar in all the acclimated cells, suggesting that also in the case of PSI, functional antenna size is constant upon acclimation to different light intensities (Fig. 6 and supplemental Fig. S2). It is important to point out that these functional measurements of PSI and PSII antenna size were performed after incubation of cells for 1 h in the dark under aerobic conditions to keep cells in state 1.

State 1 and State 2 Transitions—In addition to changes in its subunit composition and stoichiometry, the photosynthetic apparatus undergoes adaptation to light conditions by activating molecular mechanisms regulating protein-protein interactions, thus regulating light use efficiency. State 1 and state 2 transitions involve PSII Lhc proteins migrating from grana partition toward stroma membranes, thus balancing excitation between photosystems. Phosphorylation of PSII antenna proteins responsible for state transitions is mediated by STT7 kinase (10). We thus measured by Western blotting (Fig. 7A) accumulation of STT7 in the different conditions, obtaining a small increase (15 ± 5%) of the protein amount in HL and a small reduction in LL (30 ± 4%) on a chlorophyll basis with respect to CL, whereas on a cell basis STT7 level is half in HL as compared with CL (Fig. 7, B and C). To evaluate if acclimation influences the regulative capacity of C. reinhardtii, we measured the maximal capacity for state transitions in acclimated cells by treatments aimed to force state 1 or state 2, namely by incubating cells in the dark in presence of DCMU (state 1) or in anaerobiosis by addition of the Cyt-oxidase inhibitor NaN3 (state 2) and measurement of changes in PSII fluorescence emission intensity as described previously (39). Fig. 7D shows that the maximal capacity for state transitions is similar in acclimated cells and independent from light growth conditions. The activity of the STT7 kinase has been reported to be modulated by the redox state of plastids through formation of a disulfide bond between two cysteine residues (51); reducing conditions in high light was thus suggested to decrease kinase activity (51). To verify whether light acclimation did modulate the kinase activity, we recorded 77 K fluorescence emission spectra on intact cells by directly freezing cells either under growth light or upon adaptation for 1 h in the dark, a treatment effective in promoting transition to state 1. The spectra (Fig. 7, E–G) exhibited two major peaks at 685 and 715 nm, corresponding to PSII and PSI emissions, respectively. Transition to state 2 has been reported to increase the relative amplitude of the PSI peak, consistent with increased excitation from LHCII to PSI (51). Comparison of emission spectra of cells at growth light versus dark-adapted cells (in state 1) thus enables us to determine the situation of cells depending on light intensity. In the case of CL and LL, PSI emission is indeed clearly higher in light-adapted cells as compared with dark conditions. Instead, HL cells have emission spectra very similar, implying they are in state 1. This implies that despite the level of kinases up-regulated per chlorophyll depending on light intensity, and despite that the accli-
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- Mated cells have a similar capacity for state transitions upon chemical modulation of plastoquinone redox state in the dark, in physiologic high light conditions STT7 activity is inhibited by the chloroplast redox state.

Photosynthetic Function Is Modulated by Light Intensity during Growth—Biochemical analysis reported above suggests that acclimation to different irradiances implies changes in the efficiency of light conversion. We analyzed photosynthetic activity of acclimated cells by measuring oxygen production at different actinic light intensities (Fig. 8A). Indeed, on a chlorophyll basis, increasing irradiances during growth leads to an increase of $P_{\text{max}}$ in HL > CL > LL. Because PSII content per chlorophyll is similar in all conditions, an increased oxygen production with increasing light implies a more efficient electron transport at PSII level in HL > CL > LL. We verified this hypothesis by measuring the electron transport rate of PSII (Fig. 8B), which is indeed dependent on the intensity of irradiance during growth. Reduced electron transport rate in LL and CL cells as compared with HL cells is likely due to a less efficient oxidation of plastoquinone pool with respect to HL cells. The oxidation state of the first quinone acceptor in PSII, $Q_{A}$, can be investigated by measuring the qP of PSII fluorescence due to the inverse relation between $Q_{A}$ oxidation state and $1/Q_{P}$. In Fig. 8C, the maximum value of qP at different actinic light intensities is reported for acclimated cells; as expected, qP is always higher in HL cells versus CL and LL cells, and the latter shows lower qP values as compared with CL, yielding a more oxidized $Q_{A}$ in HL as compared with CL and LL conditions.

- Trans-Thylakoids Proton Gradient Formation—During the light phase of photosynthesis, electron transport across thylakoid membranes is coupled to proton accumulation into the lumen, which can be used by chloroplastic ATPase for ATP production. This movement of electrons and protons contributes to polarize the thylakoid membrane. The polarization of the membrane is well known to induce a shift in the absorption spectrum of carotenoids, called ECS (38). ECS can be used to determine the overall protonmotive force, lumen acidification by formation of trans-thylakoid $\Delta pH$, and the component of protonmotive force due to electric field formation ($\Delta \Psi$). To investigate if enhanced electron transport at the level of PSII in HL produces variation at PMF and its components, we measured the ECS at different light intensities as described previously (38). Our results, reported in Fig. 8, D–F, show that PMF formation is higher on HL compared with CL and LL, whereas $\Delta pH$ formation is similar in cells acclimated at different irradiances, indicating that the difference in PMF is solely due to $\Delta \Psi$.

- Nonphotochemical Quenching—NPQ is activated when light exceeds the capacity for electron transport to prevent ROS formation. We investigated the ability of cells to activate photoprotective excess energy dissipation, by measuring NPQ induction kinetics at different light intensities, from 100 to 1860 $\mu$E m$^{-2}$ s$^{-1}$. The NPQ induction kinetic at saturating light intensity (1860 $\mu$E m$^{-2}$ s$^{-1}$) is reported in Fig. 9A; HL cells show a maximal NPQ value of 2.7 when measured at very high light, and in LL and CL cells score below 0.5. In addition, dark recovery is almost complete in a few minutes in HL and CL cells,

FIGURE 6. Functional antenna size of photosystem I and II. Fluorescence emission kinetics of PSII from dark-adapted acclimated cells were treated with DCMU (A). The time required for reaching $2/3$ of the maximum is inversely proportional to PSII antenna size (B). Kinetics of P700 oxidation were measured as absorption differences ($\Delta$Abs) at 705 nm from dark-adapted acclimated cells treated with DCMU to inhibit electron transport from PSII; in these measurements methylviologen is used as final electron acceptor (C). The time required for reaching $2/3$ of the maximum is inversely proportional to PSII antenna size (D). a.u., arbitrary units.
whereas in LL cells, NPQ is hardly reversible, suggesting photoinhibition. The NPQ relaxation kinetics of CL and HL cells are instead very similar, suggesting photoprotection is efficient. Clearly, the fast-reversible component of NPQ (qE) is higher in HL cells and, to a lower extent, in CL cells, whereas LL cells show little activity. We obtained similar results at different actinic light intensities; maximal values of NPQ at different lights are reported in Fig. 9B, showing higher amplitude of NPQ in HL compared with CL and LL. Recently, NPQ induction was correlated to the presence of an Lhc-like protein, LhcSR3 (29, 52). We thus quantified by Western blot LhcSR accumulation in acclimated cells (Fig. 9C), obtaining a clear accumulation of this protein in HL >> CL > LL. In Fig. 9D, LhcSR3 level was plotted versus qE maximal activity, as calculated by subtracting the residual NPQ value after 3 min of dark relaxation from the maximum value of NPQ; the correlation obtained between LhcSR3 and qE is close to linearity, supporting the hypothesis that differences in qE level observed in acclimated cells depend on the level of LhcSR3 accumulation.

Alternative Electron Flows, Cyclic Electron Transport and Chlororespiration—Alternative electron flows, as cyclic electron flow around PSI and chlororespiration, have been far less studied in algae compared with higher plants. Growth in different conditions, however, requires a fine balance between phosphorylating (ATP) and reducing power (NADPH) supplies, on the basis of cell metabolic requirements (53–56). To verify if acclimation to different light conditions implies a preferential activation of CEF, we plotted in Fig. 10 the production of oxy-
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FIGURE 8. Photosynthetic properties of acclimated cells. A, oxygen production of LL (●), CL (★), and HL (△) cells normalized to chlorophyll content (milligrams)/h, measured at different actinic light intensities. B, electron transport rate (ETR) at the level of PSII of the different acclimated cells. C, photochemical quenching of acclimated cells at different light intensities. D, estimation of total proton motive force (ΔPMF) in acclimated cells upon exposure to 5 min of illumination at different light intensities, measured as difference in absorption at 520 nm, because of electrochromic shift of carotenoid absorption spectrum (ECS). E, extrapolation of trans-thylakoid ΔpH formation in acclimated cells on the basis of results reported in D; ΔpH is calculated as the portion of PMF relaxing in 20 s upon dark exposure, as described previously (38). F, extrapolation of total ΔΨ formation in acclimated cells on the basis of results reported in D and F; ΔΨ is calculated as the difference between ΔPMF and ΔpH as described previously (38).

gen with the level of PMF (Fig. 10A) and ΔpH in acclimated cells. Oxygen production gives a clear indication of LEF electron flow, whereas PMF and ΔpH are influenced by both LEF and CEF. In particular, Fig. 10, A and B, with high PMF and ΔpH at low oxygen production (left side of the graph) indicate additional proton transport into the lumen caused by CEF. Indeed LL and, to less extent, CL cells are clearly represented in Fig. 10, A and B, with high PMF and ΔpH at low oxygen production, whereas in the case of HL, high PMF and ΔpH are associated with a higher level of oxygen. These results suggest an increased activation of CEF in LL and CL as compared with HL. We independently confirmed the increased activation of CEF in LL and CL as compared with HL by measuring the level of P700 re-reduction upon illumination of cells treated with DCMU, inhibiting LEF; activation of CEF will result in a net lower oxidation of P700, because of partial re-reduction caused by electron transported by CEF. Upon normalization to overall PSI content, LL cells present a lower oxidation of P700 compared with CL, whereas HL cells oxidize P700 to the highest level, confirming the increased activation of CEF (supplemental Fig. S3) in LL > CL > HL.

A different type of electron flow alternative to LEF, previously reported on chloroplast, is the chlororespiration, by which oxygen is consumed by thylakoidal oxidase PTOX, similar to the mitochondrial alternative oxidase (57, 58). To verify the presence of changes of chlororespiratory activity in C. rein-
hardtii upon acclimation to different light intensities, we measured the accumulation of PTOX in thylakoid membrane and the O₂ evolution in the presence of propyl gallate, an inhibitor of PTOX. The accumulation of PTOX per chlorophyll is reported in Fig. 10 and supplemental Fig. S4 showing no significant differences between the different growth conditions. In Fig. 10D, the oxygen production in the presence or absence of propyl gallate is reported; the propyl gallate did not influence the production of oxygen, suggesting that PTOX activity is very low, if any, in all conditions. Thus, chlororespiratory electron transport does not significantly contribute to dissipate excess electrons in high light-acclimated cells under the conditions used in this work.

**DISCUSSION**

**Acclimation to Different Irradiance Conditions Modulates PSI and PSII Accumulation but Not Their Antenna Size**—In this study, we analyzed the effect of acclimation to different light intensities in C. reinhardtii.

The clearest effect induced by acclimation to different light intensities is a strong reduction of chlorophyll content per cell in HL; this leads to a reduction of capacity for light absorption per volume of culture when the same cell density is considered. Fig. 2 clearly shows that on a cell basis the levels of PSI and PSII are lower in HL and slightly increased in LL with respect to CL, in agreement with changes in chlorophyll content per cell. In particular, the strongest effect is exhibited by PSI, which is preferentially down-modulated versus PSII in HL, leading to a reduction of PSI/PSII ratio in HL as compared with CL. This ratio is instead slightly increased in LL. This results is consistent with higher plant response to HL acclimation, leading to a decrease of PSI/PSII ratio (6, 59), suggesting a general requirement for reduction of PSI stoichiometry versus PSII in conditions of high irradiance, possibly to avoid superoxide formation at the PSI donor site.

Besides the reaction centers, antenna protein accumulation is also down-regulated according to growth light intensity as shown in Figs. 4B and 5B. This result is in agreement with the report of transcriptional and post-transcriptional control of light-harvesting complexes upon HL treatment (28). Whatever the mechanism, accumulation of reaction center complexes and their antenna subunits appears to be strictly coordinated...
because no significant differences in functional PSI and PSII antenna size were observed by measuring the kinetics of P680 and P700 oxidation (Fig. 6) in agreement with titration of most antenna subunits of PSI and PSII (Figs. 4 and 5).

In the case of PSII, the composition and stoichiometry of the different Lhc subunit per reaction center does not show significant changes, with a ratio of Lhcb/PSII essentially conserved in all the different conditions. We observe that *C. reinhardtii* does not possess the Lhcb6 (CP24) subunit in its antenna system. Lhcb6 is a later addition to the Lhc family (60), and it is the subunit most extensively regulated during acclimation in higher plants (6) as well as during other kind of stresses, such as wounding, by the action of a specific Deg5 protease (61). Lhcb6 is a preferential site of binding for zeaxanthin, accumulated in excess light and the target of PsbS-dependent dissociation of the PSII supercomplex (62). These results suggest but do not fully prove that acclimatized modulation of PSII antenna size is a later development of plant photosynthesis upon the land colonization and recruitment of the new Lhcb6 subunit to the PSI-LHCII supercomplex. We cannot exclude that other microalgal species, such as *Dunaliella*, evolved different mechanisms for acclimating to HL, possibly by removing or accumulating specifically some light-harvesting proteins, as suggested previously (18). Our data suggest that *C. reinhardtii* evolved modulation mechanisms for the abundance of the PSII supercomplex as a whole for acclimation to different light intensities, rather than a change in its antenna size.

In the case of PSI, modulation of the relative abundance of antenna protein instead occurs depending on growth light intensity; Lhca3 is slightly increased in LL, whereas abundance of Lhca5 and Lhca8 increases in HL accompanied by a decrease in Lhca4 and Lhca7. By the way, because the antibody directed against Lhca8 partially recognizes Lhca5 as well, and the two subunits have very similar molecular weight, it was not possible to exclude that only Lhca5 was increased in HL, and the increase of Lhca8 was only due to cross-reaction with Lhca5. However, the overall PSI antenna system remains constant, implying the composition but not the size of the PSI antenna system is modulated, without changing the LHCI content per PSI reaction center. Modulation of PSI antenna composition has been previously shown upon iron deficiency (63). Our results are thus consistent with the suggested capacity of LHCI protein to replace each other in *C. reinhardtii*, demonstrating the plasticity of the PSI-LHCI complex being able to modulate the quality of its antenna subunits on the basis of the environmental signals. Recent reports have shown that Lhca5 and Lhca6 subunits, accumulated to low levels in *A. thaliana*, are required for the formation of the PSI-NDH complex involved in cyclic electron transport (64), whereas relatively high levels of this complex were detected in *C. reinhardtii* (24). Because we show that cyclic electron flow is reduced in HL acclimated cells versus LL and CL cells (Fig. 10), we tentatively suggest that Lhca4

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**FIGURE 10. Alternative electron flows in acclimated cells.** A, dependence of protonmotive force (PMF) from photosynthetic oxygen evolution in acclimated cells. Increased activation of CEF results in high PMF at lower oxygen production; both ΔPMF and oxygen evolution are normalized to chlorophyll content. B, dependence of trans-thylakoid ΔpH formation from photosynthetic oxygen evolution in acclimated cells. Increased activation of CEF results in high ΔpH at lower oxygen production-oxygen; both ΔPMF and oxygen evolution are normalized to chlorophyll content. C, PTOX protein level in acclimated cells quantified by Western blot analysis and normalized to chlorophyll or cell content. D, oxygen production in LL, CL, and HL cells normalized to chlorophyll content (milligram)/h, measured at 650 μmol m⁻² s⁻¹ in presence (+) or absence (−) of PTOX inhibitor PGAL.
and Lhca7 might be involved in the formation of the PSI-NDH complex in *C. reinhardtii*.

**Acclimation and Photosynthetic Electron Flow**—PSI and PSII are accumulated per cell inversely with respect to light intensity. On the contrary, Cyt *b*6f and chloroplastic ATPase are maintained per cell at high levels in all conditions, irrespective of growth irradiance and chlorophyll content per cell (Fig. 2). When normalized to chlorophyll content, Cyt *b*6f and ATPase are overaccumulated in HL, opposite to the reduction of PSI. This implies a higher content of Cyt *b*6f, the electron acceptor from the plastoquinone pool, and an increase of the capacity for linear electron transport in HL. In particular, an increase in proton translocation rate to the lumen and an increased proton pumping from the lumen to the stroma for ATP production occurs in HL. The increases in oxygen production and electron transport rate of PSII in HL, as well as an increase qP (Fig. 8), are in agreement with this view, leading to a higher efficiency of PSII electron transport from water to plastoquinone and an average lower reduction state of QA, efficiently oxidized by downstream electron acceptors, overaccumulated in HL. The increased ATPase content in HL, however, exploits the increased capacity for proton translocation into the lumen for ATP production, thus maintaining the lumenal pH is similar in all light conditions, despite an increased proton flux from stroma to lumen in HL (Fig. 8). In the case of higher plants and mosses, the increase of qP upon HL acclimation was related to an increase Calvin cycle activity, enabling NADPH and ATP consumption (6). This is also the case in *C. reinhardtii*, because the acclimation to HL leads to an increase of both qP and Rubisco accumulation. Increased activity of Calvin cycle enhances the release of ADP and NADP* that can be used to efficiently drain electrons from photosynthetic electron transport chain and reduce lumen acidification by activating ATPase. The efficient ATP production in HL can also be indirectly monitored from the lower level of CEF in these conditions, differently from LL and CL conditions (Fig. 10). The real importance of CEF in *C. reinhardtii* for lumen acidification and ATP production in *C. reinhardtii* is still under debate; even if evidence supports the occurrence of this electron transport cycle, the rates involved are far slower than LEF (150 e− s−1 for LEF compared with 5 e− s−1 for CEF) according to data reported by Alric and co-workers (56, 65). Our analysis indicates that upon light treatment, the kinetic of P700 oxidation are similar in all conditions (supplemental Fig. S3), whereas CEF activity becomes evident in LL and CL but not in HL (Fig. 10). This result is likely related to increased accumulation of Calvin cycle enzymes, consuming NADPH at faster rate than in CL and LL conditions, regenerating the final acceptor of electrons from PSI, NADP*. Our results confirm the relationship between CEF capability and state transitions, both increased in CL and LL, as reported previously (24), even if to a different extent, and in LL and CL cells, state transitions are similarly induced (Fig. 7), and CEF is more evident in LL conditions (Fig. 10 and supplemental Fig. S3). This result indicates that switching to state 2 promotes CEF, but the extent of state transition does not strictly correlate with the ratio between LEF and CEF. The physiological relevance of an increased ability for CEF in LL and CL conditions thus remains an open question.

**NPQ and Photoprotection Is Increased in HL Acclimated Cells**—Acclimation is a slow process requiring changes in gene expression, protein degradation, and new synthesis thus exposing photosynthetic organisms to transitory stressing conditions that can be counteracted by fast processes activated by primary signals such as the accumulation of protons in the thylakoid lumen under excess light. *Fv/Fm* is usually considered as a good indicator of the acclimation level of photosynthetic organisms, because it reflects the efficiency of PSII, one the major targets of abiotic stresses. In this study, *C. reinhardtii* cells were allowed to grow at the different light intensities for at least 10 generations with *Fv/Fm* values being stable upon the second generation. This result suggests that the acclimation process in *C. reinhardtii* occurs rather fast at least to the extent needed to prevent major damage to the PSI reaction center. Still *Fv/Fm* is somehow decreased in HL-acclimated cells as compared with LL and CL, likely because of the small level of photoinhibition, or because we observed that HL cells have a higher *Pmax* in O2 compared with LL and CL (Fig. 9), or because of activation of constitutive dissipation mechanisms in agreement with a previous suggestion (66). We have detected two major changes on cellular components related to quenching in HL cells, namely the accumulation of the two xanthophylls lutein and zeaxanthin and of the LHCSR3 protein. In Fig. 9E, the linear correlation between qE induction and LhCSR3 accumulation is reported. It should be noted that, differently from the case of higher plants, zeaxanthin is not directly related to energy quenching in *C. reinhardtii* because qE is similar in WT and in the zeaxanthin-less *npq1* strain (29). We conclude that the concomitant increase of lutein and LhCSR3 accumulation is, most likely, the factor responsible for the excess energy dissipation mechanism enhanced in *C. reinhardtii* HL-acclimated cells.

Zeaxanthin is known to act as a scavenger for reactive oxygen species, the most harmful secondary product of photosynthesis (5, 12, 67, 68). The increased carotenoid/Chl ratio detected in HL as compared with CL and LL, and in particular the higher level of zeaxanthin and lutein, suggests the occurrence of xanthophyll-dependent photoprotection mechanisms. Zeaxanthin in particular, beside ROS scavenging, is involved in quenching of singlet (11, 69) and in some cases triplet (70) chlorophyll excited states. In *C. reinhardtii* accumulation of zeaxanthin has been correlated to shortening of fluorescence lifetime imaging *in vivo* (71). Because zeaxanthin is not involved in qE, this effect is likely due to constitutive quenching of singlet excited states of Chl reported upon binding of zeaxanthin to Lhc proteins (4, 72–74), which is the molecular basis for qL component of NPQ (11). Thus, although zeaxanthin is not directly involved in qE in *C. reinhardtii*, its accumulation in HL cells provide a moderate level of excess energy dissipation and photoprotection through its binding to Lhc proteins. This scenario is different from that of higher plants where zeaxanthin plays a role in both qE, qL, ROS scavenging, and triplet quenching induction (11, 31, 62, 75–81).

HL cells thus protect themselves from potentially photo-inhibiting light through multiple mechanisms as follows. (i) zeaxanthin is accumulated, ensuring ROS scavenging and constitutive decrease of chlorophyll excited states lifetimes. (ii) LhCSR3 is accumulated, which is responsible for the fast heat
dissipation of light energy absorbed. (iii) Light-absorbing molecular structures are reduced, as evident from the decrease of pigments and photosynthetic complexes in HL on a per cell basis. The increased photoprotective capacity of HL-acclimated cells unavoidably results in a reduction in the efficiency of light energy conversion into biomass. HL cells indeed, besides a faster growth rate, are characterized by a reduced coefficient of photosynthetic efficiency (Table 1), indicating that these cells dissipate as heat a large fraction of photons absorbed. This is caused by the mode of acclimation to HL in C. reinhardtii that, differently from higher plants, dramatically up-regulates its ability to perform NPQ; LL and CL indeed show very low qE and NPQ induction even at very high light proportionally with the accumulation of LhcSR3. This is contrasting with the case of A. thaliana, where the capacity for NPQ is similar irrespective of growth light intensity (supplemental Fig. S5). It should be noted that C. reinhardtii HL cells exhibit high NPQ already at 350 μmol m⁻² s⁻¹ and saturation at 1000 μmol m⁻² s⁻¹. This is contrasting with the case of A. thaliana, where NPQ is proportional to actinic light up to 2000 μmol m⁻² s⁻¹ (supplemental Fig. S5). This result implies that LhcSR3-dependent (C. reinhardtii) and PsbS-dependent (A. thaliana) NPQ activation mechanisms in A. thaliana and C. reinhardtii are dramatically different, with strong heat dissipation activated in C. reinhardtii even with moderate light. This mechanism appears as the cause for the strong reduction of photosynthetic efficiency (coefficient of photosynthetic efficiency, Table 1) observed in HL cells in C. reinhardtii.

Conclusions—C. reinhardtii cells acclimated to different light conditions are characterized by a reduction of pigment content per cells leading to a modulation of PSI and PSII content. In particular, acclimation to HL induces a decrease of PSI/PSII ratios, whereas other protein complexes involved in the light phase, Cyt b₆f and chloroplastic ATPase, are increased. Instead, the antenna size of PSI and PSII is not modulated but for a small change in composition in the case of PSI. PSI antenna size regulation is obtained through the activation of state 1 to state 2 transitions in CL and LL conditions but not in HL. Our results strengthen the strict relationship between NPQ activation and LhcSR3 accumulation in HL, leading to strong heat dissipation even in moderate light. This effect results in reduced photosynthetic efficiency in cultures exposed to HL conditions. This suggests that domestication of C. reinhardtii strains for improvement of light energy conversion might be obtained by modulation of the heat dissipation response as much as by the reduction of cell optical density to improve the light diffusion properties of the culture.

REFERENCES
1. Alboresi, A., Caffarri, S., Nogue, F., Bassi, R., and Morosinotto, T. (2008) In silico and biochemical analysis of Physcomitrella patens photosynthetic antenna. Identification of subunits that evolved upon land adaptation. PLoS ONE 3, e2033
2. Schmid, V. H. (2008) Light-harvesting complexes of vascular plants. Cell. Mol. Life Sci. 65, 3619–3639
3. Dall’Osto, L., Fiore, A., Cazzaniga, S., Giuliano, G., and Bassi, R. (2007) Different roles of α- and β-branch xanthophylls in photosystem assembly and photoprotction. J. Biol. Chem. 282, 35056–35068
4. Ruban, A. V., and Johnson, M. P. (2010) Xanthophylls as modulators of membrane protein function. Arch. Biochem. Biophys. 504, 78–85
5. Niyogi, K. K. (1999) Photoprotection revisited. Genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 333–359
6. Ballottari, M., Dall’Osto, L., Morosinotto, T., and Bassi, R. (2007) Contrasting behavior of higher plant photosystem I and II antenna systems during acclimation. J. Biol. Chem. 282, 8947–8958
7. Pesaresi, P., Hertle, A., Pribil, M., Kleine, T., Wagner, R., Strissel, H., Ihnatovicz, A., Bonardi, V., Scharfenberg, M., Schneider, A., Pfannschmidt, T., and Leister, D. (2009) Arabidopsis STN7 kinase provides a link between short and long term photosynthetic acclimation. Plant Cell 21, 2402–2423
8. Morosinotto, T., Bassi, R., Frigerio, S., Finazzi, G., Morris, E., and Barber, J. (2006) Biochemical and structural analyses of a higher plant photosystem II supercomplex of a photosystem I-less mutant of barley. Consequences of a chronic over-reduction of the plastoquinone pool. FEBS J. 273, 4616–4630
9. Wollman, F. A. (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J. 20, 3623–3630
10. Bellafiore, S., Barneche, F., Peltier, G., and Rochaix, J. D. (2005) State transitions and light adaptation require chloroplast thylakoid protein kinase STN7. Nature 433, 892–895
11. Dall’Osto, L., Caffarri, S., and Bassi, R. (2005) A mechanism of nonphotochemical energy dissipation, independent from PsbS, revealed by a conformational change in the antenna protein CP26. Plant Cell 17, 1217–1232
12. Dall’Osto, L., Cazzaniga, S., Havaux, M., and Bassi, R. (2010) Enhanced photoprotection by protein-bound versus free xanthophyll pools. A comparative analysis of chlorophyll b and xanthophyll biosynthesis mutants. Mol. Plant 3, 576–593
13. Díaz, M., de Haro, V., Muñoz, R., and Quiles, M. J. (2007) Chlororespiration is involved in the adaptation of Brassica plants to heat and high light intensity. Plant Cell Environ. 30, 1578–1585
14. Ibáñez, H., Ballester, A., Muñoz, R., and Quiles, M. J. (2010) Chlororespiration and tolerance to drought, heat, and high illumination. J. Plant Physiol. 167, 732–738
15. Cournac, L., Josse, E. M., Joët, T., Rumeau, D., Redding, K., Kuntz, M., and Peltier, G. (2000) Flexibility in photosynthetic electron transport. A newly identified chloroplast oxidase involved in chlororespiration. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355, 1447–1454
16. Carol, P., Stevenson, D., Bisanz, C., Breitenbach, J., Sandmann, G., Mache, R., Coupland, G., and Kuntz, M. (1999) Mutations in the Arabidopsis gene IMMUTANS cause a variegated phenotype by inactivating a chloroplast terminal oxidase associated with phytoremediation. Plant Cell 11, 57–68
17. Aluru, M. R., Yu, F., Fu, A., and Rodermel, S. (2006) Arabidopsis variegation mutants. New insights into chloroplast biogenesis. J. Exp. Bot. 57, 1871–1881
18. Smith, B. M., Morrissey, P. J., Guenther, J. E., Nemson, J. A., Harrison, M. A., Allen, J. F., and Melis, A. (1990) Response of the photosynthetic apparatus in Dunaliella salina (green algae) to irradiance stress. Plant Physiol. 93, 1433–1440
19. Gordillo, F. J., Jiménez, C., Chavarria, J., and Xavier Niell, F. (2001) Photosynthetic acclimation to photon irradiance and its relation to chlorophyll fluorescence and carbon assimilation in the halotolerant green alga Dunaliella viridis. Photosynth. Res. 68, 225–235
20. Melis, A., Neidhardt, J., and Benemann, J. R. (1998) Dunaliella salina (Chlorophyta) with small chlorophyll antenna sizes exhibit higher photosynthetic productivities and photon use efficiencies than normally pigmented cells. J. Appl. Physiol. 10, 515–525
21. Falkowski, P. G., and Owens, T. G. (1980) Light-Shade Adaptation. Two strategies in marine phytoplankton. Plant Physiol. 66, 592–595
22. Diner, B. A., and Wollman, F. A. (1980) Isolation of highly active photosystem II particles from a mutant of Chlamydomonas reinhardtii. Eur. J. Biochem. 110, 521–526
23. De Vitry, C., Wollman, F. A., and Delepeule, P. (1984) Function of the polypeptides of the photosystem II reaction center in Chlamydomonas reinhardtii. Localization of the primary reactants. Biochim. Biophys. Acta 767, 415–422
24. Iwai, M., Takizawa, K., Tokutsu, R., Okamura, A., Takahashi, Y., and Minagawa, J. (2010) Isolation of the elusive supercomplex that drives cyclic
Acclimation to Light Conditions in Chlamydomonas reinhardtii

electron flow in photosynthesis. Nature 464, 1210–1213
25. Merchant, S., and Selman, B. R. (1984) Synthesis and turnover of the chloroplast coupling factor 1 in Chlamydomonas reinhardtii. Plant Physiol. 75, 781–787
26. Grossman, A. R., Karpowicz, S. I., Heinmichel, M., Dewez, D., Hamel, B., Dent, R., Niyogi, K. K., Johnson, X., Aliche, J., Wollman, F. A., Li, H., and Merchant, S. S. (2010) Phylogenomic analysis of the Chlamydomonas gene unmask proteins potentially involved in photosynthetic function and regulation. Photosyst. Res. 106, 3–17
27. Rochaix, J. D., Kuchma, M., Mayfield, S., Schirmer-Rahire, M., Girard-Bascou, J., and Bennoun, P. (1989) Nuclear and chloroplast mutations affect the synthesis or stability of the chloroplast psbC gene product in Chlamydomonas reinhardtii. EMBO J. 8, 1013–1021
28. Durnford, D. G., Price, J. A., Mckim, S. M., and Sarchfield, M. L. (2003) Light-harvesting complex gene expression is controlled by both transcriptional and post-transcriptional mechanisms during photoacclimation in Chlamydomonas reinhardtii. Physiol. Plant. 118, 193–205
29. Bonente, G., Ballottari, M., Truong, T. B., Morosinotto, T., Ahn, T. K., Fleming, G. R., Niyogi, K. K., and Bassi, R. (2011) Analysis of LhcSR3, a protein essential for feedback de-excitation in the green alga Chlamydomonas reinhardtii. PLoS Biol. 9(1): e1000577
30. Mussgnug, J. H., Klassen, V., Schlueter, A., and Kruse, O. (2010) Microalgae as substrates for fermentative biogas productions in a combined biorefinery concept. J. Biotechnol. 150, 51–56
31. Johnson, M. P., Perez-Bueno, M. L., Zia, A., Horton, P., and Ruban, A. V. (2009) The zeaxanthin-independent and zeaxanthin-dependent qE components of nonphotochemical quenching involve common conformational changes within the photosystem II antenna in Arabidopsis. Plant Physiol. 149, 1061–1075
32. Polle, J. E., Benemann, J. R., Tanaka, A., and Melis, A. (2000) Photosynthetic apparatus organization and function in the wild type and a chlorophyll b-less mutant of Chlamydomonas reinhardtii. Dependence on carbon source. Planta 211, 335–344
33. Andersson, J., Wentworth, M., Walters, R. G., Howard, C. A., Ruban, A. V., Horton, P., and Jansson, S. (2003) Absence of the Lhcb1 and Lhcb2 proteins of the light-harvesting complex of photosystem II. Effects on photosynthesis, grana stacking, and fitness. Plant J. 35, 350–361
34. Demmig-Adams, B., Adams, W. W., Barker, D. H., Logan, B. A., Bowling D. R., and Verhoeven, A. S. (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant. 98, 253–264
35. Falk, S., Leverenz, J. W., Samuelsson, G., and Oquist, G. (1992) Changes in photosystem II fluorescence in Chlamydomonas reinhardtii exposed to increasing levels of irradiance in relationship to the photosynthetic response to light. Photosyst. Res. 31, 31–40
36. Josse, E. M., Simkin, A. J., Galfé, J., Labouret, A. M., Kuntz, M., and Carol, P. (2000) A plastid terminal oxidase associated with carotenoid desaturation during chloroplast differentiation. Plant Physiol. 123, 1427–1436
37. Cruz, J. A., Kanazawa, A., Treff, N., and Kramer, D. M. (2005) Storage of in vivo photochemical instability. A useful probe to study algal photosynthesis. J. Biol. Chem. 280, 794–801
38. Slusarenko, A., Caffarri, S., Morosinotto, T., and Bassi, R. (2008) Biochemical properties of the PsbS subunit of photosystem II either purified from chloroplast or recombinant. J. Biol. Chem. 277, 22750–22758
39. Dominici, P., Caffarri, S., Armeanete, F., Ceoldo, S., Crimi, M., and Bassi, R. (2002) Biochemical properties of the PsbS subunit of xanthophylls studied in vivo and in vitro. J. Biol. Chem. 283, 8434–8445
40. Demmig-Adams, B., Adams, W. W., Barker, D. H., Logan, B. A., Bowling D. R., and Verhoeven, A. S. (1996) Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. Physiol. Plant. 98, 253–264
41. Lemesle, S., Willig, A., Depege-Fargeix, N., Delissent, C., Bassi, R., and Rochaix, J. D. (2009) Analysis of the chloroplast protein kinase Stt7 during state transitions. PLoS Biol. 7(3): e1000455 664–675
42. Alric, J. (2010) Cyclic electron flow around PSI during photosynthesis and plant stress response. Plant Physiol. 150, 19–31
43. Rumeau, D., Peltier, G., and Courlanges, L. (2010) Chlororespiration and cyclic electron flow around PSI during photorespiration. Photosyst. Res. 106, 47–56
44. Bonente, G., Howes, B. D., Caffarri, S., Smulevich, G., and Bassi, R. (2008) Interactions between the photosystem II subunit PsbS and xanthophylls studied in vivo and in vitro. J. Biol. Chem. 283, 8434–8445
45. Peltier, G., Toller, D., Billon, E., and Courlanges, L. (2010) Auxiliary electron transport pathways in chloroplasts of microalgae. Photosyst. Res. 106, 47–56
Acclimation to Light Conditions in Chlamydomonas reinhardtii

Evidence for direct carotenoid involvement in the regulation of photosynthetic light-harvesting. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4377–4382

74. Morosinotto, T., Caffarri, S., Dall’Osto, L., and Bassi, R. (2003) Mechanistic aspects of the xanthophyll dynamics in higher plant thylakoids. *Physiol. Plant.* **119**, 347–354

75. Ahn, T. K., Avenson, T. J., Ballottari, M., Cheng, Y. C., Niyogi, K. K., Bassi, R., and Fleming, G. R. (2008) Architecture of a charge-transfer state regulating light-harvesting in a plant antenna protein. *Science* **320**, 794–797

76. Avenson, T. J., Ahn, T. K., Zigmantas, D., Niyogi, K. K., Li, Z., Ballottari, M., Bassi, R., and Fleming, G. R. (2008) Zeaxanthin radical cation formation in minor light-harvesting complexes of higher plant antenna. *Physiol. Plant.* **119**, 347–354

77. Avenson, T. J., Ahn, T. K., Niyogi, K. K., Ballottari, M., Bassi, R., and Fleming, G. R. (2009) Lutein can act as a switchable charge transfer quencher in the CP26 light-harvesting complex. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 2830–2835

78. Lambrev, P. H., Nilkens, M., Miloslavina, Y., Jahns, P., and Holzwarth, A. R. (2010) Kinetic and spectral resolution of multiple nonphotochemical quenching components in *Arabidopsis* leaves. *Plant Physiol.* **152**, 1611–1624

79. Demmig-Adams, B., Adams, W. W., Heber, U., Neimanis, S., Winter, K., Krüger, A., Czygan, F. C., Bilger, W., and Björkman, O. (1990) Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. *Plant Physiol.* **92**, 293–301

80. Demmig-Adams, B., Adams, W. W., Logan, B. A., and Verhoeven, A. S. (1995) Xanthophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. *Aust. J. Plant Physiol.* **22**, 249–260

81. Ruban, A. V., and Horton, P. (1999) The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach *Plant Physiol.* **119**, 531–542

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